



TURKISH - FEPS PHYSIOLOGY CONGRESS

September 3-7, 2011

Yeditepe University



Istanbul

Abstract Submission Deadline 20 May 2011



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address: Cumhuriyet Cad. No:173/2 34373 Harbiye, Istanbul-Turkey
phone: +90 212 368 47 95 fax: +90 212 230 64 25
e-mail: feps2011@vip.com.tr



WELCOME LETTER

Dear Colleagues,

It is a great pleasure and honour for us to invite you to the joint congress of the Turkish Society of Physiological Sciences (TFBD) and European Federation of Physiological Societies (FEPS) to be held at Yeditepe University in Istanbul, Turkey between 3 to 7 September, 2011. This will be the 8th Congress of FEPS and 37th National Physiology Congress of TFBD.

The meeting will start with the traditional European Young Physiologist Symposium on September 3, organized by and for young physiologists. There will be a Masters Session on the same afternoon to welcome distinguished senior physiologists. Official opening ceremony will take place in the evening of the same day followed by the FEPS keynote lecture presented by Professor Antoon Moorman of University of Amsterdam.

The main scientific program will run from 4 to 6th September and is composed of 5 plenary lectures, 18 symposia, two workshops, 49 oral and 321 poster presentations. Symposia, workshops and plenary lectures have been carefully selected to include excellent speakers and cover all topics of physiology including teaching.

September 7 is reserved for an optional social and cultural program; the costs of transportation and cruise on the Bosphorus are included in the registration fee. There will be additional spouse programs during the meeting (September 4, 5 and 6th) for accompanying persons.

Istanbul is the former capital of three successive empires, namely the Roman, Byzantine and Ottoman, and honours and preserves the legacy of its past while looking forward to a modern future. Istanbul is the cultural capital city of Europe in 2010. It is a city where continents, cultures and history meet. Divided by the Bosphorus, Istanbul forms a link between Europe and Asia (Anatolia). No doubt that you will enjoy this beautiful city with all its colours, sounds, historic architecture and exotic markets.

We very much hope that you will have an unforgettable stay in Istanbul, enjoying science at this joint Turkish-FEPS Congress and experiencing culture and history of this remarkable city. We are looking forward to sharing this unique experience with you in Istanbul.

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Prof. Dr. Neyhan Ergene
Turkish Society of Physiological Sciences

Prof. Dr. Bayram Yılmaz
Yeditepe University

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- Res.Assist. Dr. Murat Doğan

PROGRAMME

Time	Blue Hall
All Day	Registration
09.30-09.45	Welcome & Introduction
09.45-10.00	What FEPS offers to young physiologists Ulrich Pohl
10.00-10.40	EYPS Keynote Lecture (Ahmet Korkmaz) Epigenetic mechanisms in human physiology and diseases
10.40-11.00	Coffee Break
11.00-12.45	Oral Presentations (OC 1 – OC 7)
12.45-14.00	Lunch and Poster Presentations
14.00-15.20	Oral Presentations (OC 8 – OC 13)
15.20-15.30	Coffee Break
	İnan Kırac Conference Hall
15.30-17.30	Respect to Masters Session Miklos Palkovits : New information regarding to neuronal circuits involved in the central regulation of food intake David Carpenter: New understanding of diseases caused by old chemicals Üner Tan : Üner Tan Syndrome; Human Quadrupedalism: History, Clinics, Genetics and the Self-Organized Emergent Properties
17.40-18.10	FEPS 2011 Opening Ceremony
18.10-19.00	FEPS Keynote Lecture (Antoon Moorman) Development of the building plan of the heart
19.30-22.00	Welcome Reception (Medical School)
Green Hall (Workshop I)	
Workshop I: "Lung, Macrophages, Hypoxia, Inflammation"	
08.30-09.45	Registration

09:45-10:00	Opening/welcome ceremony
10:00-10:30	(W 1) The pulmonary vasculature: Paul McLoughlin
10:30-11:00	(W 2) The alveolar barrier: Heimo Mairbäurl
11:00-11:15	Coffee Break
11:15-12.45	Short communications (W 3, W 4, OC 14, OC 15, OC 16)
12.45-14:00	Poster Session and Lunch Inflammation and hypoxia
14:00-14:30	(W 5) Inflammation and hypoxia: Stilla Frede
14:30-15:00	(W 6) Role of PHD in inflammation: Martin Schneider
15:00-15:30	Short communications (W 7, W 8)
15:30-16:00	Coffee Break Workshop: Hypoxia in the lab
16:00-16:30	(W 9) What is "tissue PO ₂ "? Heimo Mairbäurl
16:30-16:50	(W 10) Handling cells in hypoxia. Emel Baloglu
16:50-17:35	Short communications (W 11, OC 17, OC 18)
17:35-18:00	FEPS Opening Ceremony
18:00-18:50	FEPS Keynote Lecture

4th September (Sunday)

Time	Inan Kırac Conference Hall	Blue Hall
09.00-10.40	Symposium 1 Molecular physiology of chemical transmission in neuronal-glia circuits Vladimir Parpura, Peter Illes, Alexei	Symposium 2 Cardiovascular and respiratory reflexes: from physiology to clinics Alexander Gourine, Isabel Rocha, Susan A.

	Verkhatsky, Frank Kirchhoff	Deuchars, Max J. Hilz
10.40-11.10	Coffee Break	Coffee Break
11.10-11.55	Plenary Lecture (Can Ince) The pathophysiology of the septic myocardium: From microcirculation to the mitochondria	
12.00-13.00	Poster Session	Poster Session
13.00-14.00	Lunch	Lunch
14.00-15.40	Symposium 3 Purinergic signaling – coupling glia and neurons in health and disease Helmut Kettenmann, Cristina Limatola, Sergei Kirischuk, Mami Noda	Symposium 4 The increasing physiological role of brown adipose tissue Jan Nedergaard, Miguel Lopez, Michael Symonds, Francesc Villarroya
15.40-16.10	Coffee Break	
16.10-17.50	Symposium 5 Physiology of the (pro)renin receptor Agnes Prokai, Genevieve Nguyen, Jan Danser, Dominik Muller	Symposium 6 Functional alterations of membrane channels and neuronal excitability in epilepsy and muscle diseases Snezana Maljevic, Massimo Mantegazza, Karin Jurkat Rott, Matthew Walker
17.50-18.35	Plenary Lecture (Susan Wray) Uterine smooth muscle - can we tame the beast within?	

Green Hall (Workshop I)

	(Midkine Overview)
09:00-09:30	(W 12) Midkine: a multifunctional protein involved in reproduction, repair and pathogenesis of inflammatory and malignant diseases. Takashi Muramatsu
09:30-10:00	(W 13) Midkine signaling: from embryogenesis to disease. Mine Erguven
10:00-10:45	Short communications (W 14, W15, W 16)
10.45-11.00	Coffee Break
11.10-11.55	Plenary Lecture
12.00-14.00	Poster Session and Lunch Midkine, hypoxia and Inflammation

14:00-14:30	(W 17) Antimicrobial Properties of Midkine - New Roles for an old Protein. Sara Svensson
14:30-15:00	(W 18) Midkine, hypoxia and Inflammation: Signalling Pathways. Nuray Yazihan
15:00-15:30	Short communications (W 19, W 20)
15.30-16.00	Coffee Break Workshop: Visualizing hypoxic cell signaling
16:00-16:30	(W 21) Visualizing hypoxia and cellular oxygen sensing. Joachim Fandrey
16:30-17:00	(W 22) Gene/protein expression analysis: microarrays/2D, Gene/Protein Expression Analysis: Proteome, Microarrays. Duygu Ozel Demiralp
17:10-17:30	(W 23) High Throughput Experimentation for Hypothesis Generation and Testing. Bertal Huseyin Aktas
FEPS Executive Committee Meeting: 11.00 – 14.00 (# 448, Physiology Lab, Medical School)	

5th September (Monday)

Time	İnan Kırac Conference Hall	Blue Hall
09.00-10.40	Symposium 7 Erythropoietin and Brain Max Gassmann, Ertugrul Kilic, Hannelore Ehrenreich, Christian Grimm	Symposium 8 Receptors for amino acids, fatty acids and metabolites: novel detectors of metabolism Joris Robben, Graeme Milligan, Hans Brauner-Osborne, Janos Peti-Peterdi
10.40-11.10	Coffee Break	Coffee Break
11.10-11.55	Plenary Lecture (Alan North) Molecular physiology of P2X receptors	
12.00-13.00	Poster Session	Poster Session
13.00-14.00	Lunch	Lunch

14.00-15.40	Symposium 9 Hypoxia's impact on the regulation of food intake and fat metabolism Wolfgang Langhans, Margriet Westerterp, Thomas Lutz, Paul Trayhurn	Symposium 10 New insights into the physiology, pathophysiology and pharmacology of non-vascular smooth muscle Eda Kumcu, Karl Sward, Chris H. Fry, Rachel F. Floyd
15.40-16.10	Coffee Break	
16.10-17.50	Symposium 11 Oxidative stress in ischemia-reperfusion injury in skeletal muscle, in exercise and inflammation, and influence of estrogen on skeletal muscle damage. Mustafa Gül, Bernard Geny, Peter Tiidus, Sashwati Roy, Osmo Hanninen	Symposium 12 Spinal and peripheral mechanisms in nociception and chronic pain Rita Bardoni, Richard Carr, Sue Fleetwood-Walker, Valerio Magnaghi
17.50-18.30	Oral Presentations (OC 19 – OC 21)	Oral Presentations (OC 22 – OC 25)
19.30-23.00	Gala Dinner (Sabancı Öğretmenevi, Anadolu Hisarı)	

Green Hall

09.00-10.40	
10.40-11.10	
11.10-11.55	
12.00-13.00	Poster Session
13.00-14.00	Lunch
14.00-17.30	Workshop II (W 24) Integration of Physiology Learning in Clinical Settings Berrak Yegen, Mehmet Ali Gulpinar, Seref Erdogan, Levent Ozturk, Ersin Koylu, Ozgur Kasimay
17.50-18.30	Oral Presentations (OC 26 – 29)

JAFM Meeting: 09.00 – 10.45 (# 448, Physiology Lab, Medical School)

FEPS Council Meeting: 11.00 – 13.00 (Uzeyir Garip Hall)

6th September (Tuesday)

Time	İnan Kırac Conference Hall	Blue Hall	Green Hall
09.00-10.40	Symposium 13 Physiological responses to pressure Peter Norsk, Christian Aalkjaer, James Hicks, Tobias Wang	Symposium 14 Cellular dysfunction in heart disease; a paradigm of structural disorganisation and signalling failure Katharine Dibb, Ali El-Armouche, Elizabetta Cerbai, Patrick Most	Symposium 15 Neuroglial roots of neurodegenerative diseases José Julio Arellano, Daniela Rossi, Michael Heneka, Alain Privat
10.40-11.10	Coffee Break	Coffee Break	
11.10-11.55	Plenary Lecture (Gareth Leng) Neurotransmitters and peptides: whispered secrets and public announcements		
11.55-12.10	OC 30 (Bazbek Davletov) Regulation of neurotransmitter release by new botulinum molecules		
12.10-13.00	Poster Session	Poster Session	Poster Session
13.00-14.00	Lunch	Lunch	Lunch
14.00-15.40	Symposium 16 Diabetes, Obesity and GLP-1: Can a small peptide solve big problems? Jens Juul Holst, Diana Williams, Stefan Trapp, Wolfgang Langhans	Conference (Robert Carroll) Using educational research to improve your teaching Oral presentation (OC 31)	Oral Presentations (OC 32 – 40)
15.40-16.10	Coffee Break		
16.10-17.50	Symposium 17 What is new in calcium signaling? Ole H Petersen, Alexei Verkhratsky, Tullio Pozzan, Javier Garcia-Sancho	Symposium 18 Mouse models of vascular permeability control: Visualizing the Future Fitz-Roy Curry, Michaela Kuhn, Ritva Heljasvaara, Cecilie B Rygh	Oral Presentations (OC 41 - 49)
17.50-18.30	Closing Session and Award Ceremony		

Poster Presentations & Numbers

4th September (Sunday), 12.00 – 14.00h

PC 1 – PC 15 (Cellular & Molecular Physiology-I)

PC 31 – PC 45 (Muscle Physiology)

PC 76 – PC 90 (Neurophysiology –I)

PC 91 – PC 105 (Neurophysiology –II)

PC 167 – PC 181 (Endocrine Physiology-I)

PC 213 – PC 228 (Exercise Physiology-I)

PC 245 – PC 260 (Oxidative Stress – I)

5th September (Monday), 12.00 – 14.00h

PC 16 – PC 30 (Cellular & Molecular Physiology-II)

PC 46 – PC 60 (Cardiovascular & Respiratory Physiology – I)

PC 106 – PC 120 (Neurophysiology –III)

PC 121 – 135 (Neurophysiology –IV)

PC 229 - PC 244 (Exercise Physiology-II)

PC 261 – PC 275 (Oxidative Stress – II)

PC 291 – PC 305 (Reproduction)

6th September (Tuesday), 12.00 – 14.00h

PC 61 – PC 75 (Cardiovascular & Respiratory Physiology – II)

PC 136 – PC 150 (Neurophysiology –V)

PC 151 – PC 166 (Neurophysiology –VI)

PC 182 – PC 196 (Endocrine Physiology-I)

PC 197 – PC 212 (Pathophysiology)

PC 276 – PC 290 (Oxidative Stress – III)

PC 306 – PC 321 (Others)

ABSTRACTS

- Plenary Lectures (PL01-PL10)**
- Symposia (S1.1-S18.5)**
- Workshops (W01- W24)**
- Oral Communications (OC01- OC49)**
- Poster Communications (PC001- PC321)**

Plenary Lectures

PL01

Development of the building plan of the heart

Antoon F M Moorman

Department of Anatomy, Embryology & Physiology, Academic Medical Centre, Amsterdam

One of the most fascinating aspects in the formation of the heart is the very early development of the electrical patterning as can be registered by the ECG, which is the registration of the rhythmic waves of depolarizing activity over the cardiac muscle. In the mature heart, the conduction system is held responsible for the rhythmic excitations and contractions. However, in chicken embryos a sinusoidal type of ECG can already be derived from the linear heart tube stages at about two days of development onward, and less than one day later when chamber formation has just been initiated, an adult type of ECG can be monitored. The presence of an adult type of ECG in these early embryonic hearts betrays the development of fast-conducting chambers rather than the presence of a conduction system. We now know that the primary heart tube as seen in the early embryo contains the precursors for the left ventricle only, or even less, whereas the precursor cells for the remainder of the cardiac components are continuously added to both the venous and arterial pole of the heart tube during further development from a single center of growth outside the heart. Therefore, it is impossible that the straight heart tube contains the precursors for the conduction system as rings separating the purported cardiac segments. While the primary heart tube is growing by addition of cells it does not show significant cell proliferation, until chamber differentiation and expansion starts locally in the tube. The transcriptional repressors Tbx2 and Tbx3 locally repress the chamber-specific program of gene expression, by which these regions are allowed to differentiate into the distinct components of the conduction system. The cardiac building plan and the underlying mechanisms of its formation are conserved from fish to man. Detailed reconstructions of the developmental patterns of expression of Tbx3 during development in mouse and human have revealed, that Tbx3 is expressed in those areas of the heart tube that do not become chamber, i.e. in the sinu-nodal region, internodal region, atrioventricular junction, atrioventricular bundle and bundle branches. These areas comprise not only the conventional conduction system, but also the highly controversial areas of the internodal region and the entire atrioventricular junction. Also the (right) ventricular outflow tract initially expresses these transcriptional repressors, preventing it from chamber differentiation. These observations provide an embryonic basis why some areas in the heart are more arrhythmogenic than other regions.

References:

Moorman AFM & Christoffels VM (2003). *Physiol Rev* 83:1223–1267.

van den Berg G et al. (2009). *Circ Res* 104:179-188.

Christoffels VM & Moorman AFM (2009). *Circulation* 2: 195-207.

Sizarov A et al (2011). *Morphogenesis, Growth and Differentiation. Circulation* 2011; 123:1125-1135.

The pathophysiology of the septic myocardium: From the microcirculation to the mitochondria

Can Ince¹

¹Department of Translational Physiology, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

²Department of Intensive Care, Erasmus Medical Center, Erasmus University of Rotterdam, s-Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands

The past four decades have seen the mortality of sepsis increase from 0.5 to 7 per 100,000 episodes with the occurrence of severe sepsis in for example the United States today being estimated at 750,000 cases per year, resulting in 215,000 deaths annually. The majority of these sepsis patients die of hypotension unresponsive to therapy and of systemic cardiovascular failure. One of the manifestations of cardiovascular dysfunction in septic shock is myocardial depression and despite much research its cause and treatment is largely unknown. This insight into the nature of septic cardiomyopathy was achieved in the early eighties with the key note publication from the group of Parrillo who identified the presence of sepsis-induced cardiac dysfunction in patients with septic shock using portable radionuclide cardiac imaging and simultaneous thermodilution cardiac output measurements. They identified that following the onset of septic shock patients had a depression of left ventricular ejection fraction (1). Several mechanisms have been proposed as underlying this depressed contractility associated with septic cardiomyopathy. One such mechanism has been regional myocardial ischemia and hypoxemia due to a failure in myocardial autoregulatory needed to match regional oxygen need by the myocytes by adequate microcirculatory delivery. The inflammatory mediators associated with sepsis and especially increased nitric oxide are thought to underlie this aspect of cardio-(micro) vascular dysfunction (2). Such abnormalities have been shown to give rise to weak microcirculatory units becoming hypoxemic (3), emphasizing the defect in the distributive capacity of the microvasculature to provide adequate oxygen to the organs. It is this microcirculatory failure which is thought to define the pathogenesis of sepsis leading to multi organ failure (4). Microcirculatory failure in otherwise well resuscitated systemic hemodynamic parameters results in the functional shunting of the microcirculation. It is this functional shunting which has made the clinical management of sepsis so difficult because impaired microcirculatory function can occur in the presence of a normal or even elevated cardiac output hiding as it were the actual defect from conventional monitoring of systemic hemodynamic variables such as blood pressure, stroke volume and cardiac output. Such abnormal microcirculation with the presence of shunting pathways can be observed clinically by the use of bedside intravital microscopic introduced by us to observe the microcirculation during surgery and intensive care (e.g. 5, 6). Clinically, microcirculatory shunting manifests itself as a deficit in the ability of tissues to extract oxygen from macroscopically delivered oxygen resulting in microcirculatory hypoxemia in the presence high venous oxygen pressures.

Besides the (micro) vascular dysfunction described above, cellular and sub cellular pathologies have been shown to be present especially in experimental studies. These pathogenic mechanisms include electrophysiological dysfunction due to abnormal ion channel activity resulting in action potential shortening and depressed functioning of gap junctions between cardiomyocytes resulting inefficient electrical conduction (7,8). Also a defect in intracellular calcium handling has been found to result in depressed actin myosin contraction in septic cardiomyocytes (9). Mitochondrial depression has often been speculated to be associated with many of the oxygen transport abnormalities such as oxygen extraction deficit, seen in sepsis (10). This involves the idea that despite adequate oxygen delivery to the parenchymal cells, the mitochondria are unable to utilize oxygen adequately by oxidative phosphorylation to produce ATP needed for cellular energy requirements. Although not in heart, but in skeletal muscle, clinical studies studying mitochondrial bioenergetics from muscle biopsies have indeed shown depressed mitochondrial

function to exist in septic patients, a condition associated with increased mortality (11). Whether this actually occurs in vivo and to what extent this phenomena contributes to the pathogenesis of sepsis should be measurable if mitochondrial oxygen pressures could be reliably measured in vivo, a feat which to date had not been accomplished. Recently, we were successful in accomplishing this holy grail in myocardial bioenergetics by our identification of the oxygen-dependent optical properties of the endogenous mitochondrial molecule, protoporphyrin IX by which it became possible to quantitatively measure mitochondrial pO₂ in vivo by use of delayed decay of fluorescence measurements (12). After validation in single cells we showed that the technique worked in vivo and allowed us the first time quantitative measurements of mitochondrial pO₂ in liver and heart (13,14). Application in experimental models of sepsis has given new and startling insights into the mitochondrial origin of possible mechanisms of oxygen extraction deficit in the septic myocardium.

References:

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2. Avontuur JAM et al (1995). *Circ Res*, 76: 418-425.
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Uterine smooth muscle - can we tame the beast within?

Susan Wray

Department of Physiology, University of Liverpool, Liverpool, UK

The control of uterine smooth muscle remains a major challenge for clinicians, affecting preterm deliveries, dysfunctional labour and post-partum haemorrhage. The task for physiologists is to gain a better understanding of the mechanisms that lead to myometrial contractility and thus help identify novel therapeutic targets. Calcium is a key to uterine contractility and thus understanding the changes in it, at the global and local level is important. An advantage for studies of uterine physiology is the availability of both well characterized animal models and human tissue from women undergoing caesarean section operations. In addition tissues from patients who have for example diabetes or are obese or are expecting twins, allows insight into how the normal physiology of the myometrium may be altered. I will present data that sheds light on Ca signalling in the myometrium. This will include work we have done to elucidate the role of the sarcoplasmic reticulum in the uterus and its contribution to contraction and excitability. I will then show data from studies of women with altered myometrial function due to disease, lifestyle and multiple pregnancy. In my summary I will relate these findings to our knowledge of physiology and make suggestions as to how these data may be translated for patient benefit. I will also show how our knowledge of the uterus has evolved over the millennia.

Keywords: uterus, smooth muscle, calcium, contraction, myometrium, childbirth

Molecular physiology of P2X receptors

Alan North

University of Manchester

Adenosine 5'-triphosphate (ATP) was first discovered to be a transmitter at some autonomic neuroeffector junctions (1). Extracellular nucleotides were shown to have actions in several tissues, firstly mast cells, macrophages and platelets. P2X and P2Y receptors were distinguished on the basis of agonist selectivity (2). Cloning of these receptor cDNAs showed an extensive distribution of these proteins and their RNAs: for example, P2X receptors on primary afferent nerves are now known to play roles in taste, sensation from hollow viscera, and inflammatory pain (3,4). P2X receptors are ATP-gated ion channels, which form as trimeric proteins from the same or different subunits (P2X1 – P2X7). They are quite distinct from the tetrameric channels (e.g. glutamate receptors) and pentameric channels (e.g. nicotinic receptor family), but similar in membrane topology to epithelial sodium channels (ENaC) and acid-sensing ion channels (ASIC). Activation of P2X receptors by extracellular ATP signals to the cell by depolarization, calcium entry, and by direct coupling to downstream intracellular effectors. This lecture will review the physiological roles of P2X receptors, and focus on the molecular basis of channel function. This will include the structural basis for binding of ATP, the permeation of ions down the central pore, and the molecular re-arrangements that couple binding to gating (5).

References:

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Keywords: ATP, P2X, ion channel, structure/function

Neurotransmitters and peptides: whispered secrets and public announcements

Gareth Leng

University of Edinburgh, Medical School, Edinburgh UK

Peptides in the hypothalamus are not like conventional neurotransmitters; their release is not particularly associated with synapses, and their long half-lives mean that they can diffuse to distant targets. Peptides can act on their cells of origin to facilitate the development of patterned electrical activity, they can act on their neighbours to bind the collective activity of a neural population into a coherent signalling entity, and the coordinated population output can transmit waves of peptide secretion that act as a patterned hormonal analogue signal within the brain. At their distant targets, peptides can re-programme neural networks, by effects on gene expression, synaptogenesis, and by functionally rewiring connections by priming activity-dependent release. The oxytocin and vasopressin neurones of the hypothalamus fire in distinctive patterns that govern and in turn are governed by the peptide secretion that they induce. Oxytocin cells display remarkable synchronised bursts that arise through emergent properties of an interactive network; vasopressin cells also burst, but asynchronously in a very different way and for very different reasons. In their different ways, these two neuronal systems have become important “model systems in neuroscience; in this talk I will talk about what these model systems teach us about peptide communication in the brain.

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1. Leng G, Brown C, Sabatier N, Scott V (2008) Population dynamics in vasopressin neurons. *Neuroendocrinology* 88:160-72.
2. Leng G, Ludwig M (2008) Neurotransmitters and peptides: whispered secrets and public announcements. *Journal of Physiology* 586:5625-32.
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Keywords: Neurotransmitters, peptides, oxytocin and vasopressin.

Epigenetic mechanisms in human physiology and diseases

Ahmet Korkmaz

Department of Physiology, Gulhane Medical School, Ankara, Turkey

Although the sequence of human genome is known, our understanding of the complicated network that takes place inside cells is far from complete. Many questions still remain unanswered with the regard to how the complex genomic information is used by human cells. For example, how does the genome work to orchestrate changes in gene expression during development and differentiation? Are all genes expressed in every cell type in all human tissues? How many genes are coding or non-coding in a particular cell? What are the functions of non-coding genes? It seems clear that many answers to these questions will be found in a field of growing interest, i.e., the study of epigenetics. Epigenetic mechanisms are modifications that occur in the genetic material that do not change the nucleotide sequence, but instead may cause conformational modifications in DNA. There are basically three epigenetic modifications; DNA methylation, histone modification and regulation by non-coding RNA. This is a summary of epigenetic regulatory mechanisms and their effects on gene transcription.

Keywords: Epigenetic, Histone modifications, DNA methylations, micra RNA

New information regarding to neuronal circuits involved in the central regulation of food intake

Miklos Palkovits

Neuromorphological and Neuroendocrine Laboratory, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

The hypothalamus has long been considered essential in the regulation of food intake and energy homeostasis. Humoral signals from the body and afferent neuronal pathways from the brainstem and the spinal cord to the hypothalamus constitute the inputs to the hypothalamic neuronal circuits where orexigenic and anorexigenic programs are synchronized. From the hypothalamus, commands through descending neuronal pathways and further up through vagal and spinal neurons get to the responding cells in the body. A great variety of neuronal peptides, biogenic amines and endocannabinoids, as well as their receptors are involved in the different stages and time points within this complex regulatory mechanism. Here, we provide a brief neuroanatomical summary of the neurons, pathways and brain regions that constitute the food intake regulatory circuits with special interest on 1) the role of the hypothalamic arcuate nucleus as an open gate for humoral signals (ghrelin, leptin, insulin, glucose) to orexigenic and anorexigenic hypothalamic neurons, 2) the neurochemical organization of the intrahypothalamic neuronal circuit that may keep a balance in food intake by analyzing and responding to special sensory signals as, hunger and satiety, 3) recent knowledge and new experimental data for the possible role of newly recognized and characterized peptides, like prolactin-releasing peptide, glucagon-1-like neuropeptide, nesfatin-1 and urocortin III in the central regulation of food intake, 4) the sites and functional significance of interaction between neuropeptides and central catecholaminergic neurons in the food intake and energy metabolism, and 5) fine localization of neuronal pathways in the human brain through which we recognize hunger and/or satiety and respond to them appropriately, intentionally or consensually.

Keywords: Food intake, hypothalamus, orexigenic and anorexigenic

New Understanding of Diseases Caused by Old Chemicals

David O. Carpenter

University at Albany, Institute for Health and the Environment, New York

Persistent organic pollutants (POPs) are fat-soluble chemicals like dioxins, polychlorinated biphenyls (PCBs) and chlorinated pesticides that are resistant to breakdown both in the environment and in the human body. While these chemicals have been known to cause cancer and nervous system damage for many years, we now are discovering that exposure to remarkably low concentrations increases the risk of a host of additional diseases. Type II diabetes is usually thought to be a disease of obesity and inactivity. However obesity is usually a consequence of eating too much animal fat, and the animal fat contains the POPs. Studies in our and other laboratories now are finding that exposure to POPs (especially pesticides) is a major risk factor for diabetes. Lee et al. (*Diabetes Care* 29: 1638: 2006) found a 38-fold elevation in risk of diabetes in individuals with elevated levels of 3 pesticides, 2 dioxins and one PCB, and that obese persons with low POPs were not at elevated risk of diabetes. We have also studied hypertension and heart disease. For hypertension serum PCB levels are the greatest risk factor other than age, and even those with “normal” blood pressure show higher pressure in relation to serum PCB level, but not pesticides. Cardiovascular disease, on the other hand, is elevated more in relation to pesticide levels. These results indicate that POPs increase the risk of the major human chronic diseases by mechanisms not yet clear.

Keywords: PCBs, Chemicals, POPs

Üner Tan Syndrome; Human Quadrupedalism: History, Clinics, Genetics, and the Self-Organized Emergent Properties

Üner Tan

Honorary Member of the Turkish Academy of Sciences; Retired Faculty Member of Çukurova University, Medical School, Department of Physiology, Adana, Turkey

A new syndrome characterized by three main symptoms including habitual quadrupedal locomotion, dysarthric or no speech and impaired intelligence with no conscious experience was first described in 2005, called “Üner Tan Syndrome” or UTS. Since then, nine more families were found by the discoverer in various regions of Turkey. My objective is to review and interpret the Üner Tan Syndrome with history, clinical evaluations, genetics, and the dynamics of human quadrupedalism with regard to its self-organized emergent properties.

The cases exhibiting the Üner Tan syndrome were subjected to neurological examinations by one or two experienced neurologists, and blood samples were taken for genetic analysis. Sagittal and coronal MRI scans were performed to visualize the cerebro-cerebellar changes, and videos were recorded to analyze their locomotion. All of the cases exhibited habitual diagonal-sequence quadrupedal locomotion, mild to severe mental retardation without conscious experience, dysarthric or no speech with only a few sounds. In all of the affected cases, dynamic balance was impaired during upright walking, exhibiting truncal ataxia. MRI scans showed inferior cerebellar hypoplasia with mildly simplified cerebral gyri, except one case exhibiting normal brain MRI. All families showed consanguineous marriages in their pedigrees, suggesting autosomal recessive transmission. UTS was genetically heterogenous. It was suggested that the emergence of human quadrupedalism may not be explained by a single factor. Instead, the attractor, human quadrupedalism, as an adaptive self-organized motor behavior, may result from the dynamic interactions of many subsystems, such as the spinal central pattern generators, posture, balance, body constraints, muscle strength, extensor and flexor systems, perception, cognition, motivation, genetics, and the environmental constraints, not depending upon the prior existence of any genetic code or instructions embedded within the central nervous system.

Keywords: Üner Tan Syndrome, genetics and human quadrupedalism

Using educational research to improve your teaching

Robert Graham Carroll

Department of Physiology, Brody School of Medicine, East Carolina University, Greenville NC USA

There are numerous curricular approaches currently used to educate and train medical students, and faculty are often confused about their place in the curriculum. Most of these curriculum innovations are developed and supported by research on teaching and learning. The objective of this presentation is to illustrate how instructors can become better teachers by applying the results from educational research. This presentation reviews the impact of Bloom's Taxonomy, Knowles' theory of the Adult Learner, and Kolb's 4-Stage Learning Cycle on the teaching and learning environments. Bloom's Taxonomy in the cognitive domain emphasizes the differences between Knowledge, Concept and Applications, Analysis, Synthesis and Evaluation, providing insight into what should be presented to learners in different degree programs. Knowles' theory of the Adult Learner emphasizes the importance of self-direction and active learning strategies, important in curriculum design. Kolb's learning cycle shows the reinforcing power of concrete experiences, reflective observation, abstract conceptualization and active experimentation applies the scientific method to the educational setting. This research suggests effective ways to use diverse learning environments, whether they are lectures, laboratories, or small groups. Educational theory underscores the essential role of the teacher: to create an effective learning environment, to provide direction for the learner, and to model effective learning behaviors.

Keywords: Physiology, teaching, education

Symposia

S1.1

Ca²⁺ sources for the exocytotic glutamate release from astrocytes

Vladimir Parpura

University of Alabama, Birmingham

Astrocytes can exocytotically release the gliotransmitter glutamate. Increased cytosolic Ca²⁺ concentration is necessary and sufficient in this process. The source of Ca²⁺ for the Ca²⁺-dependent exocytotic release of glutamate from astrocytes predominately comes from endoplasmic reticulum (ER) stores; both inositol trisphosphate (IP₃)- and ryanodine-sensitive receptors are involved. An additional source of Ca²⁺ in this process comes from the extracellular space; canonical transient receptor potential 1 protein, which forms channels that are activated by depletion of internal Ca²⁺ stores, allows Ca²⁺ entry from the extracellular space. Mitochondria can modulate cytosolic Ca²⁺ levels by affecting two aspects of the cytosolic Ca²⁺ kinetics in astrocytes. They play a role in immediate sequestration of Ca²⁺ during the cytosolic Ca²⁺ increase in stimulated astrocytes. As cytosolic Ca²⁺ declines due to activity of pumps, such as the smooth ER Ca²⁺-ATPase, free Ca²⁺ is slowly released by mitochondria into cytosol. Furthermore, immunophilins, mitochondrial cyclophilin D and FK506-binding protein 12 of there ER, affect cytosolic Ca²⁺ dynamics and consequential exocytotic glutamate release from astrocytes. Taken together, ER, extracellular space and mitochondria, can vary concentration of cytosolic Ca²⁺ which in turn can regulate Ca²⁺- dependent vesicular glutamate release from astrocytes.

Keywords: astrocytes, glia, calium, glutamate, exocytosis, tripartite synapse

Physiological and pathophysiological potential of astroglial purinoceptors

Peter Illes

Rudolf-Boehm-Institute for Pharmacology and Toxicology, University of Leipzig, Leipzig, Germany

The aim of this study was to find out, whether P2X7 receptors are present on cortical astrocytes of the rat and mouse brain, possibly participating in neuron-glia interaction or neurodegenerative processes. Primary cell cultures and slice preparations of the rat cerebral cortex were used for immunohistochemistry and whole-cell patch clamp experiments. In primary cultures of the rat cerebral cortex, astrocytes reacted to the application of ATP or dibenzoyl-ATP (Bz-ATP) with inward currents at a holding potential of -80 mV. These current responses considerably increased in an external medium containing no Mg²⁺ and low Ca²⁺ concentrations. The use of various receptor-type selective agonists and antagonists suggested the presence of P2X7 receptors. In brain slices of the rat prefrontal cortex, astrocytes were identified on the basis of their small cell diameters, and the absence of action potentials induced by depolarizing current injection. Immunohistochemistry experiments revealed that astrocytes labelled after recording with pipettes filled with the fluorescent dye Lucifer Yellow (LY), exhibited co-localization of LY with immunoreactivity for GFAP and P2X7 receptors. Once again, all astrocytes reacted to ATP and BzATP at a holding potential of -80 mV with inward current. The current responses to ATP and BzATP were greatly potentiated in a divalent cation-poor medium. Both the agonist and antagonist profiles of the P2 receptor-ligands investigated suggested the existence of P2X7 receptors. Hence, rodent astrocytes appeared to exhibit P2X7 receptors together with a range of P2Y receptor-types. These receptors may be involved in immunological defence reactions, apoptosis/necrosis as well as long-term proliferation such as gliosis.

Keywords: Astrocytes, P2X7 receptors, neuron-glia interaction, neurodegeneration, astrogliosis

Chemical transmission in neuronal-glia networks

Alexei Verkhratsky¹, Jose Julio Rodriguez Arellano², Vladimir Parpura³

¹Faculty of Life Sciences, The University of Manchester, Manchester, UK

²IKERBASQUE, Basque Foundation for Science, 48011, Bilbao, Spain, Department of Neurosciences, University of the Basque Country UPV/EHU, 48940, Leioa, Spain

³Department of Neurobiology, Center for Glial Biology in Medicine, Civitan International Research Center, Atomic Force Microscopy & Nanotechnology Laboratories, and Evelyn F. McKnight Brain Institute, University of Alabama, Birmingham, USA

Astrocytes possess a diverse assortment of ionotropic transmitter receptors, which enable these glial cells to respond to many of the same signals that act on neurones. Ionotropic receptors mediate neurone-driven signals to astroglial cells in various brain areas including neocortex, hippocampus and cerebellum. Glutamate and ATP are the major neurotransmitters responsible for signalling in neuronal-glia networks. Recent studies have found functional NMDA receptors in brain macroglia, in astrocytes and oligodendrocytes. Glial and neuronal NMDA receptors are functionally and structurally different; the glial receptors are weakly sensitive to the extracellular magnesium block, which may indicate a predominant expression of the NR3 receptor subunit. The ionotropic purinergic neuronal-glia transmission is mediated through both P2Y metabotropic and P2X ionotropic purinoceptors. The P2Y_{1,2} receptors are ubiquitously expressed in astroglia and their activation trigger intracellular Ca²⁺ signalling. The ionotropic receptors are much more territorially restricted; P2X-mediated responses were hitherto found only in cortical astrocytes. Cortical astrocytes express P2X_{1/5} purinoceptors that are characterised by very high sensitivity to ATP (EC₅₀ ~ 50 nM) and weak desensitization. In the cortex, astroglial NMDA and P2X_{1/5} receptors are activated upon physiological synaptic transmission. Spontaneous synaptic currents were also readily recorded from cortical astrocytes, indicating the close proximity of glial membranes to the sites of neurotransmitter release from the neuronal terminals. Activation of ionotropic receptors trigger rapid signalling events in astroglia; these events, represented by local Ca²⁺ or Na⁺ signals provide the mechanism for fast neuronal-glia signalling at the level of individual synapse.

Keywords: Neuroglia; astrocytes; purinergic transmission; NMDA receptors; P2X receptors

AMPA receptors in Bergmann glia

Frank Kirchhoff

Department of Molecular Physiology, University of Saarland, Homburg, Germany

Cerebellar Bergmann glial cells (BG) enwrap synapses on Purkinje-cell spines formed by parallel or climbing fibers. On their membranes, BG expresses localized glutamate transporters (GLAST close to the synaptic cleft) and AMPA-type glutamate receptors (GluR 1 and GluR 4 close to the shaft of presynaptic terminals). While the role of GLAST in controlling extracellular glutamate levels is rather obvious, we have only a limited knowledge of glial AMPA receptor function. To investigate the impact of glial transmitter receptors in vivo, we implemented a conditional knockout approach by crossbreeding mice in which astrocytes express the tamoxifen inducible variant of the Cre recombinase CreERT2 under the control of the human GFAP promoter or from the murine GLAST gene, respectively, with genetically modified mice in which exons 11 of the GluR 1 and GluR 4 genes, respectively, were flanked by loxP sites. Here, we demonstrate the successful ablation of GluR 1 and 4 from BG processes in an inducible and timely controlled manner. The lack of GluR 1 and 4 induces ultrastructural changes in the cerebellum: the BG retract their lamellae from Purkinje-cell synapses. As a physiological consequence of these ultrastructural changes we observe altered excitatory postsynaptic currents (EPSCs) in Purkinje cells upon parallel fiber stimulation. EPSCs exhibit larger amplitudes and slower decay kinetics. These alterations of the cerebellar network lead to deficits in complex motor performance. This study shows that astroglial AMPA receptors are important mediators of neuron-glia interactions that are essential for in vivo behavior.

Keywords: cre/loxP, Bergmann glia, AMPA receptors, conditional knockout, cerebellum, astrocytes, neuron-glia interactions

Chemosensory control of breathing and autonomic rhythms

Alexander V Gourine¹, Sergey Kasparov²

¹Neuroscience, Physiology and Pharmacology, University College London, London, UK

²Physiology and Pharmacology, University of Bristol, Bristol, UK

The mechanisms of central nervous respiratory and cardiovascular chemosensory function underlying detection of blood and brain pH and PCO₂ levels are not completely understood. We hypothesised that astrocytes - the most abundant type of brain glial cells – may act as functional brain chemosensors. Indeed, by having contacts with cerebral vasculature as well as multiple neurons, astrocytes are in a position to 'taste' the chemical composition of the arterial blood entering the brain and integrate this information with that of brain parenchyma. We found that astrocytes which reside within the 'classical' brainstem chemoreceptor areas located near the ventral surface of the medulla oblongata are highly chemosensitive. They respond to small physiological decreases in pH with vigorous elevations in intracellular Ca²⁺ and release of ATP. ATP spreads astroglial Ca²⁺ excitation within the neuropil, activates key chemoreceptor neurons and respiratory neurons of the medullary rhythm-generating circuits and induces adaptive increases in breathing. During systemic hypoxia, ATP is also released and acts to maintain respiratory activity in conditions when hypoxia-induced depression of respiration occurs. Mimicking astroglial pH-evoked Ca²⁺ responses by selective light stimulation of astrocytes expressing channelrhodopsin-2 activates chemoreceptor neurons via ATP-dependent mechanism and triggers robust respiratory and sympathetic responses in vivo. Thus, medullary astrocytes appear to be highly sensitive to physiological chemosensory challenges and have the ability to impart chemosensory information onto a modified pattern of cardiorespiratory activity. This identifies astroglia as an important brain component of one of the most fundamental mammalian homeostatic mechanisms which controls breathing and cardiovascular activity during hypercapnia and hypoxia.

Keywords: glia, breathing, cardiovascular, hypoxia

Cardiovascular reflexes and circulatory control

Isabel Rocha

Faculty of Medicine of Lisbon, Portugal

The main purpose of this talk is to draw the attention to the complex mechanisms that regulate arterial blood pressure. These mechanisms involve peripheral sensors, centres in the central nervous system, cardiovascular nerves, and endothelial and humoral factors. In particular, we will focus on the basis that provide the understanding of the role of the central nervous system in cardiovascular homeostasis and in the changes that occur in developing major modifications in cardiovascular function that accompany, and support, behavioural activities.

Keywords: autonomic nervous system, baroreflex, chemoreflex, blood pressure, hypertension

Unravelling the complexities of spinal cord circuits involved in autonomic control

Susan Deuchars

Institute of Membrane and Systems Biology, University of Leeds, Leeds, UK

The control of the sympathetic nervous system is achieved at a number of levels in the central nervous system to enable rapid and precise changes in sympathetic outflow to various organs and thus maintain homeostasis. We are investigating the spinal cord circuitry regulating the activity of sympathetic preganglionic neurons (SPNs), which provide the singular gateway between the CNS and the peripheral nervous system in sympathetic control. Using a combination of electrophysiology, immunohistochemistry and electron microscopy, we have identified novel groups of local presympathetic interneurons, investigating how they may fit into circuits involved in sympathetic control. We also examine how specific gap junctions contribute to rhythmic sympathetic activity, using a number of approaches and connexin knockout mice. Our data reveal extensive interneuronal interactions within the spinal cord that extend beyond the autonomic networks to the motor outflow and may thus serve to synchronize the two systems where appropriate. Interneurons are heavily innervated by serotonergic inputs and activation of serotonergic receptors induces rhythmic activity in reduced spinal cord preparations that involves both SPNs and interneurons. Gap junctions contribute to this activity and may play a critical role in synchronizing SPNs and autonomic reflex responses. GABAergic control of sympathetic outflow is achieved through activation of both pre- and postsynaptic receptors on interneurons and SPNs. These data indicate complex levels of spinal control of sympathetic outflow. As we unravel the circuits involved in influencing sympathetic activity, we gain insight into ways in which precise control of specific pathways may be achieved.

Supported by the British Heart Foundation and the Wellcome Trust.

Keywords: Interneuron, spinal cord, integration, morphology, sympathetic, autonomic

Compromised baro-and chemo reflex function as possible cause of sudden death in Familial Dysautonomia (Riley-Day Syndrome)

Max J Hilz

Department of Neurology, University of Erlangen-Nuremberg, Erlangen, Germany, and Depts. of Neurology, Medicine, Psychiatry, New York University, New York, NY, USA

In patients with Familial Dysautonomia (FD), also called Riley-Day syndrome or hereditary sensory and autonomic neuropathy type III, compromised baroreflex function seems to account for severe orthostatic hypotension without reflex tachycardia. Moreover, FD patients have prominent respiratory abnormalities such as breath-holding or sleep apneas, probably due to impaired chemoreflex sensitivity. Most importantly, there is a high risk of unexplained sudden death in FD patients (1). Somers et al. suggested that impaired baroreflex function may predispose to excessive autonomic responses to apnea induced chemoreflex stimulation; responses may comprise non-buffered bradyarrhythmias and sympathetic activation leading to cardiovascular catastrophe and sudden death (2).

We have assessed whether FD patients have impaired cardiovascular responses to baroreflex activation and to hypoxic and hypercapnic chemoreflex stimulation (3, 4). In 21 FD patients and 22 controls, we performed 3 minutes of passive head-up tilt (HUT) and baroreceptor stimulation using sinusoidal neck suction (NS; 0 to -30 mm Hg; 0.1 Hz [LF] and 0.2 Hz [HF]). During NS, participants kept respiration at 15 breaths/minute. We recorded RR-intervals (RRI), blood pressure (BP), and respiration and assessed NS induced changes in RRI and BP by spectral analysis (3). Moreover, we assessed responses of ventilation, end-tidal carbon dioxide (CO₂-et), oxygen saturation, RRIs, and BP to progressive isocapnic hypoxia, progressive hyperoxic hypercapnia, and during recovery from moderate hyperventilation (4). In FD patients, HUT induced orthostatic hypotension without compensatory tachycardia. Only controls, but not FD patients, had augmented LF powers of RRI and BP with LF-NS and increased HF powers of RRI with HF-NS (3). In the FD patients, hyperoxic hypercapnia generated normal cardiovascular and ventilatory responses while progressive hypoxia induced an only blunted increase in ventilation and paradoxical RRI- and BP-decreases. Hyperventilation caused prolonged apneas with prominent oxygen desaturation, BP decrease, and RRI increase (4). FD patients have baroreflex dysfunction with insufficient sympathetic vasomotor responses and insufficient cardiac sympathetic and parasympathetic responses to stimulation; the baroreflex dysfunction likely accounts for abnormal BP control with supine hypertension and orthostatic hypotension as well as bradyarrhythmias (3). In FD patients, hypoxia induces central depression with hypotension, bradycardia, and hypoventilation. The combined dysfunction of the baro- and chemoreflexes may account for the increased risk of sudden death in FD patients, possibly to due bradycardia and continued respiratory arrest during hyperventilation or hypoxia (4).

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Keywords: Baroreceptors, chemorecepto reflex function, sudden death, familial dysautonomia and Riley-Day Syndrome

Purinergic signaling and microglial function

Helmut Kettenmann

Department Cellular Neurosciences, Max Delbrueck Center for Molecular Medicine (MDC), Berlin, Germany

Microglia are the immunocompetent cells of the central nervous system. They respond with a process termed 'microglial activation' to any type of pathologic event. But also under normal conditions microglial cells are highly active by constantly screening their environment with their processes. Studies over the last years have established that microglial cells express a variety of different purinergic receptors and we and others have established that these receptors are functional both in cell culture as well as in situ. Purinergic receptors control a variety of different microglial functions such as cytokine release, phagocytosis and migration. Interestingly, microglial cells express selectively defined ATP degrading enzymes namely CD39 and CD73 which were originally used as microglial cell markers. We could establish that these enzymes modulate purinergic signaling and have an impact on microglial migration and phagocytosis. Thus, purinergic signaling is complex and ATP has emerged as important signal to control microglial function.

Keywords: microglia, purinergic receptors

Cross-talk between chemokines and adenosine in neuroprotection and neuromodulation

Cristina Limatola¹, Raffaella Cipriani¹, Pia Villa², Maria Grazia De Simone², Giuseppina Chece¹, Bertil Fredholm³, Clotilde Lauro¹

¹Department of Physiology and Pharmacology, University Sapienza, Rome, Italy

²Mario Negri Institute, Milan, Italy

³Department of Physiology, Karolinska Institute, Stockholm, Sweden

The chemokine fractalkine/CX3CL1 and its receptor CX3CR1 are constitutively expressed in the nervous system where they modulate neural transmission and neuroprotection. The strategic localization of CX3CR1 on microglia and *in vitro* studies suggested that the neurotrophic and neuromodulatory actions of CX3CL1 are indirect, requiring soluble factors released by microglial cells. Among these, adenosine plays a role, being released from CX3CL1-stimulated microglia and acting on different adenosine receptors (ARs). The involvement of astrocytes and ARs on the different activities of CX3CL1 will be discussed. Neuroprotection has been investigated with *in vitro* models of excitotoxicity on hippocampal and cortical neuronal and glial cultures and, *in vivo*, in a model of permanent MCAO in rats and mice. Neuromodulation has been investigated in hippocampal primary cultures and acute slices. For both studies, either pharmacological tools (specific AR antagonists and agonists, degrading enzymes like ADA or transporters inhibitors) or specific AR-deficient mice were used. Evidence are presented for a neuroprotective activity of CX3CL1 both *in vitro* and *in vivo* models of neurotoxicity mediated by microglia, astrocytes and adenosine through A1R, and for a neuromodulatory activity on glutamatergic transmission mainly mediated by A3R. We provide evidence that adenosine is an endogenous mediator involved in the neuroprotective and neuromodulatory activities of CX3CL1 *in vitro* and *in vivo*, with mechanisms requiring the cross talk between microglia and astrocytes.

Keywords: fractalkine, adenosine, microglia, astrocytes, excitotoxicity, cerebral ischemia, neuroprotection, synaptic transmission, LTP, neuromodulation

Intracellular Na⁺ influences short-term plasticity of glutamate-transporter-mediated currents in cortical astrocytes

Sergei Kirischuk

Universal Medical Center of the Johannes Gutenberg University Mainz, Institute of Physiology and Pathophysiology, Mainz, Germany

Fast synaptic transmission requires a rapid removal of the released neurotransmitter from the extracellular space. Glial glutamate transporters strongly contribute to glutamate removal. In this work we investigated short-term plasticity of synaptically-activated, glutamate transporter-mediated currents (STCs) in cortical layer 2/3 astrocytes. STCs were elicited by local electrical stimulation in layer 4 in the presence of ionotropic glutamate, GABAA, and GABAB receptor antagonists and recorded using whole-cell patch-clamp approach. In experiments with low [Na⁺]_i (5 mM) intrapipette solution, STCs induced by paired-pulse stimulation demonstrated paired-pulse facilitation (PPF) at short (<250 ms) inter-stimulus intervals (ISIs) and paired-pulse depression (PPD) at longer (500-5000 ms) ISIs. In experiments with high [Na⁺]_i (20 mM) intrapipette solution, PPF of STCs at short ISIs was significantly reduced, while PPD at longer ISIs was not affected. In addition, STC kinetics were slowed in the presence of high [Na⁺]_i. Exogenous GABA has been shown to increase [Na⁺]_i via GABA transporters (GATs). Exogenous GABA reduced the mean STC amplitude, decreased PPF at short ISIs, and slowed STC kinetics. In experiments with the low but not the high [Na⁺]_i intrapipette solution, GAT blockers, decreased PPF at short ISIs both at room and at near physiological temperatures, suggesting that endogenous GABA is capable to modulate short-term plasticity of STCs via GATs. We conclude that 1) short-term plasticity of STCs is dependent on [Na⁺]_i and 2) GATs may influence glutamate transporter activity and thus the rate and efficacy of glutamate removal.

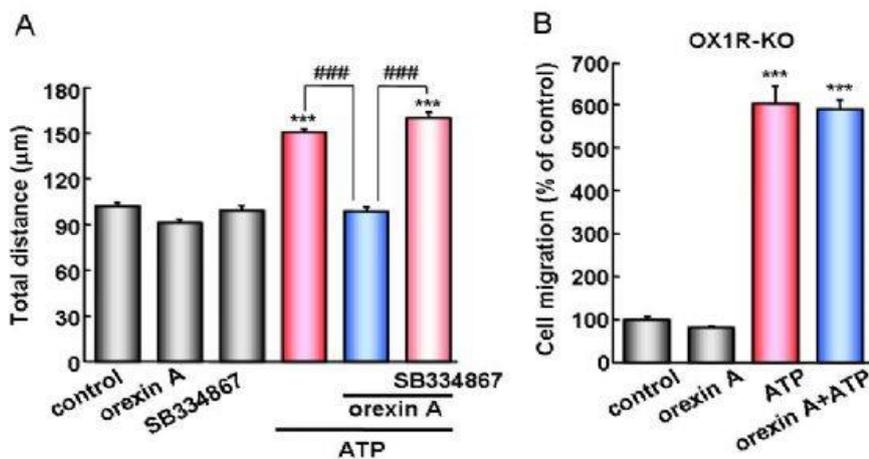
Keywords: astrocytes, glutamate transporter, GABA transporter, paired-pulse plasticity, intracellular sodium concentration

Neurotransmitter regulation of microglial motility and phagocytosis

Mami Noda, Yuko Okuno, Masataka Ifuku Laboratory of Pathophysiology, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

Microglia, the immune cells of the central nervous system (CNS), are busy and vigilant housekeepers in the adult brain. The main candidate as a chemoattractant for microglia at damaged site is adenosine triphosphate (ATP). Some neuropeptides, such as bradykinin and galanin, are also chemoattractants for microglia with different mechanism from that of ATP. In the present study, we found that ATP-induced microglial migration and morphological changes were regulated by orexin. Migration of cultured mice microglia was monitored with time lapse video microscopy system and Boyden chamber. Morphological change and the number of microglia *in vivo* were analyzed immunocyto- or immunohisto-chemically. Receptor expression was analyzed by RT-PCR and western blotting. Among various neuropeptides, orexin did not affect microglial migration by itself but inhibited ATP-induced microglial migration. Mouse microglia expresses mainly orexin receptor 1 (OXR1). From pharmacological studies and using orexin receptor (OXR1 and OXR2) knock-out (KO) mice, it was observed that orexin-A activated Gs-coupling OXR1 in microglia, competing with Gi-coupling P2Y receptors. Orexin increased intracellular cyclic adenosine monophosphate (cAMP) in cultured microglia and indeed ATP-induced migration was inhibited by membrane permeable cAMP. On the other hand, orexin-A did not affect ATP-activated PI3K/Akt signaling. From morphological analyses, orexin A also inhibited ATP-induced microglial ruffling. Using *in vivo* lesion models, microglial accumulation around the lesion was inhibited in OXR2-KO mice, where OXR1 was somehow up-regulated. Microglial motility and morphological change induced by ATP, one of the neurotransmitters, was inhibited by orexin. These results may help understanding unknown function of neuropeptides in microglia and their physiological and pathophysiological roles.

Keywords: microglia, migration, ATP, orexin, cAMP



Abst Noda Fig 1: Orexin inhibits ATP-induced microglial motility by activation of orexin receptor 1 (OX1R). A. Orexin A inhibits ATP-induced microglial motility. In the presence of SB334867, an OX1R selective antagonist, orexin A does not have inhibitory effect on ATP-induced microglial motility. B. Orexin A fails to inhibit ATP-induced motility in microglia from OX1R-knock out (KO) mice.

Brown adipose tissue is essential for cold- and diet-induced nonshivering thermogenesis

Jan Nedergaard

The Wenner-Gren Institute, Stockholm University, Sweden.

Adaptive adrenergic thermogenesis is induced in two physiological conditions. One form develops as an effect of acclimation to cold ("classical nonshivering thermogenesis"); the other (the existence of which is still somewhat controversial) develops as an effect of the intake of certain diets (e.g. "cafeteria diet"). Thermogenesis in brown adipose tissue is fully dependent on the activity of uncoupling protein 1 (UCP1). Thus, the importance of brown adipose function for different forms of thermogenesis can be established in UCP1-ablated mice. In the absence of UCP1, classical thermoregulatory nonshivering thermogenesis is fully obliterated - but the animals can compensate for the absence of nonshivering thermogenesis by shivering. Upon exposure to a palatable diet, the mice without UCP1 have no alternative to the metaboloregulatory thermogenesis occurring in brown adipose tissue, and they will therefore become obese. The present realization that brown adipose tissue is present and active even in adult humans raises evidently questions as to the significance of brown-fat thermogenesis in the protection against obesity (as well as against diabetes) and opens new possibilities for development of therapeutic agents against obesity and the metabolic syndrome in general.

Keywords: brown adipose tissue, UCP1, nonshivering thermogenesis, diet-induced thermogenesis, obesity

Thyroid hormone thermogenesis is mediated by brown adipose tissue

Miguel López

University of Santiago de Compostela, Spain

Thyroid hormones have widespread cellular effects; however it is unclear whether their effects on the central nervous system (CNS) contribute to global energy balance. The aim of our study was to characterize central action of thyroid hormones on energy balance. Here, we demonstrate that either whole body hyperthyroidism or central administration of triiodothyronine (T3) decrease the activity hypothalamic AMP-activated protein kinase (AMPK), resulting in activation of acetyl-CoA carboxylase (ACC) and increased expression of fatty acid synthase (FAS). Such induction of hypothalamic de novo lipogenesis increases activity in the sympathetic nervous system (SNS) and upregulates thermogenic markers in brown adipose tissue (BAT). Pharmacological and genetic manipulation provide evidence that inhibition of the lipogenic pathway in the ventromedial nucleus of the hypothalamus (VMH) prevents CNS-mediated activation of BAT by thyroid hormone and reverses the weight loss associated with hyperthyroidism. Furthermore inhibition of thyroid hormone receptors (TRs) in the VMH reverses the weight loss associated with hyperthyroidism, which underlines the importance of the hypothalamic effects of thyroid hormone in controlling energy balance. This regulatory mechanism depends on AMPK inactivation as genetic ablation of this enzyme in the VMH of euthyroid rats induces feeding-independent weight loss and increased expression of thermogenic markers in BAT. These effects are reversed by pharmacological blockage of the SNS. Overall, these findings demonstrate that thyroid-hormone-induced modulation of AMPK activity and lipid metabolism in the hypothalamus is an important regulator of energy homeostasis. Furthermore, our data indicates that central targeting of AMPK leading to increased activation of BAT thermogenic program may represent a novel therapeutic approach to treat obesity.

Keywords: AMPK, brown adipose tissue, thyroid hormones, UPC1

New insights into brown adipose tissue function across the life cycle

Michael Symonds, Lindsay Elvidge, Helen Budge

Early Life Nutrition Research Unit, Academic Child Health, School of Clinical Sciences, Queens Medical Centre, University Hospitals, Nottingham, NG7 2UH, United Kingdom.

One of the main roles of brown adipose tissue (BAT) is the rapid generation of heat at birth in response to cold exposure to the extra-uterine environment. This is mediated by the rapid increase in a range of neuro-endocrine stimulators within the fetal circulation which includes noradrenaline, cortisol, thyroid hormones and prolactin. The rate of decline in these factors may then start to initiate the gradual decline in BAT in a depot specific manner. This could contribute to the divergent molecular signatures of different adipose tissue depots which are dependent on whether they were either white or brown in origin. It is now established that BAT is present throughout the life cycle in humans although the challenge is to establish on a population wide basis the main factors that regulate this process. We have thus developed the use of thermal imaging in order to assess the physiological regulation of BAT and establish the critical time points at which it is lost. We have also shown in a large human cohort that the presence of BAT is more closely related to the prevailing photoperiod rather than ambient temperature. The extent to which photoperiod, as opposed to ambient temperature, is a primary regulator of BAT function remains uncertain. The prolactin receptor is necessary for normal BAT function and in the newborn sheep, direct stimulation of the prolactin receptor promotes BAT thermogenesis. Changes in maternal diet can determine the amount of BAT in the newborn, an adaptation mediated through changes in prolactin receptor abundance.

Keywords: brown adipose tissue, development, endocrine

Novel factors involved in the regulation of brown fat thermogenesis

Francesc Villarroya, Elayne Hondares, Roser Iglesias, Teresa Mampel, Marta Giralt
Institute of Biomedicine, University of Barcelona and CIBER Fisiopatología de la Obesidad y Nutrición

The identification of factors distinct from the classical beta-adrenergic regulation of brown adipose tissue thermogenic activity is of interest in order to better understand brown fat physiology and to advance in the development of novel strategies to influence brown fat thermogenesis. Recently, the so-called "hormonal FGFs" (and specially fibroblast growth factor-21, FGF21) have emerged as relevant in the control of brown fat thermogenesis. FGF21 is secreted by the liver and by brown fat itself, being able to act in an endocrine and autocrine manner. FGF21 induces the expression of UCP1 and genes involved in the provision of metabolic foodstuff to sustain thermogenesis (i.e. GLUT1) and in mitochondrial oxidation (cytochrome c) both in vivo and in vitro. In brown adipocytes, FGF21 increases cell respiration and induces significantly the oxidation of glucose and palmitate. Injection of FGF21 to mouse neonates increases local temperature in the dorsal area, corresponding to interscapular brown fat location. The action of FGF21 takes place through the interaction of the hormone with a receptor complex in the brown adipocyte surface including conventional FGF receptors (mainly FGFR1) and the co-receptor beta-Klotho, essential for the FGF21 effects. The expression of beta-Klotho is strongly induced during brown adipocyte differentiation. The interaction of FGF21 with the receptor-co-receptor complex causes the activation of a set of intracellular kinases, mainly p38 MAP-kinase, that lead ultimately to the pattern of induction of gene expression associated with the promotion of thermogenesis. The hormonal FGF system appears as a novel factor regulating brown fat thermogenic activity, which deserves further interest for basic and biomedical application purposes.

Keywords: brown adipose tissue, thermogenesis, fibroblast growth factor-21, beta-Klotho

A direct link between metabolism and the tissue renin-angiotensin system in the collecting duct

Agnes Prokai, Jim Burford, Haykanush Gevorgyan, Zalán Péterfi, Sarah L. Vargas, Janos Peti Peterdi
Department of Physiology and Biophysics and Department of Medicine, Zilkha Neurogenetic Institute,
University of Southern California, Los Angeles, California, USA.

Renin release, the rate-limiting step of RAS is controlled by GPR91, a novel metabolic receptor for succinate. Renin activates a new element of RAS, the (pro)renin receptor [(P)RR]. Furthermore, the renal collecting duct (CD) is the major source of (pro)renin in diabetes. Since the highest renal GPR91 and (P)RR expression was found in the CD, this study investigated whether succinate via GPR91 regulates the local CD RAS including the (P)RR. Western blot analysis of succinate-treated M1 cells (CD cell line) showed a dose-dependent, 2-2.5-fold elevation in pERK^{1/2}, pp38, COX2, renin, (P)RR. In WT and GPR91^{-/-} control and STZ-diabetic (DM) mice, CD pERK^{1/2} (by immunofluorescence) and urinary PGE2 excretion (measured by ELISA) increased 4-20 fold in DM mice in a GPR91-dependent manner. Medullary (P)RR and (pro)renin protein expression (by immunoblotting) and renin activity in the CD tubular fluid (visualized in vivo using multiphoton microscopy and a fluorogenic renin substrate delivered by renal micropuncture) increased 4-5 fold in WT DM vs. control mice, which was completely abolished in GPR91^{-/-} DM mice. CD (pro)renin activity was reduced by 40% after aliskiren (direct renin inhibitor) and by 70% following handle region peptide (PRR antagonist) administration. This is the first, direct demonstration of CD renin activity in vivo. Succinate accumulation, and the consequent GPR91-(P)RR signaling are novel (patho)physiological regulatory mechanisms that activate the local RAS in the CD via MAP kinases and COX2, PGE2 release, and increased (pro)renin synthesis. Succinate, GPR91 and (P)RR may be important regulators of the local CD RAS in DM and new therapeutic targets in diabetic nephropathy.

Keywords: diabetic nephropathy, (pro)renin receptor, renin-angiotensin system, GPR91

The (Pro)Renin Receptor Functions

Genevieve Nguyen

Center for Interdisciplinary Research in Biology (CIRB), INSERM U1050 UMR 7241

The (Pro)Renin Receptor, PRR, was identified as a component of the renin-angiotensin system but studies have revealed that it is a multifunctional molecule. The unique gene called *ATP6ap2/PRR* located on the X chromosome encodes a unique splice variant. However the protein undergoes intracellular processing generating a soluble form of PRR that can be measured in plasma and a membrane-cytoplasmic form associated with the vacuolar proton-ATPase. It was initially shown that PRR-bound renin and prorenin, increasing their enzymatic activity and activating intracellular signaling upregulating the expression of profibrotic proteins. Therefore most studies focused on the role of PRR in hypertension, cardiovascular and renal diseases, organ damage trying to identify PRR as a therapeutic target to optimize RAS blockade. Recent data have now confirmed that renin bound to PRR more efficiently catalyzes the conversion of angiotensinogen to angiotensin, based on crystal structure analysis, and that PRR is linked to blood pressure and plasma aldosterone levels, based on gene polymorphism studies. However, specific PRR deletion in cardiomyocytes and in podocytes draw our attention to a functional link between PRR and the vacuolar proton-ATPase and, in *Xenopus* and in *Drosophila*, ubiquitous or tissue-specific knock-down is responsible for embryonic lethality and severe epithelia abnormalities attributed to a defect in Wnt signaling pathways, independently of (pro)renin. The use of PRR conditional deletion to study the effect of tissue-specific ablation in adult and in embryo will clearly help deciphering the pathophysiological roles of PRR in development and in diseases.

Keywords: (pro)renin receptor, hypertension, organ damage, development

(Pro)renin receptor blockade on top of renin inhibition: focus on the vascular wall in diabetic rats

Jan Danser
Erasmus MC

We have studied whether the elevated prorenin levels in diabetes exert detrimental vascular effects via binding to the (pro)renin receptor ((P)RR). Diabetic TGR(mREN2)27 rats were treated with vehicle, the renin inhibitor aliskiren, or aliskiren plus the (P)RR antagonist HRP. Blood pressure and heart rate were monitored with telemetry transmitters. After 3 weeks rats were sacrificed, and mesenteric arteries (MA) were removed. Aliskiren lowered blood pressure by maximally 40 mmHg, without altering heart rate. HRP did not change this. Acetylcholine (ACh) fully relaxed precontracted MA of vehicle-treated rats. The NO synthase inhibitor L-NAME partially blocked the effect of ACh, whereas adding Tram34 and apamin (inhibitors of intermediate and small conductance Ca²⁺-dependent K⁺ channels) on top of L-NAME reversed the relaxant ACh response into a contractile effect. COX2 inhibition with NS398 and removal of the endothelium abolished this contractile response. Aliskiren did not alter the relaxant effect of ACh, nor the degree of blockade by L-NAME, but prevented the contractile response to ACh in the presence of L-NAME+Tram34+apamin. Yet, following co-treatment with HRP, the contractile response returned. Treatment did not alter the NO-responsiveness of the vascular smooth muscle cells, evaluated with the NO donor SNAP. Endothelin-1 constricted MA identically with and without treatment. Yet, the ETA receptor antagonist BQ123 inhibited this effect in aliskiren+HRP-treated rats only, suggesting selective upregulation of ETA receptors by HRP. HRP upregulates vascular ETA receptors and the generation of an endothelial contractile factor by COX2, thereby counteracting the beneficial vascular effects of aliskiren. This occurs in a blood pressure-independent manner, and argues against detrimental effects of (P)RR-prorenin interaction.

Keywords: prorenin, handle region peptide, (pro)renin receptor, renin inhibition, angiotensin, COX2

Prorenin receptor is essential for podocyte autophagy and survival

Dominik N Muller¹

¹Department of Experimental Medicine I, Nikolaus-Fiebiger-Center for Molecular Medicine, Friedrich-Alexander-University Erlangen-Nürnberg, Germany

²Experimental and Clinical Research Center (ECRC), an institutional cooperation between the Charité Medical Faculty and the MDC, Berlin, Germany

We investigated the role of the PRR in the maintenance of podocyte function. Generation of podocyte-specific PRR knockout mice (cKO) with the help of cre-loxP technique and phenotypical analyses. Generation of podocyte-specific PRR knockout mice (cKO) resulted in death of the animals between 2-3 weeks after birth. Within 14 days, cKO developed nephrotic syndrome, albuminuria with podocyte foot process fusion, and cytoskeletal changes. Podocyte-specific PRR deletion also led to disturbed processing of multivesicular bodies and enrichment of autophagosomal (LC3) and lysosomal (LAMP2) markers. The findings indicated a functional block in autophagosome-lysosome fusion and overload of the proteasome protein degradation machinery, as supported by p62 accumulation. In vitro PRR knockdown and pharmacological v-ATPase activity blockade increased vesicular pH with accumulation of LC3-positive and LAMP2-positive vesicles and altered cytoskeleton. Our findings suggest that the PRR is essential for podocyte function and survival by maintaining autophagy and protein turnover machinery. PRR plays a pivotal role in lysosomal pH control, which is important for podocyte survival and cytoskeletal integrity.

Keywords: prorenin receptor, podocytes

KV7 channelopathies

Holger Lerche, [Snezana Maljevic](#)

University of Tübingen, Hertie Institute for Clinical Brain Research, Germany

KV7 channels have raised an increasing interest in the last years for their important role in brain and heart physiology, and since four of its five so far identified members are related to different hereditary diseases. These channels are expressed in brain, heart, muscle, inner ear, and intestines. They give rise to functionally important potassium currents, reduction of which results in pathologies such as long QT syndrome, neonatal epilepsy or progressive deafness. The talk will be focused on mechanisms of neuronal KV7 channel mutations causing neonatal seizures or peripheral nerve hyperexcitability. Moreover, KV7 channels present attractive pharmacological targets for treatment of diseases characterized by membrane hyperexcitability and the first compound enhancing the activity of neuronal KV7 channels has just been launched for the add-on treatment of focal epilepsy.

Keywords: KV7 channels, brain, epilepsy

Nav channelopathies in epilepsy

Massimo Mantegazza¹, Massimo Mantegazza²

¹Institute of Molecular and Cellular Pharmacology, CNRS & University of Nice Sophia Antipolis, Valbonne France

²Besta Neurological Institute, Milano, Italy

Mutations of Nav1.1 (SCN1A) voltage-gated Na⁺ channel are the most common known cause of genetically determined epilepsy, causing epileptic syndromes that range in severity from relatively mild disorders such as simple febrile seizures (sFS) and generalized epilepsy with febrile seizures plus (GEFS+) to the epileptic encephalopathy termed Dravet syndrome (DS) or severe myoclonic epilepsy of infancy (SMEI). They can cause also familial hemiplegic migraine (FHM), a severe inherited subtype of migraine with aura. The functional effects of Nav1.1 mutations have not been completely clarified yet, impairing the identification of their pathogenic mechanism and the development of targeted therapies. We have studied an animal model of DS, Nav1.1 knock out mice, and Nav1.1 missense mutations engineered in the human clone and expressed in cell lines or cultured neurons. We have shown that in Nav1.1 knock out mice loss of function of Nav1.1 causes reduced excitability of GABAergic interneurons and reduced network inhibition in hippocampal slices. We have also obtained evidences that Nav1.1 epileptogenic missense mutations can cause loss of function of Nav1.1 by inducing folding defects that can be rescued by interacting proteins and drugs. Moreover, our results show that FHM mutations cause gain of function both in cell lines and in neurons. Our data point to a loss of function as the main effect of epileptogenic Nav1.1 mutations, in some cases because of folding defects, and to a gain of function as the effect of FHM mutations.

Keywords: Epilepsy, sodium channel, excitability, familial hemiplegic migraine

Omega currents and paradoxical depolarization in periodic paralysis

Karin Jurkat Rott¹, James Groome², Boris Holzherr¹, Chunxiang Fan¹, Frank Lehmann Horn¹

¹Division of Neurophysiology, Ulm University, Ulm, Germany

²Dept. Biological Sciences, Idaho State University, USA

Hypokalemic periodic paralysis is caused by mutations in two voltage-gated cation channels in skeletal muscle, Cav1.1 and Nav1.4. The mutations neutralize positively charged amino acid in the voltage-sensing transmembrane segments S4 of the channels. The gating defects induced by these mutations reveal loss-of-function which does not explain the phenotype. Cut-open measurements on *Xenopus* oocytes and current-voltage relationships on native muscle preparations of patients are performed. A cation leak current that does not flow through the ion-conducting pore but rather through a crevice formed by the mutations called omega current is present in the oocytes. A decrease of extracellular potassium decreases the conductance of the resting potential-maintaining rectifying potassium channels in native muscle. Weakness is associated with depolarization in native muscle. Omega current depolarize muscle membrane. Reduction of extracellular potassium reduces rectifying potassium currents. This process will lead to further depolarization resulting in inexcitability and weakness of muscle (hypokalemic periodic paralysis). Because a hyperpolarization according to Nernst is expected, the depolarization is termed paradoxical.

Keywords: periodic paralysis paradoxical depolarization omega currents voltage sensor

A focus on tonic GABA: How extrasynaptic receptors regulate excitability

Matthew Charles Walker
University College London

In addition to synaptic inhibition, GABA(A) receptors can mediate a “tonic” form of signaling that is not time-locked to presynaptic action potentials, and which is mediated by specific extrasynaptic GABA(A) receptor subtypes. As a result, it is sensitive to certain modulators, such as endogenous neurosteroids, which undergo changes related to hormonal status and stress. Here I review the mechanisms by which tonic currents regulate neuronal and network excitability. We have measured tonic currents in the hippocampi from healthy and epileptic rats following status epilepticus or traumatic brain injury, and determined the neuronal and network effects of modulating these tonic currents. Importantly, tonic currents show cell type specificity; for example, in the hippocampus, they are larger in interneurons than in pyramidal cells. Tonic currents are preserved or increased in models of focal epilepsy, even in the face of a profound loss of synaptic inhibition. This may represent a compensatory change that prevents seizure generation. In contrast to synaptic currents, tonic currents in the hippocampus markedly change the offset of the neuronal input-output function without affecting neuronal gain. The compensation of decreased synaptic inhibition by tonic inhibition may lead to networks composed of neurons with higher gain. This along with the inability of tonic currents to respond rapidly to increases in network activity, can lead to compensated networks that rapidly decompensate in the face of an increasing input. Cell type specificity further complicates the network effects, so that increasing tonic currents can decrease synaptic inhibition onto pyramidal cells (through inhibiting interneurons) or can promote spike-wave discharges through thalamocortical hyperpolarization.

Keywords: GABA, tonic, GABA(A) receptors, epilepsy, network excitability

Epo's impact on hypoxic ventilation and exercise performance in mice and men

Max Gassmann, Jorge Soliz, Beat Schuler
University of Zurich

Acclimatization to hypoxia relies on elevated ventilation and erythropoietic activity. We recently showed that erythropoietin (Epo) links both responses: apart from erythropoiesis, cerebral and plasma Epo interferes with the central (brainstem) and peripheral (carotid body) respiratory centers. Knowing that women cope better than men to reduced oxygen supply we analyzed the hypoxic ventilatory response in transgenic female mice with elevated Epo levels in brain only (Tg21), or in brain and plasma (Tg6) or in wild type animals injected with recombinant human Epo. Exposure to moderate and severe hypoxia revealed that the presence of transgenic or recombinant human Epo extensively increases the hypoxic ventilatory response in female mice compared to their male siblings. Finally, human volunteers were injected with recombinant human Epo and subsequently exposed to 10% oxygen. Compared to men, the hypoxic ventilatory response was significantly increased in women. We conclude that Epo exerts a gender-dependent impact on hypoxic ventilation that involves sexual hormones. In another line of investigation, we show that the optimal hematocrit for maximal performance is 0.57 to 0.65. On the other hand it is known that high doses of injected Epo crosses the blood brain barrier causing neurologic effects. We present a new role by which Epo augments exercise performance without altering the hematological parameters. Tg21 or wild-type mice (WT) treated with a high dose of rhEpo (WT+rhEpo) demonstrate a dramatic improvement in maximal exercise performance independent of changes in blood and cardiovascular parameters. This novel finding builds a more complete understanding regarding the central effects of endogenously produced and exogenously applied Epo on exercise performance.

Keywords: erythropoietin, hypoxia, ventilation, exercise performance, gender differences

The roles of Epo in neuroprotection and repair after brain injury

Ertugrul Kilic

Yeditepe University, Faculty of Medicine, Department of Physiology, Istanbul

Neuroprotection therapies have made limited progress in recent years. Several compounds shown to be efficacious in animals were tested in humans in cost-expensive trials. Unfortunately none of these studies were able to demonstrate efficacy under clinical conditions in patients. In order to establish treatments that are of benefit not only in animals but also humans, new strategies are clearly needed, comprising (i) new factors mimicking intrinsic mechanisms that the brain itself makes use of, (ii) novel delivery techniques allowing drugs to pass the blood-brain barrier more efficaciously than before, (iii) better, functionally relevant readouts of brain recovery and (iv) strategies that are of usefulness not only in the acute, but also post-acute stroke phase. According to these strategies, EPO's neuroprotective and restorative effects on ischemic brain injury will be reviewed.

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Keywords: Erythropoietin, cerebral ischemia, stroke, axonal reorganization

Erythropoietin improves cognition: models and mechanisms

Hannelore Ehrenreich

Max Planck Institute of Experimental Medicine, Göttingen, Germany

Erythropoietin (EPO) effects on cognition were for ages attributed to increased hemoglobin. Even after discovery of EPO/EPO receptor (EPOR) in brain, preclinical studies employed EPO mainly as neuroprotectant. Clinical trials (schizophrenia, multiple sclerosis, preterm infants) found brain matter protection and superior cognition upon EPO, particularly of processing speed/working memory. EPO treatment of juvenile mice achieved better learning/memory/attention, paralleled by enhanced hippocampal long-term potentiation. For mechanistic insight, we created a mouse model, overexpressing continuously active EPOR under α -calcium/calmodulin-dependent protein kinase II (α -CaMKII) promoter, i.e. in pyramidal neurons (cortex/hippocampus). In this model, we found a boost of higher cognition. Together, these results imply a role for EPO in neuroplasticity/higher cognition. We hypothesized that a respective relevance of the EPO/EPOR system would also be reflected by genetic variation of encoding genes. For addressing this hypothesis, we took advantage of the GRAS (Göttingen Research Association for Schizophrenia) data collection, providing a unique ground for phenotype-based genetic association studies (PGAS). GRAS comprises >1,000 patients with schizophrenia (DSM-IV-TR). DNA samples of GRAS patients were genotyped for genetic polymorphisms of EPO/EPOR. For statistical analyses, age, sex, negative symptoms, and duration of disease were used as covariates. Genotype-phenotype analysis, targeting higher cognition revealed significant associations of EPO/EPOR variants - alone and combined - with processing speed/working memory but not with blood readouts. The data show that genetic variants of the EPO/EPOR system influence cognition of schizophrenic individuals. Their interaction will help providing mechanistic insight into the molecular interplay between EPO and EPOR regarding cognition.

Keywords: Genetic, hematopoietic growth factor, learning, working memory, processing speed

The HIF-EPO system in retinal development and neuroprotection

Christian Grimm, Christian Caprara, Christina Lange, Markus Thiersch

Lab for Retinal Cell Biology, Dept Ophthalmology; Center for Integrative Human Physiology; Neuroscience Center; University of Zurich, Zurich, Switzerland

Hypoxic preconditioning induces expression of Epo in the retina and protects photoreceptor cells against degeneration. We investigated the influence of the HIF-EPO system on retinal development, physiology, and neuroprotection. Photoreceptor-specific and retina-specific knockdowns of HIF1A and VHL were generated using the cre-lox system. Retinal morphology was analyzed by light microscopy. Gene and protein expression was studied by qPCR, Western blotting and immunofluorescence. The knockdown of HIF1A in adult photoreceptors reduced hypoxic expression of HIF1 target genes, but not of Epo. Photoreceptors lacking HIF1A tolerated hypoxic exposure which also protected them against degeneration by a subsequent insult. Knockdown of HIF1A in most cells of the retina already during retinal development resulted in elevated levels of HIF2A protein and of Epo mRNA and in a vascular phenotype which lacked the intermediate plexus. Normal layering of retinal cells was not affected. Knockdown of the upstream regulator VHL in photoreceptor cells activated the hypoxia response pathway in normoxic conditions. Levels of HIF1A and HIF2A were elevated and expression of several hypoxia-related genes was increased. Epo, however, remained at basal levels. Hypoxic preconditioning transiently protected photoreceptors in young adults. Prolonged activation of this pathway (1 year) resulted in retinal degeneration. Knockdown of VHL in most retinal cells resulted in severe developmental defects of the retinal vasculature and strong retinal degeneration despite a 30-fold induction of Epo gene expression. The HIF-EPO system influences retinal development, the formation of the retinal vasculature and the protection of neuronal cells.

Keywords: Erythropoietin, HIF, retina, neuroprotection, development, vasculature

Regulation of renal sodium transport by the succinate receptor GPR91

Joris Robben

Radboud Universtiy, Nijmegen Medical Centre, The Netherlands

The succinate receptor GPR91 is a known regulator of a number of adaptive processes in response to a (locally) altered metabolic state. These include regulation of profibrotic factors, neovascularization, cell survival and cellular function. GPR91 is expressed in several tissues, including kidney, heart, liver, retina and blood cells. In kidney, it likely acts as an important direct and indirect regulator of the renin-angiotensin system, blood pressure, renal sodium transport and fibrosis. We set out to determine succinate release in response to oxidative stress in vivo and in vitro, and the effects of SUCNR1 on renal sodium handling and blood pressure. To gain insight in the effects of succinate on the kidney, succinate was administered to mice via injection of minipumps. Blood pressure was measured, and urine and blood collected for measurement of succinate levels, osmolality and electrolytes. In addition, expression levels of major renal sodium transporters were determined. Since oxidative stress is known to trigger succinate release, we tested whether plasma and succinate levels were increased in mice subjected to renal ischemia/reperfusion. Succinate administration for three days did not affect blood pressure. Interestingly, urinary and plasma levels decreased when high concentrations of succinate were given, suggesting that succinate is rapidly cleared from the circulation. Succinate administration affected major apical sodium transporters in the distal nephron, including the sodium-chloride cotransporter NCC and the epithelial sodium channel ENaC. Moreover, we found that renal ischemia caused increased plasma and urine succinate levels. Succinate represents a novel mediator in the body's sodium and water balance in renal ischemia/reperfusion.

Keywords: succinate, kidney, sodium transport, oxidative stress

Developing novel ligands for free fatty acid receptors

Graeme Milligan, Brian D. Hudson

College of Medical, Veterinary and Life Sciences, University of Glasgow

Our objective was to identify novel ligands for members of the family of G protein-coupled receptors that responds to free fatty acids, to define their selectivity and mode of binding and to explore their use to define the specific roles of the individual receptors. A series of small carboxylic acids were assessed for their capacity to activate human FFA2 and FFA3 using transfected cell lines and the mode of binding of these further assessed following expression of mutated receptors. These were then tested for activity at each of rat, mouse and bovine orthologues of the receptors. Cells lines endogenously expressing either or both FFA2 and FFA3 were identified via RT-PCR and the selectivity of the ligands then assessed in such cells. A series of small carboxylic acids with selectivity for either human FFA2 or FFA3 were identified and shown to maintain this selectivity at a range of species orthologues as well as in cell lines endogenously expressing these receptors. These ligands will be useful tools in exploring the biology and function of these two closely related receptors in both ex vivo and in vivo models of diabetes, adiposity and inflammation.

Keywords: Free fatty acid, drug screening, G protein-coupled receptor, diabetes, inflammation

Deorphanization and characterization of GPRC6A – a promiscuous G protein-coupled receptor activated by basic L-amino acids

Christoffer Clemmensen, Sanela Smajilovic, David Gloriam, Petrine Wellendorph, Lars Dan Johansen, Vikram Kjølner Bhatia, Alex Rojas Bie Thomsen, Daniel Sejer Pedersen, Hans Bräuner Osborne
Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Denmark

In 2004 we reported the cloning of a novel mammalian family C G-protein coupled receptor, termed GPRC6A. The subsequent objective was to identify the endogenous ligands for the receptor, develop the first selective antagonist and unravel its physiological function. The molecular pharmacology of the receptor was determined by expression in *Xenopus* oocytes and HEK293 cells. Pharmacological responses were measured by electrophysiology and inositol phosphate generation, respectively. The first selective antagonists were discovered using a chemogenomic approach which identified 2-phenyl-indoles as potential antagonists, which were subsequently purchased/synthesized and tested pharmacologically. The physiological function of the receptor was determined by generation and analyses of a GPRC6 receptor knockout mouse. Measurement of calcium-dependent chloride currents in *Xenopus laevis* oocytes and inositol phosphate generation in HEK293 cells facilitated the deorphanization of GPRC6A and identification of L-alpha-amino acids as agonists. The most active agonists were basic L-alpha-amino acids, L-arginine, L-lysine and L-ornithine. The receptor is widely expressed in human and mouse tissue, including a range of tissues relevant to metabolism regulation. Based on chemogenomic predictions, 25 2-phenyl-indole analogs were purchased/synthesized and tested pharmacologically. 3 compounds were found to be selective GPRC6A receptor antagonists. The GPRC6A mouse was generated by ablation of exon 6 coding the 7-transmembrane domain and was backcrossed to C57BL/6. The mice show no obvious phenotype on regular chow, but do show a phenotype related to exercise/metabolism in more complex scenarios. We have identified a novel receptor promiscuously activate by L-amino acids. We have developed the first selective antagonists, and shown that the receptor is potentially involved in regulating functions related to exercise/metabolism.

Keywords: GPRC6A receptro, L-amino acid, metabolism, exercise

(Patho)physiology of the succinate receptor GPR91

Janos Peti Peterdi

Depts. of Physiology and Biophysics, and Medicine, University of Southern California, Los Angeles, USA

GPR91 is a recently identified cell membrane receptor for the tricarboxylic acid (TCA)-cycle intermediate succinate and it is highly expressed in various organs including the kidney, liver, and adipose tissue. This novel, unconventional role of succinate (i.e. acting as a signaling molecule) via GPR91 can signal mitochondrial stress, a local mismatch between tissue energy supply and demand, and can activate a hypoxia response independent of the hypoxia inducible factor (HIF). Within the kidney, GPR91 is predominantly localized in the luminal cell membrane of the distal nephron-collecting duct system which is located in the most hypoxic part of the organ. Succinate accumulation and GPR91 signaling in diabetes have been linked to the increased synthesis and release of renin, and the activation of the pathogenic renin-angiotensin system (RAS). GPR91 signaling involves Gi and Gq, activation of MAP kinases ERK1/2 and p38, COX-2, and the generation of prostaglandins (e.g. PGE₂) which are highly relevant to the control of renal salt and water transport and blood pressure. In the diabetic kidney, the activation of several pathogenic pathways including RAS/hypoxia/fibrosis mediators (pro)renin, VEGF, TGF- β , MCP-1 are GPR91 dependent. GPR91 deficiency has protective effects in the development of albuminuria in a mouse model of diabetic nephropathy and also reduces blood pressure. Due to its diverse tissue expression, GPR91 may play an important physiological role in many organs as well as in complications of diabetes (e.g. in retina, neurons) and other metabolic diseases.

Keywords: tissue metabolism, succinate, GPR91, renin-angiotensin system, diabetes mellitus

The roles of gut peptides and inflammation in the control of eating

Wolfgang Langhans

Institute of Food, Nutrition and Health, ETH Zurich

The control of eating is part of the homeostatic regulation of energy stores, metabolites and essential nutrients. Meal initiation and termination result from signals originating in the oral cavity (orosensory stimuli), in the gastrointestinal tract (stomach distension, nutrients, osmolality), and from the metabolization of absorbed nutrients. In addition, signals reflecting the amount of fat stored in the body supposedly modulate the central nervous system (CNS) integration of the meal-related neural (afferent nerve activity) and humoral (e.g. gut peptides) signals, which ultimately shapes the behavioural (eating) and autonomic (metabolism) response. Gut peptides released from enteroendocrine cells in response to luminal nutrient stimulation can influence eating through an endocrine effect or through a paracrine action on afferent nerves in the gut. Some gut peptides also have potent insulinotropic and gluco-regulatory effects, making them attractive candidates for the pharmacotherapy of obesity and type II-diabetes. Besides stimulating gut peptide release, eating causes an immune reaction in the gut. Thus, fat intake can trigger inflammatory reactions that may change the response to satiating gut peptides. Systemic immune challenges inhibit eating through pro-inflammatory cytokines that can act on CNS neurons through vagal afferent signalling, by stimulating the release of neuromodulators from blood brain barrier (BBB) epithelial cells, or after active transport across the BBB. In any case, the signalling pathways converge on the same CNS circuits that control normal eating. Whether the immune reaction induced by a meal is strong enough to inhibit eating is unknown. Here I review the various gut peptide and inflammation-related mechanisms that affect eating and their potential interactions.

Keywords: Satiating, Cytokines, Vagus, Hindbrain, Immune reaction, Small intestine

Reduction of food intake at high altitude

Margriet S Westerterp Plantenga

Department of Human Biology, Nutrim, FHML, Maastricht University, Maastricht, The Netherlands

Progressive loss of body-mass during high-altitude sojourns may be largely caused by decreased food intake, possibly due to hypobaric hypoxia. Therefore we assessed the effect of long-term hypobaric hypoxia per se on appetite and food intake. Eight men were exposed to a 31-day simulated stay at several altitudes up to the peak of Mt. Everest (8,848 m). Palatable food was provided ad libitum, and stresses such as cold exposure and exercise were avoided. At each altitude, body-mass, energy-expenditure (EE), energy and macronutrient intake, attitude toward eating and appetite profiles during and between meals were assessed by using questionnaires. Body-mass reduction of an average of 5 +/- 2 kg was mainly due to a reduction in energy intake of 4.2 +/- 2 MJ/day ($P < 0.01$). EE had slightly decreased. At 5,000- and 6,000-m altitudes, subjects had hardly any acute mountain sickness symptoms and meal size reductions ($P < 0.01$) were related to a more rapid increase in satiety ($P < 0.01$). Meal frequency was increased from 4 +/- 1 to 7 +/- 1 eating occasions per day ($P < 0.01$). At 7,000 m, when acute mountain sickness symptoms were present, uncoupling between hunger and desire to eat occurred and prevented a food intake necessary to meet energy balance requirements. On recovery, body mass was restored up to 63%; this suggests physiological fluid retention with the return to sea level. Exposure to hypobaric hypoxia per se was associated with a change in the attitude toward eating and with a decreased appetite and food intake, resulting in body-mass loss.

Keywords: Hypobaric hypoxia, body-mass loss, food intake, energy expenditure, Mt Everest simulation.

Reduced body weight in hypoxic rats and mountaineers

Thomas Alexander Lutz

Institute of Veterinary Physiology, University of Zurich, Zurich, Switzerland

Physiological exposure to low oxygen levels (hypoxia) occurs at high altitude. Hypoxia leads to reduced eating and body weight loss which depend mainly on the duration of exposure and level of hypoxia. Various mechanisms have been proposed to explain these phenomena (e.g. altered leptin release from fat tissue), but there is no general consensus on the underlying causes. Based on the hypothesis that eating in hypoxia is reduced because of increased release of gastrointestinal (GI) hormones that inhibit eating and a local inflammatory response in the GI tract, we performed in-vitro and in-vivo studies in rats and a field study in mountaineers at 500m and 4550m; tests were done on day 2 and 4 at 4550m. We replicated previous findings of reduced eating in hypoxia but this appeared to be unrelated to altered GI hormone release (e.g., CCK, amylin, glucagon) or local GI inflammation. Gastric emptying was rather accelerated in hypoxia. In the field study, some mountaineers required dexamethasone (dex) treatment due to symptoms of acute mountain sickness (AMS). Reduced energy intake in hypoxia appeared to be associated with the severity of AMS. Surprisingly, retrospective analysis of these individuals' data revealed that mountaineers that required dex had reduced insulin sensitivity and lower erythropoietin levels already under baseline conditions. In conclusion, reduced eating at high altitude was not associated with changes in the release of anorectic GI hormones or local GI inflammation. Further, individuals with lower insulin sensitivity may be at higher susceptibility to develop AMS.

Keywords: hypoxia, food intake, gastric emptying, dexamethasone, insulin sensitivity

Hypoxia and adipose tissue dysfunction in obesity

Paul Trayhurn

Obesity Biology Research Unit, University of Liverpool, Liverpool, UK

White adipose tissue is a major endocrine and secretory organ, white adipocytes releasing a multiplicity of protein hormones and factors - adipokines. Many adipokines are linked to the inflammatory response, and inflammation within the enlarged adipose tissue underlies the development of the disorders linked to obesity. Local hypoxia develops as adipose mass expands, large adipocytes becoming O₂-deprived. Candidate gene studies with adipocytes in culture have shown that the expression and secretion of key inflammation-related adipokines, including IL-6, leptin and VEGF, are stimulated by low pO₂; the production of adiponectin with its anti-inflammatory action is, however, inhibited. Microarray studies have demonstrated that the expression of >650 genes is up- and >600 down-regulated in response to hypoxia. These include the facilitative glucose transporter GLUT1, expression of which increases, and there is a rise in GLUT1 protein linked to a hypoxia-stimulated increase in glucose uptake. Lactate release is also increased, indicative of a switch to glycolytic metabolism. Synthesis of the monocarboxylate transporter, MCT1, is stimulated by hypoxia, enabling increased lactate removal from hypoxic adipocytes. Recent studies have shown that even small reductions in oxygen tension induce changes in the production of key adipokines, including leptin and VEGF, as well as increases in glucose utilisation. Other cells within adipose tissue also respond to hypoxia, the synthesis of leptin being induced in preadipocytes, for example. Hypoxia has a pervasive effect on adipocyte function and is central to the dysregulation of adipose tissue in obesity that underlies insulin resistance and the metabolic syndrome.

Keywords: hypoxia, adipose tissue, adipocytes, inflammation, adipokines, leptin, obesity, oxygen

Latest findings on bladder, beta receptors and physiology and pharmacology

Eda Kumcu

Department of Pharmacology, Çukurova University, Adana, Turkey

The urinary bladder has physiological functions of urine storage and voluntary micturition. These functions are regulated through by complex interaction between neural and myogenic mechanisms. Alterations in these mechanisms may cause dysfunction of bladder such as overactive bladder. Overactive bladder is a common condition characterised by the symptoms of urinary frequency, urgency and incontinence. Acetylcholine, a cholinergic agent, causes the initiation of micturition via muscarinic receptors, especially M3 receptors and to some extent M2. Antimuscarinic drugs are generally thought to exert their therapeutic action on detrusor overactivity by reducing the ability of the detrusor muscle to contract. Although atropine can almost completely block parasympathetic-nerve mediated contraction of human detrusor muscle, there is still atropine-resistant response resulted from purinergic signaling in human detrusor muscle. There is also evidence that abnormal purinergic transmission in patients with detrusor instability. It was also shown that abnormal purinergic transmission may cause the symptoms seen in overactive bladder and urothelial ATP release is augmented under pathophysiological conditions. Also, in recent years, there is further evidence that a third subtype, beta3-adrenergic receptor, mediates relaxation in human detrusor muscle and discusses the potential use of beta3-adrenergic receptor agonists for the treatment of overactive bladder. It was also shown that phosphodiesterase inhibitors such as rolipram induced a significant decrease on the cyclophosphamid-induced spontaneous contractions. Thiols also may play a differential role in regulating bladder contractility depending on age. Based on these recent advances, ATP, purinergic receptors, beta3 adrenergic receptors, phosphodiesterase and thiols are become to be new therapeutic targets to management of detrusor dysfunction.

Keywords: bladder, bladder dysfunction, purinergic receptors, beta3 adrenergic receptors, phosphodiesterase inhibitors, new therapeutic targets

Association of M3 muscarinic receptors and Kir6.1 with caveolae facilitates human detrusor activation

Mari Ekman, Catarina Rippe, Mardjaneh Karbalaeei Sadegh, Saeed Dabestani, Matthias Mörgelin, Bengt Uvelius, Karl Sward

Department of Experimental Medical Science, Lund University, Lund, Sweden

Caveolae are 50-100 nm large membrane invaginations found in many cell types. A considerable body of information has accumulated pointing towards an essential functional role of these structures in smooth muscle from experimental animals, but comparative data in humans is scarce. Here we aimed to assess whether M3 and Kir6.1 are associated with human detrusor caveolae and to probe the functional relevance of this organization. Smooth muscle strips were dissected from human detrusors and used in ultrastructural, biochemical and mechanical studies. Caveolae were disrupted by cholesterol desorption using methyl- β -cyclodextrin (m β cd). Quantitative RT-PCR (RT-qPCR) was used to assess subunit composition of human detrusor KATP channels. The subcellular distribution of M3 muscarinic receptors and Kir6.1 was examined using sucrose density fractionation and immunoelectron microscopy. Desorption of cholesterol right-shifted the concentration-response curve for the muscarinic receptor agonist carbachol ~3-fold. This effect was inhibited by glibenclamide, PNU-37883 and chelerythrine, and mimicked by levcromakalim, suggesting involvement of KATP channels. RT-qPCR demonstrated expression of Kir6.1, Kir6.2, and SUR2A. Sucrose density fractionation and Western blotting revealed co-fractionation of detrusor M3 receptors and Kir6.1, in partial overlap with caveolin-1. Immunoelectron microscopy showed M3 and Kir6.1 to be enriched 6-fold in caveolae. Thus, ultrastructural imaging, unique in its kind using the human urinary bladder, demonstrates that M3 receptors co-localize with Kir6.1 in caveolae. Functional assays moreover reveal that this organization facilitates cholinergic detrusor activation. Changes in caveolae-associated signaling may thus underlie functional bladder disturbances in man.

Keywords: Smooth muscle, M3 muscarinic receptors, Kir6.1, caveolae and human detrusor activation

From bench-side to bed-side: progress in understanding urethral physiology

Christopher Fry

Postgraduate Medical School, University of Surrey, Guildford, UK

The urethra and bladder neck act (bladder outflow) together as a variable resistor, controlling the rate of urine outflow from the bladder. During urine storage the resistance is high and when voiding occurs the resistance is low. A pathological increase of resistance may cause retention of urine; resulting in renal damage if sustained. If a high filling resistance cannot be maintained, urinary incontinence can result. Urethral resistance is determined by several factors: the tone of urethral smooth muscle; the activity of associated skeletal muscle (the rhabdosphincter); and basal turgidity provided by mucosal blood flow. This talk will consider advances in understanding the regulation of smooth contraction and relaxation, in particular via autonomic and nitroergic mechanisms as well as the potential role of interstitial cells in regulating muscle tone. In addition the influence of prostatic smooth muscle tone in regulating urethral resistance will be discussed; of importance to men with benign or malignant growth of the prostate gland. Of particular interest is the synergistic interaction between sympathetic and parasympathetic nervous systems in regulating smooth muscle tone. Finally, the control of rhabdosphincter function and its importance to regulating urethral function will be described. Examples will be provided as to the translation of this fundamental knowledge to management of patients with bladder outflow conditions.

Keywords: Urethra, smooth muscle, autonomic nervous system, bladder

Physiology and pathology of human ureter: effects of clinically relevant *E. coli* infections

Rachel Victoria Floyd¹, Susan Wray¹, Theodor Burdyga¹, Craig Winstanley²

¹Dept of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Crown Street, Liverpool L69 3BX

²Dept of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Daulby Street, Liverpool, L69 3GA.

Urinary tract infections (UTI) are the second most common infectious disease, incurring significant medical costs and resulting in widespread morbidity and mortality worldwide. Most UTI are caused by *Escherichia coli* colonization of the urethra and ascension into the bladder causing cystitis. Pyelonephritis can develop as a consequence of bacteria ascending the ureters leading to kidney scarring and kidney failure. Efficient peristalsis is therefore vital in maintaining renal health. *E. coli* colonization can impair ureteric contractility and cause dilation which in turn potentiates infection due to urinary stasis and/or vesicoureteric reflux but little is known about the mechanisms of impaired ureteric function. In this presentation I will show how exposure of rat and human ureters to uropathogenic *E. coli* (UPEC) causes changes in phasic contractions and Ca²⁺ transients evoked by electrical field stimulation and suggest underlying mechanisms. The physiological effects of blocking type-1 fimbrial binding by pre-incubation of UPEC with mannose will be presented and strain-specific differences will be discussed in detail in light of current understanding of how host and pathogen interact in the urinary tract. Recent studies in animal bladder have given insight into the invasive potential of sub-types of UPEC, which increases their capacity to cause recurrent disease. These findings will be reviewed and preliminary data from our group investigating these mechanisms in ureter which show that UPEC have the capacity to invade and replicate within urothelial cells, will be presented. These multidisciplinary approaches allow us to further understand ureter physiology and pathophysiology which can be translated into clinical benefit for patients affected by recurrent and persistent UTI.

Keywords: Ureter, UTI, *E. coli*, peristalsis

Introduction to the pathophysiology of ischemia-reperfusion injury

Mustafa Gül

Department of Physiology, Faculty of Medicine, Atatürk University, Erzurum, Turkey.

The degree of the damage due to ischemia depends on the delay between sudden artery occlusion and reopening. Inhibition of adenosine triphosphate (ATP) synthesis and accumulation of metabolic products due to lack of washout are the two mechanisms causing cell death. Reperfusion before all cells die does not always mean saving the cells, which are slightly injured but yet alive. Abrupt reperfusion can kill severely ischaemic but viable muscle cells. The three main harmful phenomena leading to cellular reperfusion injury are: oxidative stress, energy paradox and Inflammation. Reactive oxygen species are produced so much that the cells can manage without being damaged. The reperfusion energy scenario results in cell death by two mechanisms: one leading to broken membranes and the second resulting in new damage to the mitochondria. The reason behind the latter is the opening of the mitochondrial permeability transition pore (mPTP), which remains closed during ischaemia and opens early during reperfusion. This increases calcium overload and further contraction, and also leads to collapsing the mitochondrial membrane potential and uncoupling the respiratory chain, which may stop ATP production. Swelling of the mitochondrion causes cytochrome c release, which in turn induces the caspase cascade and promotes apoptosis. Ischaemic injury also initiates an acute inflammatory response, which is augmented by reperfusion and can contribute to the muscle damage. Reperfusion is necessary, but that “off-on” reperfusion seems to be over. Preconditioning or postconditioning (i.e., ischemia-reperfusion cycles before and after ischemia) or pharmacologic interventions can reduce the cell death due to ischemia-reperfusion injury.

Keywords: ischaemia, reperfusion, ischaemia-reperfusion injury, pathophysiology

Skeletal muscle mitochondrial dysfunction during ischemia reperfusion: implication of oxidative stress and protective effects of ischemic pre- and post-conditioning

Bernard Geny¹, Anne Laure Charles¹, Jamal Bouitbir¹, Anne Sophie Guilbert¹, Ziad Mansour³, Max Guillot², Julien Pottecher², Anne Lejay¹, Joffrey Zoll¹, François Piquard¹

¹Physiologie et d'Explorations Fonctionnelles, Hôpitaux Universitaires de Strasbourg et Université de Strasbourg, Equipe d'Accueil 3072, France

²Pôle Anesthésie Réanimation Chirurgicale, Hôpitaux Universitaires de Strasbourg et Université de Strasbourg, Equipe d'Accueil 3072, France

³Service de chirurgie cardiovasculaire, Hôpitaux Universitaires de Strasbourg et Université de Strasbourg, Equipe d'Accueil 3072, France

Mitochondria, main energy sources of the cells, are causes and targets of increased oxidative stress which plays a key role in peripheral artery disease and ischemia-reperfusion(IR)-induced muscular impairments. Here, we will synthesize data from several experiments. Rats were randomized to control, IR (clamping of the infra-renal aorta for 3 h followed by 2 h of reperfusion), and IR+ pre- or postconditioning groups. Complexes I, II, III and IV activities of the mitochondrial respiratory chain were measured using glutamate-malate (V_{max}), succinate (V_{succ}) and TMPD-ascorbate(V_{TMPD}) in gastrocnemius permeabilized fibers. Superoxide anion production was assessed by dihydroethidium (DHE) staining. IR significantly reduced maximal oxydative capacity (V_{max}, -26%, p<0.05), complexe II, III and IV activities (V_{succ}, -28%, p<0.05) and complexe IV activity (V_{TMPD}, -25%). IR increased dihydroethidium (DHE) staining (+200%, p<0.05). Pre- and post-conditioning similarly counteracted these deleterious effects, increasing mitochondrial complexes activities and restoring muscle DHE staining. Aortic cross clamping induces muscle mitochondrial dysfunction and reactive oxygen species overproduction. Both pre- and post-conditioning protect skeletal muscle mitochondria, likely by reducing oxidative stress and preserving antioxidant defence. Whether this approach targeted toward mitochondria might reduce patients morbidity during major vascular surgeries deserve further investigations.

Keywords: reperfusion injury, skeletal muscle, mitochondria, reactive oxygen species, ischemic pre-conditioning, ischemic post-conditioning

Estrogen influence on skeletal muscle damage and repair

Peter Tiidus

Department of Kinesiology & PE, Wilfrid Laurier University, Waterloo, ON, Canada
Department of Kinesiology & PE, Wilfrid Laurier University, Waterloo, ON, Canada

To present an overview of current research dealing with estrogen influence on skeletal muscle damage and repair. A review of research using animal models and humans will be presented. Research primarily in rodents has shown that estrogen will significantly attenuate exercise induced muscle damage as well as markers of inflammation such as leukocyte infiltration of muscle following exercise induced muscle damage. Further animal research has also demonstrated that estrogen will also enhance factors associated with muscle repair such as satellite cell activation and proliferation consequent to muscle damage. Research involving post-menopausal females has also recently confirmed that estrogen and hormone replacement therapy (HRT) will enhance skeletal muscle function, strength, recovery from damaging exercise and reduce markers of muscle damage. A number of possible mechanisms may be involved in these effects including effects mediated by estrogen receptor-alpha and subsequent signaling as well as possible non-receptor mediated effects. Emerging research has demonstrated in both animals and humans that estrogen can reduce exercise induced muscle damage and inflammation and enhance muscle repair. Estrogen can also enhance muscle strength and functioning and thereby contribute to improved muscular health and functioning in post-menopausal females. The use of HRT in older females should be considered in light of these potentially positive findings of estrogen effect on muscle function and health.

Keywords: estrogen, muscle damage, muscle repair, estrogen receptors, exercise

Resolution of Inflammation

Sashwati Roy

Comprehensive Wound Center, Department of Surgery, The Ohio State University Medical Center, Columbus, OH

Acute inflammation resolves by mechanisms that are not fully understood. Persistence of the inflammatory response (chronic inflammation) can lead to scarring and loss of organ function. An actively coordinated program of resolution initiates in the first few hours after an inflammatory response is triggered by tissue injury. Resolution of inflammation is enabled if granulocytes are eliminated via efferocytosis and the tissue mononuclear cell population (macrophages, m ϕ) returns to baseline count and phenotypes. We observed that compared to non-diabetic animals, dead cell clearance activity (efferocytosis) was markedly impaired in wound macrophages harvested from diabetic mice. Oxidants produced by NADPH oxidase are directly involved in the oxidation and externalization of phosphatidyl serine (PS), a process that is critical for dead cell recognition. Compromised NADPH oxidase activity was observed in macrophages harvested from diabetic wounds. Milk fat globule EGF factor 8 (MFG-E8; also known as lactadherin) is secreted by activated m ϕ . MFG-E8 acts as a bridging molecule that is capable of binding to phosphatidyl serine on apoptotic cells as well as $\alpha v \beta 3$ or $\alpha v \beta 5$ integrin receptors on m ϕ . The presence of arginine residues renders this protein susceptible to glycation. Using ELISA and Biacor assays we demonstrate that glyoxal, a product of glucose oxidation, can glycate MFG-E8. Once glycated the affinity of MFG-E8 for binding with PS markedly diminished. Diabetic wound tissue showed presence of glycated MFG-E8. These data suggest compromised NADPH oxidase activity in diabetic wound cells result in diminished phosphatidylserine oxidation impairing the process of dead cell recognition by m ϕ . Glycation of MFG-E8 diabetic macrophages represents another major mechanism that impairs dead cell recognition and clearance in diabetic wounds. Such dysfunction in macrophage activity is in direct conflict with resolution of inflammation resulting in chronic inflammation noted in diabetic wounds.

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Keywords: Wound Healing, Diabetes, Macrophages

Exercise, impaired glucose metabolism and heat shock proteins

Osmo Hänninen, Mika Eerik Venojärvi, Mustafa Atalay
Institute of Biomedicine, Physiology, University of Eastern Finland

Diabetes exerts a negative impact on tissue protection through a number of mechanisms including impaired heat shock protein (HSP) defence. In insulin resistant states and diabetes, heat shock factor 1 (HSF-1) is low in insulin sensitive tissues, resulting in low HSP60, HSP 70 and HSP90 levels. Strategies to decrease oxidative stress and to modulate stress proteins: expression of heat shock proteins, which are important components of protein homeostasis and cell survival may have important implications for reducing insulin resistance, improving impaired glucose regulation and increasing the protection against diabetes and its complications. After the 2-years exercise-diet intervention oxidative stress was reduced as shown by decreased serum levels of uric acid and protein carbonyls in subjects with impaired glucose tolerance (IGT). In addition cytoprotection was improved in the skeletal muscle tissue, observed as the increased expression of mitochondrial HSP60 and GRP75 in the IGT subjects while no response was found in cytoplasmic chaperones HSP72 and HSP90. The adaptive changes in the expression of GRP75 and HSP60 could support mitochondrial protein import, protein folding and enhance tissue protection in insulin-resistant muscle tissue. These adaptive changes of mitochondrial HSPs and increased oxidative capacity are mainly due to increased contractile activity of skeletal muscle. Exercise has both direct and indirect positive effects on muscle and whole body metabolism. The benefits can be obtained at exercise levels, which are safe and possible in everyday life in middle aged subjects.

Keywords: Exercise, glucose metabolism, oxidative stress, heat shock protein.

Presynaptic AMPA receptors modulate glutamate release in mouse spinal cord dorsal horn

Rita Bardoni¹, Amy B. Macdermott², Rohini Kuner³

¹Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy

²Department of Physiology, Columbia University, New York, USA

³Department of Pharmacology, University of Heidelberg, Heidelberg, Germany

Purpose of the study was the investigation of the role of AMPA receptors, expressed on nociceptive fiber terminals, in modulating glutamate release. We also intended to characterize the contribution of different AMPA receptor subunits to presynaptic modulation. We used the patch-clamp technique on dorsal horn neurons in mouse spinal cord slices. Excitatory postsynaptic currents (EPSCs), recorded from lamina II neurons, were evoked by stimulation of the attached dorsal root. Recordings were performed from both wild-type animals and transgenic mice carrying a nociceptor-specific deletion of GluA1 or GluA2 subunit. We observed that application of AMPA receptor agonists (AMPA or kainate) caused a significant depression of evoked glutamatergic EPSCs in wild type mice. The effect was reversible in wash. The inhibition of EPSCs was accompanied, in most neurons, by an increase of synaptic failures and a change in the coefficient of variation (CV) of EPSC peak amplitudes. Both effects are considered as indicators of presynaptic modulation. We observed similar effects, in the presence of AMPA, also in mice with nociceptor-specific deletion of the GluA2 subunit. However, in mice with deletion of the GluA1 subunit, presynaptic modulation exerted by AMPA (expressed as a change of CV) was significantly reduced. We showed that functional AMPA receptors are expressed on nociceptive primary afferents in mouse spinal cord. Their exogenous activation causes the depression of glutamate release. The decrease of presynaptic modulation observed in mice carrying a deletion of the GluA1 subunit, but not of GluA2, suggests that calcium-permeable AMPA receptors are particularly important in regulating glutamate release from primary afferents.

Keywords: pain, glutamate, presynaptic modulation, electrophysiology

Presynaptic regulation of nociceptive inputs mediated by peripheral unmyelinated axons and the role of axonal chemosensitivity

Richard W Carr¹, Johannes Fleckenstein²

¹Institute of Physiological Genomics, Ludwig-Maximilians University, Munich, Germany

²Department of Anaesthesiology, Ludwig-Maximilians University, Munich, Germany

A proportion of small diameter primary sensory neurones innervating human skin are chemosensitive. They respond in a receptor dependent manner to a range of substances including neurotransmitters, inflammatory mediators, algogens, thermogens and pruritogens. Interestingly, both the terminals and axons of unmyelinated C-fibres are chemosensitive and exposure of either to chemical agents may result in the generation of action potentials and/or a more subtle modulation of excitability. Using electrophysiological techniques, the chemosensitivity of human peripheral axons has recently been examined. In vitro assessment of human sural nerve indicates that a distinct population of C-fibre afferents is depolarized by GABA acting via GABAA receptors. This provides supportive evidence from human tissue for the idea that GABA may presynaptically modulate peripheral C-fibre input to the spinal dorsal horn and potentially regulate the onset and progression of chronic neuropathic pain states. Electrophysiological techniques have also recently been employed to compare human sural nerve A-fibre responses in vitro A-fibre recordings from the median nerve in healthy subjects before and after an oral dose of the analgesic compound flupirtine. Flupirtine enhances the opening of slowly-activating KV7 channels and the effects of a single clinical dose can be detected in peripheral A-fibres as a shortening of the relative refractory period and a curtailing of ectopic induced following ischaemia. Electrophysiological assessment of A- and C-fibre axons in human peripheral nerve provides insight into both the physiological and therapeutic potential of axonal chemosensitivity.

Keywords: Axonal chemosensitivity, Pain, C-fibre

Plasticity of spinal sensory neurons in chronic pain states

Sue M Fleetwood Walker, Emer M Garry, Liting Sun, Rory Mitchell
Edinburgh University, Edinburgh, UK

Dorsal horn neurons receive powerful excitatory inputs from sensory primary afferents. In chronic pain states maladaptive changes occur that lead to hypersensitivity and receptive field expansion – manifest as hyperalgesia (increased response to noxious stimuli) and allodynia (perception of previously innocuous stimuli as noxious). A key component of this functional plasticity and the focus of our work here is the reorganisation of proteins associated with excitatory glutamate receptors in the postsynaptic density of spinal sensory neurons. Behavioral reflex sensitivity was assessed in models of chronic neuropathic and inflammatory pain and spinal cord tissue analyzed by biochemical approaches to assess protein: protein interactions. We have identified changes in synaptic trafficking of AMPA receptor subunits, their association with key partner proteins and differential linkage of glutamate receptors with downstream signaling enzymes that could participate differentially in neuropathic or inflammatory pain states. The functional hypersensitivity that occurs in chronic pain states is associated with marked dynamic plasticity in the location, interactions and downstream signaling of excitatory glutamate receptors.

Keywords: pain, plasticity, glutamate receptor, hypersensitivity

GABA receptors in Schwann cells, emphasizing their cell non-autonomous contribution to the peripheral myelination process and pain sensitivity

Valerio Magnaghi¹, Lawrence Wrabetz², Bernhard Bettler³, Alessandro Faroni⁴, Egle Maria Mambretti¹, Patrizia Procacci⁵, Marcella Motta¹

¹Dept. Endocrinology, Physiopathology, Applied Biology, University of Milan, Italy

²San Raffaele Scientific Institute, Division of Genetics and Cell Biology, Milan, Italy

³Dept. Biomedicine Institute of Physiology, University of Basel, Switzerland

⁴Blond McIndoe Laboratories, Tissue Injury and Repair Group, The University of Manchester, UK

⁵Dept. Human Morphology Citta Studi, University of Milan, Italy

The GABAergic system in the peripheral nervous system (PNS) is functionally active. New evidence shows that Schwann cells express the GABA receptors (GABA-A and GABA-B) and are able to synthesize and release GABA. Interestingly, GABA-B receptor activation modulates Schwann cell proliferation and myelination. Accordingly, the GABA-B1 $-/-$ knockout mice show morphological and molecular changes in peripheral nerves, including an increased number of small myelinated fibers and small neurons of the lumbar dorsal root ganglia. These mice are hyperalgesic, show gait alterations and reduced allodynic sensitivity. In order to study whether these changes may be the result of a Schwann cell autonomous GABA-B mediated effect, By means of different methodologic approaches we analyzed conditional-null mice with a specific deletion of GABA-B1 in Schwann cells. The PNS morphology of these mice appears to be different from that previously observed in GABA-B1 $-/-$ total knockout mice. Indeed, preliminary data reveal a high number of myelin abnormalities, delaminations and apoptotic Schwann cells. The increase in Remak bundles and unmyelinated fibers correlate with the hyperalgesic and allodynic state. Moreover, NF200 and CGRP immunostaining is altered in GABA-B1 conditional-null mice. Our studies provide novel evidence for a physiological role of GABA and/or GABA-B receptors in the PNS, and may reveal new therapeutic strategies for peripheral neuropathies and associated chronic pain.

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Keywords: Myelin, neuropathy, peripheral pain

Human cardiovascular adaptation to weightlessness

Peter Norsk

Element Scientist, NASA-Human Research Program USRA/NASA-Johnson Space Center

Entering weightlessness (0 G) induces immediately a shift of blood and fluid from the lower to the upper parts of the body inducing expansion of the cardiac chambers (Videbaek & Norsk 1997). For many years the effects of sudden 0 G on central venous pressure (CVP) was discussed, and it puzzled researchers that CVP compared to the 1-G supine position decreased during the initial hours of spaceflight, when at the same time left atrial diameter increased (Buckey et al. 1996). By measuring esophageal pressure as an estimate of inter-pleural pressure, it was later shown that this pressure decreases more than CVP does during 0 G induced by parabolic flights (Videbaek & Norsk 1997). Thus, transmural CVP is increased, which distends the cardiac chambers. This unique lung-heart interaction whereby 1) inter-pleural pressure decreases and 2) central blood volume is expanded is unique for 0 G. Because transmural CVP is increased, stroke volume increases according to the law of Frank-Starling leading to an increase in cardiac output, which is maintained increased during months of 0 G in space to levels of some 25 % above that of the 1-G seated position (Norsk unpublished). Simultaneously, sympathetic nervous activity is at the level of the upright 1-G posture, which is difficult to explain based on the high stroke volume and decreased blood pressure and systemic vascular resistance. This paradox should be explored and the mechanisms revealed, because it might have implications for estimating the cardiovascular risk of travelling in space.

Keywords: weightlessness, space, cardiovascular

Adaptations in resistance arteries to pressure

Christian Aalkjaer

Department of Biomedicin, Aarhus University, 8000 Aarhus C, Denmark

In hypertension the media thickness to lumen diameter ratio of resistance arteries is increased. This structural adaptation is important for the increased peripheral resistance and for the increased pressures for cerebral autoregulation in hypertension. To provide additional information on the impact of pressure on the resistance vasculature we investigated resistance arteries and the main arteries of the legs of giraffes. Muscular resistance arteries from the lower leg, the lower neck and the neck close to the head were mounted in wire myographs for measurements of structure and contractile function. Small arteries from the brain, tongue, muscle close to the head and rete mirabilis were mounted in pressure myographs and the myogenic response determined. Intraarterial pressure was measured at the top and bottom of the main artery of the leg and the vessel visualized with ultrasound. The media thickness to lumen diameter ratio and the contractility of the muscular arteries increased the higher the transmural pressures were (i.e. the closer to the ground the arteries were taken). Arteries from rete mirabilis had no myogenic response. The other vessels had a substantial myogenic response which was maximal around 180 mmHg for the extracranial arteries and around 100 for the intracranial arteries. The main artery of the leg is equipped with a sphincter-like structure and constriction of this structure and the artery either spontaneously or to injected noradrenaline results in a viscous resistance, which likely protects the vascular system of the lower leg against an excessive transmural pressure.

Keywords: resistance arteries, gravity, pressure

The influence of gravity on the evolution of the cardiovascular system

James Hicks

Department of Ecology and Evolutionary Biology, University of California, Irvine, Irvine, CA 92697, USA

Natural selection drives the evolution of both form and function, yet the biophysical world can influence and/or constrain evolutionary pathways. For example, the convergent fusiform body shape of actively swimming aquatic organisms is most likely the common evolutionary solution to the influences of hydrodynamic drag. In terrestrial vertebrates, the effects of gravity may have influenced the interactions of body size and shape, with cardiovascular morphology and function. Specifically, in long bodied animals (snakes), in which large hydrostatic columns exist, the evolution of the cardiovascular system may have been influenced by organismic features (length of animal), behavior (climbing and non-climbing), and habitat occupied (terrestrial, aquatic). A previous study (Lillywhite, H., *Amer Zool*, 1987) found the heart in terrestrial and arboreal species of snakes was located near the head, while in aquatic species the heart was located near the middle of the animal's body. Based on the concept that the vertebrate heart must do additional work to overcome gravity, the anterior heart in arboreal species served to reduce the hydrostatic blood pressure when these animals adopt vertical postures during climbing. A recent study (Gartner et al, *Physiol Biochem Zool*, 2009) analyzed a new data set of 155 species from five major families of snakes. This analysis indicated that heart position is influenced both by gravity as well as a strong phylogenetic signal.

Keywords: gravity, pressure

Haemodynamic consequences of the long neck in giraffes and ostriches

Tobias Wang¹, Morten Smerup², Jonas Funder², Erik Sloth¹, Steen Buus¹, Christian Aalkjaer¹, Emil Toft Brøndum¹, Niels H Secher³, Peter Bie⁴, Mads Damkjaer⁴, Mads F Bertelsen⁵, Geoffrey Candy⁶

¹Aarhus University, Aarhus, Denmark

²Clinical Institute, Aarhus Universitet, Aarhus, Denmark

³Copenhagen University, Copenhagen, Denmark

⁴SD University, Odense, Denmark

⁵Copenhagen Zoo, Copenhagen, Denmark

⁶University of Witwatersrand, Johannesburg, South Africa

Animals with long necks, where the brain may be situated more than a meter above heart level, have high arterial blood pressures to maintain adequate cerebral perfusion. In both giraffes and ostriches, the tallest living mammals and birds on the earth today, the pressure in the carotid arteries declines with the vertical distance above the heart according to gravity, and results in a normal inflow pressure at the brain. In anaesthetised giraffes and ostriches, the pressure generated by the heart is reduced when the head is lowered below heart level, which may be an important mechanism protecting the brain. In both animals, a large volume of blood also pools within the jugular vein when the head is lowered and this seems to reduce cardiac filling and hence ventricular pressure development on the giraffe heart, but a similar mechanism does not appear to apply to the ostriches. In line with these observations, arterial pressure of giraffes is exquisitely sensitive to volume depletion by bleeding, while the ostrich is less affected. Surprisingly, the relative mass of giraffe heart is not bigger than other mammals, and we believe that its capacity to generate high pressures resides with relatively small end-diastolic and end-systolic volumes providing a normal mammalian wall tension. This may entail an evolutionary scenario where the ability to generate high blood pressures has evolved at the expense of a reduction in cardiac output.

Keywords: Haemodynamic, heart, giraffes and ostriches

The control of atrial contraction in health and disease

Katharine Dibb, Jessica Clarke, Mark Richards, David Eisner, Andrew Trafford
Unit of Cardiac Physiology, University of Manchester, Manchester, UK

Despite atrial fibrillation (AF) being the commonest cardiac arrhythmia, much less is known regarding atrial physiology in comparison to the well studied ventricle. The transverse (t)-tubule is a deep invagination of the surface membrane which forms a regular network in mammalian ventricular myocytes but is generally absent from the atria of small mammals e.g. rat. Proteins that couple excitation to the rise in intracellular Ca^{2+} are concentrated around the t-tubule membrane and thus intracellular Ca^{2+} rises rapidly and synchronously throughout the ventricular myocyte but much more slowly in the rat atrial myocyte. We have used confocal microscopy and electrophysiological experiments to investigate the role of atrial t-tubules in health and disease. Unlike the rat, large mammalian atria (including human) possess t-tubules resulting in a more rapid rise in intracellular Ca^{2+} in sheep atria. In a sheep model of heart failure (HF) we have shown atrial t-tubules are almost completely lost and this is associated with a slowing in the rise of intracellular Ca^{2+} . Electrophysiological experiments have highlighted how t-tubule loss and slowed Ca^{2+} release affect atrial Ca^{2+} homeostasis in failing hearts. Firstly, peak L-type Ca^{2+} current and amplitude of the systolic Ca^{2+} transient were reduced. We were able to mimic this result by artificially removing t-tubules from control atrial myocytes. Secondly we observed an increase in SR Ca^{2+} content in atrial cells from the failing heart despite a reduction in SR mediated uptake. Both increased SR Ca^{2+} content, and decreased SR uptake have the potential to be arrhythmogenic and therefore may contribute to the propensity for AF in HF.

Keywords: atria, calcium, t-tubule

Phosphatase-1-inhibitor-1 in physiological and pathological beta-adrenoceptor signaling

Ali El Armouche

Department of Pharmacology, University Goettingen, Germany

Phosphatase-inhibitor-1 (I-1) is a distal amplifier element of beta-adrenergic signaling by preventing dephosphorylation of its downstream targets. I-1 is downregulated in failing hearts and overexpression of a constitutively active mutant form (I-1c) reversed contractile dysfunction in failing hearts, suggesting that I-1c may be a candidate for gene therapy. Here we generated conditional double-transgenic mice (Tet-Off system) with cardiomyocyte-restricted expression of I-1c (dTG-I-1c) on an I-1 knockout (KO) background. Transactivator only mice on a KO background (tTA) served as controls. Young adult dTG-I-1c mice exhibited enhanced cardiac contractility as determined by echocardiography. However, this phenotype turned into contractile dysfunction and ventricular dilatation when mice were subjected to chronic catecholamine infusion. Telemetric ECG recordings revealed typical catecholamine-induced ventricular tachycardia in dTG-I-1c and sudden death. Abnormalities in dTG-I-1c were reversed by shutting-off I-1c expression and were absent in tTA. Hearts from dTG-I-1c showed hyperphosphorylation of phospholamban and the ryanodine receptor, which could account for the hypercontractile phenotype and may increase the propensity for spontaneous Ca²⁺-release, respectively. Indeed, isolated myocytes from dTG-I-1c showed a higher frequency of catecholamine-induced Ca²⁺-sparks, consistent with a higher susceptibility for triggered arrhythmia. Notably, aging dTG-I-1c mice developed a cardiomyopathic phenotype. Taken together, transgenic expression of I-1c enhanced contractile function in young animals, but promoted catecholamine/stress-induced arrhythmias and structural cardiac pathology. Longterm expression of dTG-I-1c induced cardiomyopathy. These data point to fundamental risks of I-1c gene therapy in chronic heart failure.

Keywords: phosphatases, beta-adrenergic signaling, contractile function, heart failure

Electrical alterations in heart failure: focus on electrophysiological and excitation-contraction coupling mechanisms

Elisabetta Cerbai¹, Raffaele Coppini¹, Cecilia Ferrantini¹, Leonardo Sacconi², Francesco Pavone², Alessandro Mugelli¹, Corrado Poggesi¹

¹Center of Molecular Medicine, University of Florence, Italy

²European Laboratory of Non-Linear Spectroscopy, Florence, Italy

The main features of heart failure(HF) pathophysiology are the impaired contraction of ventricular muscle and arrhythmogenic risk. Loss or disorganization of the network of transverse tubules has turned out as an early feature of cardiomyocyte remodeling in heart failure. However, the impact of t-tubular remodelling on the mechanics or on the arrhythmogenic potential of the myocardium was never before investigated. We first developed and validated a novel method to achieve acute disconnection of t-tubules from the surface sarcolemma in ventricular trabeculae with formamide-induced osmotic shock and evaluated the effects of detubulation on force development. We then introduced a novel optical technique to simultaneously record electrical activity from multiple small regions of the t-tubular network and surface sarcolemma employing voltage sensitive membrane dyes. The electrical function of t-tubules was evaluated on control myocytes and cells from rat with post-ischemic heart failure induced by coronary ligation. Acute loss of t-tubules in trabeculae significantly reduces myocardial force and impairs the ability to increase contraction in response to high frequency. Heart failure myocytes show a reduced t-tubular density with patchy areas devoid of t-tubules and display smaller global calcium transients with areas of delayed and incomplete calcium release. Local optical recordings show that action potentials occur simultaneously throughout the whole membrane in control myocytes; however, in HF, part of remodelled t-tubules does not show a regular electrical activity and often display local spontaneous depolarizations. Altered t-tubular electrical function is an additional mechanism besides orphaned ryanodine receptors contributing to reduced force in HF and spontaneous activity of altered tubules may produce arrhythmias.

Keywords: heart failure, t-tubules

Targeting abnormal G-protein-coupled receptor kinase and Gbeta-gamma protein activity in heart failure: Translation of molecular therapeutic innovations beyond beta-AR blockade

Patrick Most

Institute of Molecular and Translational Cardiology, Department of Internal Medicine III, University of Heidelberg, Germany

Heart failure (HF) is a major worldwide health problem and its incidence in the Western World is projected to rise steadily as median life expectancy increases. Abnormal beta-adrenergic receptor (b-AR) signalling is considered a hallmark of the clinical syndrome driving fatal progression towards contractile failure and lethal tachyarrhythmias. Although prolonging life expectancy in HF patients, b-AR blockade is considered an indispensable but increasingly blunt weapon in light of our advanced understanding of underlying molecular abnormalities in b-AR signalling. To this end, therapeutic innovations targeting dysfunctional G-protein-coupled receptor kinase (GRK) activity beyond b-AR blockade and novel strategies gleaned from unexpected molecular insights in dysfunctional b-AR signalling will be discussed, and translational strategies embarking on proof-of-concept studies in small and large animal HF models for targeted modulation of the GRK2 isoform and Gbeta-gamma protein dependent signalling will be presented.

Keywords: beta-adrenergic signaling, heart failure, G-protein coupled receptors

The forgotten and dark side of Alzheimer's Disease: Neuroglial Pathology

Jose Julio Rodriguez Arellano

1. IKERBASQUE, Basque Foundation for Science, 48011, Bilbao, Spain; 2. Department of Neurosciences, University of the Basque Country UPV/EHU, Leioa 48940, Spain; 3. Institute of Experimental Medicine, ASCR, Prague, Czech Republic.

Neuroglial cells are fundamental for brain homeostasis and they represent the intrinsic brain defence system. Therefore, all forms of neuropathological processes inevitably involve glial cells. Neurodegenerative diseases, including Alzheimer's disease (AD) disrupt brain connectivity affecting neuronal-neuronal, neuronal-glial and glial-glial contacts and interaction. In addition neurodegenerative processes trigger universal and conserved glial reactions represented by astrogliosis and microglial activation. The complex of recently acquired knowledge allows us to regard the neurodegenerative diseases as primarily gliodegenerative processes, in which glial cells determine the progression and outcome of neuropathological processes such as AD. We have recently probed this active pathological role, by showing: (i) an astroglial generalised atrophy with a concomitant astrogliosis just restricted to A β plaques presence ii) alterations in glutamate glial metabolism and (iii) an early resting microglial recruitment in the affected areas, even before the presence of activated/macrophagic microglial cells. These glial alterations are fundamental for the disruption of neural networks connectivity as well as with the neurotransmitters imbalance that underlie the mnesic deficits associated with AD.

Keywords: Alzheimer disease, neuroglia, neurodegeneration, glutamate, plasticity,

Aberrant intracellular Ca²⁺ signalling correlates with astrocyte degeneration in Amyotrophic Lateral Sclerosis

Francesca Martorana¹, Liliana Brambilla¹, Chiara F. Valori², Chiara Roncoroni², Chiara Bergamaschi¹, Andrea Volterra³, Paola Bezzi³, Daniela Rossi¹

¹Salvatore Maugeri Foundation

²University of Milan

³University of Lausanne

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder characterized by motor neuron degeneration. About 2% of cases are associated with mutations in the gene encoding the enzyme superoxide dismutase 1 (SOD1). Recent evidence indicates that motor neuron death is a non-cell-autonomous event that involves glial cells, particularly astrocytes. Recently, we reported that a subpopulation of astrocytes degenerates in the spinal cord of SOD1G93A transgenic mouse model of ALS. Mechanistic studies in cultured astrocytes revealed that such effect is mediated by the transmitter glutamate via the activation of its inositol 1,4,5 triphosphate (IP₃)-generating metabotropic receptor 5 (mGluR5). Since non-physiological formation of IP₃ can prompt Ca²⁺ release from the intracellular stores and trigger cell death, we investigated the intracellular Ca²⁺ signalling that occurs downstream of mGluR5 in hSOD1G93A-expressing astrocytes. Primary astroglial cultures were prepared from spinal cord of newborn (0-24 hrs) mice. Contrary to wild-type cells, we found that stimulation of mGluR5 in hSOD1G93A-expressing astrocytes causes unusual rises in the intracellular Ca²⁺ concentrations that correlate with cell death. Based on these observations, we next screened the glioprotective effect of innovative drugs, namely cell-permeable therapeutics. These consist of peptidic effector moieties coupled to the selective intracellular peptide transporter TAT protein. We initially validated the usefulness of these molecules demonstrating that a control fluorescent peptide enters astrocytes in culture and is retained within the cells. We then tested the impact of specific intracellular peptides with anti-apoptotic properties on glutamate-treated hSOD1G93A-expressing astrocytes. We identify one molecule that rescue the aberrant Ca²⁺ signaling and protects the mutant cells.

Keywords: Amyotrophic Lateral Sclerosis, astrocyte, glutamate, calcium

Microglial response in neurodegeneration and Alzheimer's disease

Michael Thomas Heneka

Department of Neurology, University of Bonn, Germany

Generation of neurotoxic amyloid- β peptides and their deposition along with neurofibrillary tangle formation represent key pathological hallmarks in Alzheimer's disease (AD). Recent evidence suggests that inflammation may be a third important component which, once initiated in response to neurodegeneration or dysfunction, may actively contribute to disease progression and chronicity. Various neuroinflammatory mediators including complement activators and inhibitors, chemokines, cytokines, radical oxygen species and inflammatory enzyme systems are expressed and released by microglia in the AD brain. Degeneration of aminergic brain stem nuclei including the locus ceruleus and the nucleus basalis of Meynert may facilitate the occurrence of inflammation in their projection areas given the antiinflammatory and neuroprotective action of their key transmitters norepinephrine and acetylcholine. While inflammation has been thought to arise secondary to degeneration, recent experiments demonstrated that inflammatory mediators may stimulate amyloid precursor protein processing by various means and therefore can establish a vicious cycle. Despite the fact that some aspects of inflammation may even be protective for bystander neurons, antiinflammatory treatment strategies should therefore be considered. Non-steroidal anti-inflammatory drugs have been shown to reduce the risk and delay the onset to develop AD. While, the precise molecular mechanism underlying this effect is still unknown, a number of possible mechanisms including cyclooxygenase 2 or c-secretase inhibition and activation of the peroxisome proliferator activated receptor c may alone or, more likely, in concert account for the epidemiologically observed protection. Data on microglial activation in AD along with suggestions to modify and alter the pro- into an antiinflammatory phenotype will be reviewed and discussed.

Keywords: neuroinflammation, systemic immune challenge, microglia, innate immunity, neurodegeneration, local immune reaction, chronic,

Astrocytes in spinal cord injury: Friend or foe?

Alain Privat

University of Bilbao, Spain

Astrocytes have been considered over decades as accessory cells in the physiology and pathophysiology of the central nervous system. More specifically, their possible role in the pathophysiology of neurotrauma has never been considered until the beginning of the eighties, when Aguayo and colleagues demonstrated that the absence of axonal regeneration in the CNS of adult mammals was not an intrinsic property of neurons, but rather depended upon their cellular environment. Using oxysterols, which had been shown to block the reactivity of astrocytes, we found that the two major proteins of the astrocyte cytoskeleton, GFAP and Vimentin, were major actors of this reactivity in spinal cord injury. We managed then to obtain mice knock-out for the genes coding for these two proteins. Using co-cultures of newborn spinal cord KO astrocytes, with embryonic cortical neurons, we found that these astrocytes were more permissive for neuron survival and neuritic extensions than wild type astrocytes. Moreover, these KO astrocytes presented many biochemical characteristics of the so-called radial glia which operate as guides for neuronal migration and neuritic extension during embryogenesis. KO mice underwent lateral hemi-section of the spinal cord, and we found that at variance with wild type they did not develop astrocytic scars, sprouting of corticospinal and raphespinal axons was extensive, and they regained function of the paralyzed hindlimb. We have recently replicated these results both on in vitro and in vivo models with lentiviral vectors carrying siRNA for GFAP and Vimentin, on wild type mice. It appears then that reactive astrocytes, when adequately manipulated can be converted into permissive substrates for axonal regeneration and functional recovery after spinal cord injury.

Supported by INSERM, IRME, Verticale and Ikerbasque

Keywords: Astrocytes, GFAP and Vimentin

Diabetes, Obesity and GLP-1: Lessons from bariatric surgery

Jens Juul Holst

Department of Biomedical Sciences, University of Copenhagen, Denmark

Bariatric surgery mainly includes gastric banding, GB, and Roux-en Y Gastric Bypass, RYGB and both cause weight loss and diabetes (T2DM) resolution. Whereas after GB, diabetes resolution develops gradually in parallel with weight loss and in only about 50% of patients. With T2DM, resolution is almost instantaneous and obtained in 84% after RYGB, prompting a search for associated mechanisms. The abnormal exposure of more distal parts of the small intestine to nutrients and digestive secretions after RYGB suggested that secretion of distal gut hormones could be responsible. Indeed, the secretion of GLP-1 and PYY may be up to 20-fold elevated after RYGB but is unchanged after GB. Both hormones decrease food intake but GLP-1 is strongly insulinotropic. Attempts to block the secretion (somatostatin) or GLP-1 actions (Exendin 9-39) are associated with increased appetite and impaired glucose tolerance. But what causes the exaggerated release. The density of L-cells in the ileum is much higher than in the upper jejunum and this provides part of the explanation. In addition, the passage of nutrients through the bypass and to the ileum may be greatly exaggerated because of bypassing of the upper intestinal mechanisms normally inhibiting gastric emptying (explaining the tendency to dumping after RYGB). Finally, recent studies have revealed expression on the L-cells secreting PYY and GLP-1 of stimulatory receptors for bile acids and lipids to which these cells are now abnormally exposed. Thus, the mechanism is most likely to represent exaggerated activation of the normal “ileal brake” mechanism, with GLP-1 playing a predominant role for the resolution of diabetes.

Keywords: gastric bypass, PYY, glucagon, exendin,

Role of rostral forebrain GLP-1 receptors in the control of food intake

Diana L Williams

Department of Psychology & Program in Neuroscience, Florida State University, Tallahassee, FL, USA

Glucagon-like peptide 1 (GLP-1) is produced in the intestine and in neurons of the caudal brainstem, and GLP-1 can act at distinct sites in the periphery and in the brain to affect food intake. Recent studies in our laboratory have focused on the role of GLP-1 neurons in the control of feeding in the rat. Hindbrain GLP-1 neurons project to many brain areas known to be involved in the control of energy balance. One of these projection sites is the Nucleus Accumbens (NAc), known for its role in reward and motivated behavior. We characterized this projection using retrograde neuronal tracers injected into the NAc combined with immunofluorescent histochemical staining for GLP-1 in the caudal brainstem. In a series of studies, we examined rats' behavioral responses to direct injection of GLP-1 or GLP-1R antagonist into the NAc. Stimulation of GLP-1 receptors (GLP-1R) in the NAc Core, but not the Shell, suppressed chow intake for up to 24 h after an acute treatment. Blockade of GLP-1R in the NAc Core increased food intake, providing strong support for the hypothesis that endogenous GLP-1 release at this site contributes to physiologic control of feeding. Ongoing research is focused on the mechanisms through which NAc GLP-1R activity affects food intake. These studies identify a novel site for endogenous central GLP-1 action and raise the possibility that exogenous pharmacologic GLP-1R agonist treatments exert their anorexic effects in part by acting in the NAc.

Keywords: GLP-1, satiety, Nucleus Accumbens, hindbrain

Satiety signals stimulate preproglucagon neurons in the nucleus tractus solitarius: Functional properties of GLP-1 producing neurons

Stefan Trapp

Department of Surgery and Cancer, Imperial College London, Lundo, United Kingdom

Glucagon-Like Peptide-1 (GLP-1) is an incretin released from enteroendocrine L-cells postprandially and has glucoregulatory and satiety effects. Mounting evidence implicates GLP-1 receptors within the brain in these effects, but the short half-life of GLP-1 in the circulation makes it questionable whether peripheral GLP-1 reaches brain receptors. Within the brain, preproglucagon (PPG) neurons, found mainly in the nucleus tractus solitarius, produce GLP-1. Little is known about what governs the activity of these neurons, primarily because of difficulties identifying these cells *in vitro*. We have addressed this issue with transgenic mice expressing yellow fluorescent protein (YFP) under PPG promoter control. We showed that PPG neurons are spontaneously active and their electrical activity is enhanced by peripheral satiety factors such as leptin and cholecystokinin (CCK), but not GLP-1 or peptide YY. PPG neurons do not actually express the GLP-1 receptor. They receive glutamatergic input from the solitary tract, reflecting vagal afferent activity. A proportion of PPG cells respond to changes in ambient glucose levels with altered electrical activity. Our immunohistochemical studies showed that PPG neurons project widely to central autonomic regions, including various brainstem nuclei. Recent *in vivo* studies have highlighted the importance of hindbrain receptors for GLP-1's anorexic effects, and our results might indicate that GLP-1 released from PPG cells, rather than peripheral GLP-1 entering the brain, interacts with these receptors. We hypothesise that PPG neurons are central to integrating peripheral satiety signals and brain energy status into a feeding response. Further studies should target these neurons *in vivo* to test this hypothesis.

Supported by the Medical Research Council, UK

Keywords: NTS, brainstem, electrophysiology, gut peptides, glucose

Paracrine and endocrine effects of peripheral GLP-1 on eating

Wolfgang Langhans

Institute of Food, Nutrition and Health, ETH Zurich

Enteroendocrine L-cells release GLP-1 in response to luminal nutrient (primarily carbohydrate and fat) stimulation, GLP-1 receptors (GLP-1R) are expressed in the periphery and in brain areas implicated in the regulation of energy homeostasis. GLP-1 has potent insulinotropic and gluco-regulatory effects, and peripheral administration of GLP-1 inhibits eating. This may reflect a physiological satiating function of endogenous GLP-1, but the exact stimuli of GLP-1's release and the site(s) and mechanism(s) of its action are unresolved. Here I present data indicating that: 1) high fat meals are a more potent stimulus for intestinal GLP-1 release in rats than isocaloric low fat meals, as measured by postprandial changes in active GLP-1 levels in intestinal lymph; 2) intestinal triglyceride re-synthesis directly or indirectly modulates dietary fat-induced GLP-1 release; 3) hepatic degradation appears to prevent a systemic increase in endogenous GLP-1 during chow meals in rats; 4) the area postrema and hindbrain GLP-1R activation are involved in mediating a possible endocrine eating-inhibitory effect of GLP-1, whereas abdominal vagal afferents are involved in its putative paracrine satiating action; and 5) GLP-1 can promote adipocyte proliferation and triglyceride synthesis through a direct, GLP-1R-mediated effect. Further studies should a) examine under which conditions a systemic endocrine or a local paracrine action of GLP-1 in the intestine is physiologically relevant for satiation, b) identify the neural mechanisms mediating these effects, and c) test whether GLP-1 released into the intestinal lymph may have direct access to intra-abdominal adipose tissue.

Keywords: Gut peptides, Eating, Satiation, Glucose homeostasis, Vagus, Hindbrain, Intestinal lymph

Acid calcium stores and their role in cellular pathophysiology

Ole Holger Petersen

School of Biosciences, Cardiff University, UK

The role of acid Ca^{2+} pools in both physiology and pathophysiology has been controversial, but much evidence has recently accumulated indicating that these pools are functionally important for cellular regulation. The pancreatic acinar cell, which is a classical object for Ca^{2+} signalling studies provides a good example. High-resolution confocal and two-photon studies of the dynamics of $[\text{Ca}^{2+}]$ in the cytosol and the major organelles have shown that physiological stimuli (acetylcholine and cholecystokinin) elicit repetitive release of tiny quantities of Ca^{2+} from the endoplasmic reticulum (ER) resulting in spiking Ca^{2+} signal patterns in the apical part of the cytosol, driving fluid and enzyme secretion (Petersen & Tepikin *Annu Rev Physiol* 70, 273-299, 2008). In contrast, pathological stimuli (alcohol/alcohol metabolites/bile acids) initiating the human disease acute pancreatitis, generate sustained, global and toxic elevations of the cytosolic $[\text{Ca}^{2+}]$ due to massive release of Ca^{2+} from both the ER and acid stores (Petersen et al *Cell Calcium* 45, 634-642, 2009; Petersen et al *Cell Calcium* in press 2011). It is the excessive Ca^{2+} release from the acid stores, rather than from the ER, that activates intracellular proteases leading to auto-digestion. The Ca^{2+} release from the acid stores occurs principally via IP_3 receptors of sub-types 2 and 3 (Gerasimenko et al *PNAS* 106, 10758-10763, 2009). Very recently we have discovered that intracellular calmodulin has a protective effect against toxic Ca^{2+} signal generation and the resulting intracellular protease activation. We have also shown that this protective effect can be boosted significantly by application of a membrane-permeable Ca^{2+} -like peptide (Gerasimenko et al *PNAS* 108, 5873-5878, 2011).

Keywords: Calcium, toxicity, proteases, inositol trisphosphate receptors, calmodulin

Calcium signalling in neuroglia

Alexei Verkhratsky¹, Jose Julio Rodriguez Arellano², Vladimir Parpura³

¹Faculty of Life Sciences, The University of Manchester, Manchester, UK

²IKERBASQUE, Basque Foundation for Science, 48011, Bilbao, Spain

³Department of Neurobiology, Center for Glial Biology in Medicine, Civitan International Research Center, Atomic Force Microscopy & Nanotechnology Laboratories, and Evelyn F. McKnight Brain Institute, University of Alabama, Birmingham, USA

Glial cells respond to various electrical, mechanical and chemical stimuli, including neurotransmitters, neuromodulators and hormones, with an increase in $[Ca^{2+}]_i$. These glial $[Ca^{2+}]_i$ signals exhibit a variety of temporal and spatial patterns. Glial $[Ca^{2+}]_i$ signals can traverse gap junctions between glial cells without decrement and travel over a great distances within glial networks. The predominant source of Ca^{2+} for Ca^{2+} signal generation in astrocytes resides within the endoplasmic reticulum (ER). Inositol 1,4,5-trisphosphate and ryanodine receptors of the ER provide a conduit for the release of Ca^{2+} to the cytosol. The ER store is (re)filled by the ER-specific Ca^{2+} -ATPase of SERCA type. Ultimately, the depleted ER is replenished by Ca^{2+} which enters from the extracellular space to the cytosol via store-operated Ca^{2+} entry; the TRPC1 protein has been implicated in this part of the astrocytic exocytotic process. Voltage-gated Ca^{2+} channels and plasma membrane Na^+/Ca^{2+} exchangers are additional means for cytosolic Ca^{2+} entry. Cytosolic Ca^{2+} levels can be modulated by mitochondria, which can take-up cytosolic Ca^{2+} via the Ca^{2+} uniporter and release Ca^{2+} into cytosol via the mitochondrial Na^+ /Ca^{2+} exchanger, as well as by the formation of the mitochondrial permeability transition pore. The interplay between various Ca^{2+} sources determines cytosolic Ca^{2+} dynamics that differentially drives multiple Ca^{2+} -depenent cytoplasmic processes. The highly specialised glial Ca^{2+} signals provide means for information encoding within glial networks, integrating them with neuronal circuits. An understanding of this process in vivo will reveal some of the astrocytic functions in health and disease of the brain.

Keywords: Neuroglia, calcium signalling; astrocytes; ionotropic receptors, metabotropic receptors

Heterogeneity of Ca²⁺ homeostasis in the Golgi apparatus

Tullio Pozzan, Valentina Lissandron, Paola Capitanio, Paola Pizzo
University of Padova, Department of Biomedical Sciences and CNR Neuroscience Institute, Padova, Italy

The Golgi apparatus plays a key role in protein sorting and their post translational modifications. All proteins synthesized in the endoplasmic reticulum and targeted to the plasma membrane, lysosomes or to be secreted in the medium are somehow modified in the Golgi apparatus before they reach their final destination. Such post-translational modifications require the concerted action of numerous enzymes that are selectively localized in the three major sub-compartments of the Golgi, i.e. the Cis- Intermediate and Trans-Golgi. In addition, it has been demonstrated that the Golgi not only can accumulate Ca²⁺ within its lumen in an ATP dependent way, but also that it can release it into the cytoplasm during cell activation. Finally evidence has been provided by different groups indicating that the luminal Ca²⁺ concentration in the Golgi is pivotal for several of the organelle functions. We have recently demonstrated that the trans-Golgi does not express significant amounts neither of the Sarco-Endoplasmic-Reticulum Ca²⁺ ATPase nor of the IP3 receptors, but rather it is endowed with another Ca²⁺ ATPase, the Secretory Pathway Ca²⁺ ATPase (SPCA1) and ryanodine receptors. Here I will briefly discuss these characteristics of the Trans-Golgi compartment and I will then focus on a few recent results obtained with a new GFP-based Ca²⁺ indicator selectively localized in the Cis- and Intermediate Golgi regions. I will show that these two compartments behave, in terms of Ca²⁺ handling, very differently from the trans Golgi and that within these two subregions the different cisternae appear quite heterogeneous in terms of both IP3 sensitivity and Ca²⁺ uptake mechanisms.

Keywords: Golgi calcium store, Ca²⁺ homeostasis and signaling

Ca²⁺ stores and Ca²⁺ entry: SERCA provides the link

Javier Garcia Sancho

Instituto de Biología y Genética Molecular, University of Valladolid, Spain

Cross-talk between plasma membrane (PM) and subcellular organelles is essential for cellular Ca²⁺ homeostasis. Emptying the endoplasmic reticulum (ER) calcium stores increases Ca²⁺ entry through PM (store operated Ca²⁺ entry, SOCE). STIM1 and Orai1 are the principal players in SOCE. STIM1 senses the Ca²⁺ content inside ER, and, when it decreases, activates Orai1, a PM store-operated calcium channel (SOC) that promotes Ca²⁺ entry and increases cytosolic Ca²⁺. We have studied here the role of cytoplasmic organelles. We monitored the fate of Ca²⁺ entering through SOC and its redistribution among different organelles (ER, mitochondria, nucleus, cytosol) using selectively targeted Ca²⁺ probes. On depletion of the ER Ca²⁺ stores SERCA colocalizes with STIM1 and Orai1 at puncta. ER Ca²⁺ refilling is tightly coupled to SOCE when the proportions of STIM1, Orai1 and SERCA are adequate, and little Ca²⁺ reaches the cell core under these conditions. The tightness of this calcium entry-calcium refilling (CECR) coupling, as measured by the slope of the stimulus-signal strength function, was comparable to classic excitation-response coupling mechanisms. Mitochondrial Ca²⁺ uptake was very minor. SERCA is the third element of SOCE. It colocalizes with STIM1 and Orai1 at puncta, where it is tightly coupled to plasma membrane SOC. This allows extremely efficient refilling of the ER stores. Whereas ER takes up most of the SOCE calcium load, mitochondria take very little. These differences in behaviour depend on: i) spatial positioning with regard to SOC, ii) amplitude of the high Ca²⁺ microdomains, and iii) differences in the Ca²⁺ affinity of the uptake mechanisms present in both organelles.

Keywords: Calcium signalling, calcium microdomains, STIM1, endoplasmic reticulum, aequorin, chemiluminescence, Store-operated calcium entry

Mouse models of vascular permeability control: Visualizing the Future

Rolf K. Reed¹, Fitz Roy E. Curry²

¹Department of Biomedicine, University of Bergen, Norway

²Department of Physiology and Membrane Biology, University of California at Davis, USA

The symposium will focus on the use of genetically modified mice to investigate the control of vascular permeability and microcirculatory exchange by applying appropriate methods for phenotyping and physiological studies. Recent developments allow multiple, non-invasive measurements of blood to tissue exchanges using advanced techniques based on magnetic resonance imaging, micro-positron emission tomography, and long wavelength fluorescence imaging. The symposium will begin with an overview of new approaches enabling investigations of common mechanisms controlling vascular permeability at the molecular and cellular level, in individually perfused microvessels, and in whole organs. The symposium will then focus on more detailed presentations of recent investigation of several different models. First, genetically modified mice and new imaging techniques are now opening new and unexplored avenues for studying the in vivo function of the role of the family of natriuretic peptides which control the plasma volume by modifying vasodilation, vascular permeability, and renal excretion of water.

Keywords: Mouse models

Measurement of vascular permeability in genetic mouse models

Fitz Roy E. Curry¹, Rolf K. Reed², Cecilie B. Rygh², Scott Simon¹, Min Ho Kim¹

¹University of California, Davis, California, USA

²University of Bergen, Bergen, Norway

New methods to measure vascular permeability in mice enable longitudinal investigation of changes in permeability in the same animal, and distinguish tissue uptake, due to increased microvessel permeability from increased surface area for exchange. One method measures fluorescent intensity (FI) in skin after macromolecules labeled with long-wavelength fluorescent tracers are rapidly injected iv. Permeability is quantified within sub-regions of images of the fluorescent tracer distribution using the initial step increase in FI (measures local intravascular volume) and the subsequent increase of FI (due to blood to tissue efflux of tracer). If increased, the ratio of efflux to local vascular volume is a better measure of real changes in permeability than tissue uptake alone (e.g. using the Miles Assay: see Bates, Cardiovascular Research, 2010). With narrow-band filters, solutes labeled with different fluorescent tracers are injected sequentially for longitudinal measurement of permeability, or used in combination with cells expressing fluorescent protein markers (Kim et al, Am. J. Physiol., 2009). The approach has been extended for internal organs using macromolecules with tracers appropriate for MRI (Curry et al, J. Physiol, 2010; Cardiovascular Research, 2010) or micro-PET (Rygh et al, Clinical Cancer Research, 2011) and tested using two-tracer standards (Lin et al, J. Physiol, 2011). Collaborations using these methods facilitate investigations of normal and dysfunctional endothelial barriers in multiple mouse organs. HL28607

Keywords: Vascular permeability, fluorescent tracer methods, Miles Assay.

Mouse models to decipher the vascular functions and signaling networks of atrial natriuretic peptide

Michaela Kuhn, Wen Chen

Institute of Physiology, University of Wuerzburg, Germany

Cardiac atrial natriuretic peptide (ANP) participates in the maintenance of arterial blood pressure and intravascular volume homeostasis by concerted renal, adrenal, vascular and central nervous actions. These various cellular effects are mediated by the guanylyl cyclase-A (GC-A) receptor and intracellular cGMP. Within the vascular system the GC-A receptor is densely expressed both in smooth muscle and endothelial cells. To dissect the functions of these vascular action sites of ANP in vivo, we inactivated the GC-A gene selectively in one of both cell types, using Cre-lox technology. Remarkably, smooth muscle-restricted deletion of GC-A in mice completely abolished the direct vasodilating effect of ANP but did not affect resting arterial blood pressure and volume. In contrast, mice with endothelial GC-A deletion, despite the preservation of the diuretic and vasodilating effects of ANP, have marked chronic hypervolemic hypertension. Furthermore, acute vascular volume expansion, which caused release of endogenous cardiac ANP, did not affect resting central venous pressure of control mice but rapidly and significantly increased central venous pressure of mice with endothelial GC-A deletion. Our intravital microscopy studies showed that ANP, via endothelial GC-A and the downstream cGMP-dependent kinase (cGK I), increases the microvascular extravasation of albumin in the skin and skeletal muscle. Investigations in vivo and in cultured microvascular endothelial cells indicated that caveolae-mediated albumin transcytosis is one mechanism mediating this effect. The ANP/GC-A-induced plasma proteins escape across capillary walls increases interstitial oncotic pressure and ultimately can shift fluid from the intravascular to the interstitial compartment to maintain intravascular volume homeostasis.

Keywords: natriuretic peptides, cyclic GMP, endothelium, microcirculation, permeability

Mouse models reveal diverse roles for basement membrane collagens XV and XVIII in vascular development, integrity and function

Ritva Heljasvaara, Taina Pihlajaniemi

Biocenter Oulu and Department of Medical Biochemistry and Molecular Biology, Institute of Biomedicine, University of Oulu, Finland

The vascular basement membrane (BM) contributes substantially to blood vessel formation and function. Collagens XV and XVIII, both with properties of collagens and proteoglycans and containing an anti-angiogenic endostatin domain, are common components of vascular BMs. Our mouse models for these collagens have revealed that although they share many structural features, their biological functions are essentially different. For example, lack of collagen XVIII leads to abnormal outgrowth of retinal vasculature and delayed regression of hyaloid vessels, while collagen XV appears to regulate capillary structure and function in the heart and skeletal muscle and lack of this collagen leads to mild myopathic phenotype and may also predispose the mice to cardiomyopathy. Collagen XVIII/endostatin affects both tumor angiogenesis and lymphangiogenesis, and regulates inflammatory response in skin tumors. Finally, our recent mouse studies point out the importance of these collagen molecules in maintaining normal vascular permeability.

Keywords: basement membrane, collagen, vascular permeability, vascular development

Longitudinal and quantitative assessment of tumor microcirculation using positron emission tomography

Cecilie B. Rygh¹, Shengping Qin², Jai W Seo², Lisa M Mahakian², Katherine W Ferrara²

¹Department of Biomedicine, University of Bergen, Bergen, Norway

²Department of Biomedical Engineering, University of California Davis, California, USA

Imaging techniques such as positron emission tomography (PET) and magnetic resonance imaging (MRI) are valuable tools for non-invasive in vivo longitudinal assessment and quantification of physiological processes. Microcirculatory changes are an important component in the pathophysiological cascade in cancers, and detection of changes over time provides insight in progression of the disease. We wanted to investigate microcirculatory changes as well as the utility of nano-sized particles as the lesions progressed from premalignant to malignant in a mouse model of ductal carcinoma in situ (DCIS) using PET. Liposomes and albumin were labeled with ⁶⁴Cu, which allowed us to follow the fate of the tracer up to 48 hrs after injection. PET scanning was performed at multiple time-points up to 7 weeks after transplantation of premalignant tissue into the mammary pad. An image-driven pharmacokinetic model was applied to quantify microvascular parameters as the lesions progressed. Vascular volume fraction and vessel permeability of both tracers increased with tumor transition but albumin uptake preceded liposomal uptake. A comparison of the accumulation of the two tracers emphasized the heterogeneity in the effective permeability size cutoff. Further, PET facilitated direct visualization of tumor spatial heterogeneity of tracer distribution. One of the greatest challenges in developing therapeutic regimens is the inability to rapidly and objectively assess tumor response due to treatment. In vivo imaging may provide invaluable insight in early responses and longitudinal progression.

Keywords: PET, imaging, longitudinal, microcirculation, quantitative, cancer

Workshops

W01

The unique hypoxic responses of the pulmonary vasculature: underlying mechanisms

Paul Mcloughlin

University College Dublin, School of Medicine and Medical Sciences, Dublin, Ireland

Chronic pulmonary hypoxia causes pulmonary hypertension, which results from a sustained increase in pulmonary vascular resistance, a response to hypoxia that is not seen in other vascular beds. The changes in the vasculature that accompany this include arterial remodelling, an altered contractile state and angiogenesis (3). These pulmonary specific responses suggest that there are genes whose expression is altered selectively in the lung in response to alveolar hypoxia, controlled by specific transcriptional mechanisms (4). The bone morphogenetic antagonist (BMP), gremlin 1, is selectively upregulated in the hypoxic lung in vivo, but not in other organs (1, 2). This gene is of particular interest because it has previously been found that heterozygous BMP receptor mutations associated with reduced receptor signalling were the underlying defect in families with a rare heritable form of pulmonary arterial hypertension. BMP signaling is also reduced in hypoxic pulmonary hypertension. Evidence for the role of the BMP antagonists in hypoxic pulmonary hypertension and in human disease will be reviewed, the transcriptional mechanisms underlying their lung selective hypoxic regulation and their actions on vascular structure and function.

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Keywords: Hypoxia and pulmonary vasculature

The alveolar barrier

Heimo Mairbaurl

Medical Clinic VII, Sports Medicine, University of Heidelberg, Germany

The alveolar barrier is formed by monolayers of alveolar epithelium (AE) and endothelium. It separates distal air spaces from blood. A thin fluid film on its surface is required for gas exchange. Any disturbance of barrier function and thickening of the fluid film impairs gas exchange and causes hypoxemia and tissue hypoxia; whereas the layer of endothelial cells is quite leaky, alveolar epithelium forms a tight monolayer which prevents permeation of plasma water into alveolar space. Another protective mechanism is alveolar reabsorption: active reabsorption of Na⁺ from the alveolar surface, which is mediated by apical epithelial Na-channels and basolateral Na/K-ATPase in alveolar epithelial cells, generates the osmotic driving force for water. Hypoxia of the alveolar barrier occurs when fluid accumulates at its surface subsequent to increased capillary filtration pressure or upon inflammation-induced malfunction of junction proteins. It increases its permeability and also inhibits reabsorption thus impeding potentially protective mechanisms. Although pathogens can also directly affect alveolar epithelial cells to modify their function, most negative effects are mediated or enhanced by signaling molecules released from alveolar macrophages. Amongst those are TNF-alpha, TGF-beta, interleukins, and NO. The mechanisms involved in the impairment of barrier function are only poorly understood. They appear to involve enzyme-mediated processes such as ERK1/2, MAP kinases, but also direct protein nitrosylation. Reabsorption and barrier tightness can be improved by beta2-adrenergic and glucocorticoid stimulation, which affect protein compartmentalization and biosynthesis. Although hypoxia and inflammation seem to impair signaling as well, stimulation still occurs and can restore normal barrier function.

Keywords: lung, gas exchange, fluid balance, ion transport, hypoxia, inflammation

Mechanisms of LPS induced impairment of alveolar Na-transport by stimulated macrophages and effects of hypoxia

Emel Baloglu, Peter Bärtsch, Heimo Mairbaurl

Medical Clinic VII, Sports Medicine, University of Heidelberg, Heidelberg, Germany

Lung inflammation increases alveolar permeability, inhibits alveolar fluid reabsorption, and causes edema. The signaling pathways mediating inhibition of transport in alveolar epithelial cells are not clear. We aimed to identify by which mechanisms inflammation- and hypoxia- activated alveolar macrophages, propagate signals to alveolar epithelium and affect ion transport of alveolar epithelial cells. Primary rat alveolar epithelial cells (A₁II) were cultured on filters in absence and presence of alveolar macrophages (MA) and were exposed to hypoxia (1.5% O₂) for 24h with LPS (1µg/ml). Cells were also treated with NO-donors and inhibitors of NOS, ERK1/2, MAPK and JunK to identify signaling pathways. To test, whether the presence of macrophages is required for transport inhibition, A₁II cell monolayers were also exposed to conditioned media from LPS-stimulated macrophages. Transepithelial transport was measured in Ussing chambers. Nitrite in culture media was measured with the Griess reagent. LPS-stimulation of macrophages inhibited A₁II cell ion transport. Hypoxia enhanced this effect. Inhibition of NOS and ERK1/2 prevented transport inhibition caused by LPS stimulation of macrophages when the inhibitors were added either to the macrophages or to A₁II cells exposed to conditioned media from LPS treated macrophages. Inhibitors of NOS and ERK1/2 but not MAPK and JunK prevented the increase in nitrite in macrophages exposed to normoxia and hypoxia. Stimulation of alveolar macrophages inhibited alveolar epithelial ion transport by NOS and ERK1/2 dependent mechanisms which points to NO and cytokines as possible mediators. Hypoxia aggravates the inhibitory effect by not yet identified mechanisms.

Keywords: Macrophages, inflammation, alveolar ion transport

Effects of Hypoxia, LPS, and Macrophages on Alveolar Epithelial Permeability

Dan Way¹, Emel Baloglu¹, Nuray Yazihan², Peter Bärtsch¹, Heimo Mairbaurl¹

¹Medical Clinic VII, Sports Medicine, University of Heidelberg, Germany

²Molecular Biology Research Unit, University of Ankara, Turkey

Lung inflammation such as in ARDS and ALI causes accumulation of alveolar fluid, which hampers oxygen diffusion to alveolar epithelium. Hypoxia and inflammation have been shown to increase alveolar permeability. The mechanisms are poorly understood. We tested whether presence of alveolar macrophages is required to increase alveolar permeability in LPS-induced inflammation and whether hypoxia aggravates this effect. Primary rat alveolar epithelial cells (ATII) were cultured on filters in absence and presence of alveolar macrophages (MA) and were exposed to hypoxia (1.5% O₂) for 24h with and without treatment with LPS (1µg/ml). The permeability to fluorescein and FITC-albumin and the transepithelial electrical resistance (TEER) were measured as indicator of barrier tightness. TEER was decreased significantly by LPS. The effect was increased by the presence of MA and by hypoxia. MA, LPS, and hypoxia alone did not affect AT2 cell fluorescein and FITC-albumin permeability. LPS in presence of MA significantly increased permeability (+ 70%). Hypoxia did not aggravate the LPS effect in absence and presence of MA but increased the permeability of MA-AT2 co-cultures in absence of LPS. Effects of LPS and MA were more pronounced when applied to the basolateral than to the apical side. Together these results indicate that LPS-induced signals from MA increased alveolar permeability to small and large molecules. In contrast to our hypothesis, hypoxia does not aggravate the MA/LPS- induced increase in alveolar epithelial permeability.

Keywords: Hypoxia, inflammation, alveolar epithelial permeability

Inflammation and Hypoxia

Stilla Frede

Clinic of Anaesthesiology, University of Bonn, Bonn, Germany

The oxygen sensitive transcription factor hypoxia inducible factor-1 (HIF-1) is known as the key regulator of hypoxia induced gene expression. The microenvironment of inflamed and injured tissue is characterized by low levels of oxygen and glucose and high levels of inflammatory cytokines, reactive oxygen and metabolites. In addition to hypoxia, a broad variety of inflammatory mediators like the proinflammatory cytokines IL-1-beta and TNF-alpha, as well as bacterial lipopolysaccharides (LPS) have been shown to induce HIF-1 suggesting an integrative role for HIF-1 in conditions of hypoxia and inflammation. While hypoxia post-translationally stabilizes HIF-1alpha protein bacterial stimuli like LPS increase HIF-1alpha by transcriptional processes mediated by NF-κB. Since NF-κB could also be activated under conditions of severe hypoxia / ischemia activation NF-κB is able to enhance the inflammatory upregulation of HIF and HIF target genes. Using siRNA approaches we could also discriminate between HIF and NF-κB dependent hypoxic responses. Survival and migration of cells of the innate and adaptive immune system in a hypoxic microenvironment seems to be HIF-1 dependent whereas ICAM-1 mediated adhesion of leukocytes to endothelial cells depends on the hypoxic induction of NF-κB. Once extravasated from the vasculature the activity of immune cells could be further enhanced by stimulation of HIF-1 by proinflammatory cytokines and locally expressed tissue factors. Cross-talk between hypoxic induction of HIF-1, NF-κB and other signalling pathways activated by inflammation ensures a cell-type specific and stimulus adequate cellular response.

Keywords: hypoxia, inflammation, NF-κB, leukocyte invasion

Role of the oxygen sensor PHD3 in macrophage-mediated inflammation

Judit Kiss¹, Martin Mollenhauer¹, Sarah R Walmsley², Moira K B Whyte², Peter Carmeliet³, Massimiliano Mazzone³, Jürgen Weitz¹, Martin Schneider¹

¹Department of General and Transplantation Surgery, University of Heidelberg, Germany

²The Department of Infection and Immunity, The Medical School, University of Sheffield, United Kingdom

³Vesalius Research Center, K.U. Leuven, Leuven, B-3000, Belgium

Hypoxia and hypoxia-inducible factors (HIF-1 α and HIF-2 α) modulate innate immune responses in the setting of systemic inflammatory responses and sepsis. The HIF-prolyl hydroxylase enzymes, PHD1, PHD2 and PHD3 regulate the mammalian adaptive response to hypoxia. However, their significance in the innate immune response is poorly understood. We found that genetic loss of PHD3 specifically shortens the survival of mice subjected to abdominal sepsis. In vivo, plasma levels of pro-inflammatory cytokines were enhanced, and recruitment of macrophages to internal organs was increased in septic PHD3-deficient mice, altogether indicating enhanced innate immune functions. Reciprocal bone marrow transplantation in sublethally irradiated mice revealed that the enhanced susceptibility of PHD3-deficient mice to sepsis-related lethality was specifically caused by loss of PHD3 in myeloid cells. In vitro assays revealed enhanced cytokine-production, migration, phagocytic capacity, and pro-inflammatory activation of PHD3-deficient macrophages. Increased pro-inflammatory activity of PHD3-deficient macrophages occurred concomitantly with enhanced HIF-1 α protein stabilization and increased NF- κ B activity, and interference with the expression of HIF-1 α or NF- κ B blunted their pro-inflammatory phenotype. It is concluded that loss or impairment of PHD3 enzyme function aggravates the innate immune response to septic stimuli via enhanced macrophage pro-inflammatory activity.

Keywords: Oxygen sensor, PHD3 and macrophage-mediated inflammation

Endotoxin and hypoxia increase the expression of pro-inflammatory cytokines from alveolar macrophages, ATII cells, and endothelial cells in-vitro

Emel Baloglu¹, Nuray Yazihan², Peter Bärtsch¹, Heimo Mairbaurl²

¹Medical Clinic VII, Sports Medicine, University of Heidelberg, Germany

²Molecular Biology Research Unit, University of Ankara, Turkey

Stimulation of alveolar macrophages leads to impaired function of the alveolar barrier with subsequent edema formation, which causes hypoxia of the alveolar cells. Here we tested, whether release of pro-inflammatory mediators upon macrophage stimulation with LPS is aggravated by hypoxia, and whether other cells of the alveolar wall also release these mediators, and whether stimulation has consequences for barrier tightness. The alveolar wall was modelled by co-culturing rat alveolar macrophages, primary rat alveolar type II cells (ATII), and rat lung microvascular endothelial cells on transwell filters. Cells were stimulated with LPS (1µg/ml) in normoxia and hypoxia (1.5% O₂). mRNA expression was measured by qRT-PCR using 28S rRNA for normalization. LPS (4h) increased TNF-α and IL-6 mRNA in the co-culture, which resulted largely from macrophages. However, LPS also caused cytokine-expression in ATII and endothelial cells. In all cell types, LPS-induced TNF-α and IL-6 mRNA had almost returned to baseline values 24h and 48h after stimulation. Hypoxia caused a slow increase in TNF-α and IL-6 mRNA in macrophages, ATII cells, and endothelial cells. Effects of LPS and hypoxia were not additive. Expression of MCP-1, MMP-12, and COX-1 was not affected. In presence of macrophages, both 24h and 48h exposure to LPS and hypoxia decreased the electrical resistance of the co-cultures, whereas ATII cell mono-cultures were not affected. These results indicate a pronounced release of pro-inflammatory mediators upon LPS-stimulation in normoxia and hypoxia mainly from alveolar macrophages, which seems involved in increasing alveolar permeability.

Keywords: Endotoxin, hypoxia, alveolar permeability, alveolar macrophages, TNF-α

Hypoxia decreases mRNA-expression of mitochondrial electron transfer chain (mETC) enzymes in lung A549 cells

Heimo Mairbaurl, Carolin Schmieg, Peter Bärtsch
Medical Clinic VII, Sports Medicine, University of Heidelberg, Germany

Hypoxia has been shown to decrease ATP-consuming processes such as Na/K-ATPase and protein synthesis. We have shown previously that this was associated with decreased mitochondrial O₂-consumption and decreased activity of mETC complexes 1, 2 and 3 (Heerlein et al. AJRCMB 32:44 (2005)). Here we tested, whether decreased respiratory activity is associated with altered expression of mETC enzymes encoded on the nuclear and mitochondrial genome, as well as expression of potential regulators. Confluent human A549 cells were exposed to hypoxia (5% and 1.5% O₂) for 4h, 24h and 48h. mRNA expression was measured by qRT-PCR using 28S rRNA for normalization. Severe hypoxia for 24h and 48h caused a decreased mRNA expression of the nuclear-encoded NDUFS8 of complex 1 and SDHB of complex 2. Also the messages for the mitochondrial encoded cytochrome B, cytochrome oxidase-1, and ATPase-6 were decreased. At 5% O₂, there was a decrease in mRNA expression only after 24h but recovery thereafter. These changes were paralleled by a transient decrease (5% O₂, 24h) and pronounced decrease (1.5% O₂, 24h, 48h) of mRNA and protein of the key activator of mitochondrial transcription, TFAM. 1.5% O₂ had no effect on the mRNA expression of AMP-kinase, PPAR, NRF-1 and NRF-2. Their expression appeared up-regulated after 48h at 5% O₂. Our results indicate that decreased mitochondrial respiration in severe hypoxia is associated with a decreased expression of key enzymes of the mETC, which is probably caused by decreased TFAM consistent with mitochondrial autophagy.

Keywords: Hypoxia, mitochondrial autophagy, TFAM, metabolic depression

What is “tissue PO₂”?

Heimo Mairbaurl

Medical Clinic VII, Sports Medicine, University of Heidelberg, Germany

Cells use oxygen for aerobic metabolism localized in mitochondria but also for a variety of other oxygen dependent biochemical reactions. Thus all cells have a need for oxygen. There is a gradient of oxygen from the inspired air to the cells. Its steepness determines cellular oxygen supply. Determinants are the PO₂ of inspired air, diffusion capacity of the alveolar wall, capacity of blood to bind oxygen, circulation of oxygen containing blood, distribution of blood to specific organs and to specific locations within the organ by the number of capillaries supplying cells, by the distance between cells and blood capillaries, and by the oxygen demand of the cell. Another determinant is the affinity of O₂-binding and utilizing systems for oxygen. Thus, lung cells and cells in arterial blood are exposed to the highest PO₂ whereas cells in bone marrow and exercising heart and skeletal muscle probably see the lowest level of oxygen. In this review various aspects of these systems will be discussed.

Keywords: Tissue PO₂, oxygen, cell and aerobic metabolism

Handling cells in hypoxia

Emel Baloglu, Heimo Mairbaurl

Medical Clinic VII, Sports Medicine, University of Heidelberg, Germany

Culturing mammalian cells in a hypoxic environment is a useful model to study mechanisms of hypoxia associated diseases. Low solubility of O₂ in aqueous solutions and slow diffusion from the gas phase through the culture medium to cells is the major barrier to rapid changes in O₂ concentration. The oxygen concentration to which cells are actually exposed results from the balance between O₂ diffusion through culture medium and rate of O₂ consumption by the cells. It may take several hours to fully equilibrate culture media with a gas of defined composition when regular culture dishes are used. Time depends on thickness of the layer of medium and surface area. Mixing or use of gas-permeable flasks shortens equilibration. For hypoxic exposure cells can be kept in a plastic box flooded with gas of the required composition, or in oxygen- and CO₂ controlled incubators or glove boxes. If fast responses to hypoxia are studied, culture medium should be replaced with one equilibrated to gas of the required composition. An important aspect is handling of cells that were exposed to hypoxia for harvesting or further experimentation. The procedure depends on the readout. If changes in parameters to be tested are readily reversible upon reoxygenation (e.g. ROS, nuclear HIF) then cells have to be handled in an hypoxic environment such as in an environmental glove box or under a stream of gas. If changes are stable (e.g. cell protein content and slow turnover) cells might even be handled in normoxia.

Keywords: hypoxia, methods, tissue culture

Role of HIF and CREB on the activity and expression of ion transporters in primary rat lung alveolar epithelial cells

Gabriel Nonnenmacher, Emel Baloglu, Peter Bärtsch, Heimo Mairbaurl
Medical Clinic VII, Sports Medicine, University of Heidelberg, Heidelberg, Germany

Hypoxia impairs alveolar reabsorption by decreasing ion transport, whereas β 2-adrenergics stimulate. Both effects are caused by modulating expression and activity of transporters. Typically, hypoxia mediated gene expression depends on hypoxia-inducible factors (HIF), whereas β 2AR-stimulated gene expression requires CREB. Yet, their role in the expression of ion transporters is unclear. We tested whether effects of hypoxia on alveolar transport are prevented by silencing HIF- α , and whether CREB is involved. Interaction between both transcription factors is likely due to requirement for p300 and CBP of both. Adenoviruses expressing shRNA sequences were generated to decrease the expression of HIF-1 α , HIF-2 α , and CREB1 in primary rat alveolar epithelial cells. Transfected cells were exposed to normoxia and hypoxia (1.5% O₂) with or without terbutaline for 24h and 48h. mRNA expression was measured by qRT-PCR. Ion transport activity was measured in Ussing chambers. Silencing of HIF-1 α and CREB1 decreased the mRNA expression of α , β and γ ENaC. HIF-1 α and CREB1-silencing decreased total short circuit current (ISC_{tot}) and its amiloride sensitive component (ISC Δ amil) in normoxia and hypoxia after 24h stimulation with terbutalin. In HIF-2 α silenced cells no ion transport activity was detectable when cells were exposed to hypoxia (48h) in absence and presence of terbutaline. HIF-1 α and CREB1 are involved in controlling expression and activity of ion transporters in alveolar epithelium. Silencing CREB1 prevented the terbutalin induced stimulation of ion transport activity. Inhibition of transport by hypoxia on ion transport activity can not be prevented by silencing HIF-1 α and HIF-2 α .

Keywords: HIF, CREB, alveolar epithelium, Na⁺ transport

Midkine: a multifunctional protein involved in reproduction, repair and pathogenesis of inflammatory and malignant diseases

Takashi Muramatsu

Department of Health Science, Aichi Gakuin University, Nisshin, Japan

Midkine is a heparin-binding cytokine or a growth factor with a molecular weight of 13 kDa, and is rich in both basic amino acids and cysteine. Here, I summarize general information on midkine. Midkine binds to oversulfated structures in heparan sulfate and chondroitin sulfate. The midkine receptor is a molecular complex containing proteoglycans and transmembrane proteins such as low density lipoprotein receptor-related protein and integrins. Receptor-like protein tyrosine phosphatase-zeta is a chondroitin sulfate proteoglycan and serves as the key receptor component in the majority of cases. Intracellular tyrosine phosphorylation is increased as the result of midkine action. Midkine promotes migration, survival and other activities of target cells. Midkine has about 50 % sequence identity with pleiotrophin. Mice deficient in both factors exhibit severe abnormalities including female infertility and developmental deficits. In adults, midkine is expressed in damaged tissues and involved in the reparative process. It is also involved in inflammatory reactions by promoting the migration of inflammatory leukocytes, induction of chemokines and suppression of regulatory T cells. Midkine is expressed in a variety of malignant tumors and promotes their growth and invasion. Midkine appears to be helpful for the treatment of injuries in the heart, brain, spinal cord and retina. Midkine inhibitors (siRNAs, antisense oligoDNAs, aptamers, antibodies, peptides, low molecular weight compounds etc) are expected to be effective in the treatment of malignancies, rheumatoid arthritis, multiple sclerosis, renal diseases, restenosis, hypertension and adhesion after surgery. Midkine is an interesting molecule upon investigating pathogenesis and therapy of various diseases.

Keywords: midkine, cell survival, inflammation, malignancy

Midkine signaling: from embryogenesis to disease

Mine Erguven¹, Nuray Yazihan², Ayhan Bilir³

¹Department of Biochemistry, Yeni Yuzyıl University, Istanbul, Turkey

²Department of Pathophysiology, Ankara University, Ankara, Turkey

³Department of Histology and Embryology, Istanbul University, Istanbul, Turkey

Midkine (MK) is a growth factor with cytokine action playing a central role in survival, growth, migration. The expression of MK is strictly controlled during embryogenesis that means high MK levels are detected in midgestation, but these levels were decreased after. MK regulates lung development, alveolar sacs formation and pulmonar vascular remodelling. Recent reports indicate that autophagy (lysosomal degradation pathway) is a tumor suppressor mechanism, which is connected to its role in the clearance of the scaffold protein, prevention of oxidative stress and genomic instability, however because autophagy is also expected as a survival mechanism, cancer cells can also exploit it to survive nutrient limitation and hypoxia. It was shown that hypoxia via hypoxia inducible factor-1 alpha enhanced the transcription of MK in developing lungs in vivo. Eventually, MK increased muscularization of small pulmonary arteries, increasing alpha-smooth muscle actin and caldesmon staining and the expression of myocardin, and consequently lung acquired a shape which they can function. MK involved in infammatory reactions and there is a complex reciprocal relationship between the autophagy pathway/proteins and immunity and inflammation; the autophagy proteins function in both the induction and suppression of immune and inflammatory responses, and immune and inflammatory signals function in both the induction and suppression of autophagy. In our recent study, we determined the significant role of MK in the switch from autophagy to apoptosis in human glioblastoma cells and spheroids. It can be speculated that MK determines the fate of autophagy as cell death or survival, or as the induction or supression of immune/inflamation responses.

Keywords: Midkine, autophagy, embryogenesis, lung modelling, immune response, inflammatory response, apoptosis

Dietary Aflatoxins and Hepatitis B Virus Infection with Respect to Hepatocellular Carcinoma and Inflammation

Nuray Yazihan¹, Cihan Yurdaydin², Burcu Sirin³, Asli Belen³, Ethem Akcil¹, Yildirim Cesaretli⁴, Ozden Uzunalimoglu⁵

¹Ankara University, Faculty of Medicine, Pathophysiology Department, Ankara, Turkey

²Ankara University, Faculty of Medicine, Gastroenterology Department, Ankara, Turkey

³Ankara University, Faculty of Medicine, Molecular Biology R&D Unit Ankara, Turkey

⁴Refik Saydam National Public Health Agency Ankara, Turkey

⁵Turkish Hepatology Foundation, Ankara, Turkey

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and has an extremely poor prognosis. The major risk factors are chronic infection with hepatitis B viruses (HBV) as well as dietary exposure to aflatoxins. Prospective epidemiological studies have shown that there is a synergic interaction between HBV and aflatoxins in terms of HCC risk. However, the biology underlying this interaction is not fully understood. Midkine has mitogenic, antiapoptotic function in cancerogenesis and is also one of the inflammatory mediators. In this study, we aimed to evaluate underlying mechanisms of role of HBV infection in liver immune responses to carcinogenic/toxic substance aflatoxin B1. HBV infected (Hep3B) and noninfected (HepG2) human hepatocellular cancer cells cocultured with monocytic cells (THP-1) and are exposed to aflatoxin B1 for 24 h. Inflammatory responses of each cells defined with NF κ B activation, I κ B, TNF- α , IL-10, IFN- γ and midkine secretion levels. Toll Like Receptor (TLRs) expression levels are evaluated by western blot. Exposure to aflatoxin results in activation of NF κ B. The activation was higher in HBV infected group. HBV infection inhibits IFN- γ secretion responses to aflatoxin in hepatocytes. TLR expression increased in cocultured hepatocytes in normal and aflatoxin exposed group. We found an increased inflammatory response that could be associated with the hepatotoxic effects of aflatoxin B1 in hepatocytes and monocytes. The predisposition of HBV-infected hepatocytes to aflatoxin-induced inflammatory damage is higher than non-infected hepatocytes. HBV infection suppresses immune responses via inhibiting IFN- γ production in liver cells. The increase in midkine secretion might be one of the underlying mechanisms of HBV induced carcinogenesis in aflatoxin exposure. This study was supported by Ankara University.

Keywords: Aflatoxin, Hepatitis B Virus (HBV), hepatocellular carcinoma, inflammation, midkine, NF κ B, Toll Like receptors (TLR), IFN γ

Cell Signaling and Cytokine Secretion Response Differences in Midkine Silenced and Overexpressed Rat Alveolar Macrophages

Nuray Yazihan¹, Fikret Sahin², Ezgi Ermis³, Evin Yilmaz³, Mehtap Kacar Kocak⁴, Ethem Akcil¹, Emel Baloglu⁵, Heimo Mairbaurl⁵

¹Ankara University, Faculty of Medicine, Pathophysiology Department, Ankara, Turkey

²Ankara University, Faculty of Medicine, Microbiology Department, Ankara, Turkey

³Ankara University, Faculty of Medicine, Molecular Biology R&D Unit Ankara, Turkey

⁴Yeditepe University, Faculty of Medicine, Pathophysiology Department Istanbul, Turkey

⁵Heidelberg University, Sport Medicine Division, Heidelberg, Germany

Hypoxia, which commonly associates with respiratory and cardiovascular diseases, provokes an acute inflammatory response. Molecular mechanisms of the inflammatory reaction in hypoxia-induced lung injury are not well defined. Evidence obtained in intact animals and in primary cell cultures indicate that alveolar macrophages activated by hypoxia release a mediator(s) into the circulation. Midkine is one of the molecules playing a significant role in the control of inflammatory processes. It is a heparin-binding growth factor which promotes cell adhesion, migration, proliferation and survival. In this study, we aimed to evaluate effect of midkine in cytokine secretion responses of alveolar macrophages in normoxia and hypoxia. Midkine expression in rat alveolar macrophage cell line NR8383 was silenced using midkine siRNA sequence primers and overexpressed. Quantitative RT-PCR assay was performed to quantify the mRNA expression changes of midkine. Cells were exposed to K pneumonia lipopolysaccharide (LPS) in normoxic and hypoxic conditions. TNF- α , IL-4, IL-5, IL-6, IL-10 levels were measured by ELISA. Cell proliferation rate was higher in midkine overexpressed cells. Basal cytokine secretion levels and cytokine response to LPS increased in midkine overexpressed cells whereas it was decreased in midkine silenced cells. Midkine overexpressed cells showed increased NF κ b, p38/MAPK, JNK, protein kinase C and erk activation compared to control and silenced cells. Our results showed that midkine is important for cytokine secretion, intracellular signalling functions and survival of alveolar macrophage cells. This study was supported by TUBITAK-BMBF (SBAG108S262).

Keywords: Alveolar macrophages, midkine overexpression, midkine silencing, hypoxia, TNF- α , IL-4, IL-5, IL-6, IL-10, NF κ b, p38/MAPK, JNK, protein kinase C and erk

Immune Suppressor Effect of Cadmium in Lung Alveolar Macrophage Cells

Nuray Yazihan¹, Fikret Sahin³, Burcu Sirin², Ezgi Ermis², Asli Belen², Mehtap Kacar Kocak⁴, Yildirim Cesaretli⁵, Ethem Akcil¹

¹Ankara University, Faculty of Medicine, Pathophysiology Department, Ankara, Turkey

²Ankara University, Faculty of Medicine Molecular Biology R&D Unit Ankara, Turkey

³Ankara University, Faculty of Medicine, Microbiology Department, Ankara, Turkey

⁴Yeditepe University, Faculty of Medicine, Pathophysiology Department Istanbul, Turkey

⁵Refik Saydam National Public Health Agency Ankara, Turkey

Inhalation is an important route of Cadmium (Cd) exposure, and the lung is considered to be one of the main target organs of Cd toxicity, which is present in both air pollution and cigarette smoke. Pulmonary inflammation seems to be involved in development of many lung diseases also in Cd toxicity. In the present study, we aimed to evaluate effect of different dosages of Cd (1-100 μ M) in normoxic, hypoxic and inflammatory conditions in normal and midkine overexpressed rat alveolar macrophage cell line NR8383. Midkine expression was controlled by RT-PCR. Total/phospho Nfkb, Ikb, p38/MAPK levels measured in cell lysates and midkine, TNF- α and IL-6 levels were evaluated by ELISA. K. Pneumonia LPS (10 μ g/ml) stimulation induced NF κ B activation, TNF- α and IL-6 secretions. Similar results were observed with TNF- α and midkine. Midkine overexpressed cells showed higher inflammatory response and TNF- α secretion. Our results showed that Cd at fairly low concentrations (1-2.5 μ M) induced inflammation via p38/MAPK and NF κ B activation and results in an increased secretion of cytokines in rat alveolar macrophage cell line ($p < 0.05$). Cd causes cytotoxicity and suppresses inflammatory responses in a dose and time dependent manner. In conclusion, our results suggest that inflammation may contribute in Cd-induced lung damage in lower dosages, midkine is one of the important mediators of immune function. Moreover, with increasing dose of Cd, cytokine secretion response of alveolar macrophages is inhibited. Cd exposure might be one of the causes of the immune suppressor results of cigarette smoking. This study was supported by TUBITAK-BMBF (SBAG108S262).

Keywords: Alveolar macrophages, cadmium, midkine, Nfkb, inflammation, TNF- α , IL-6

Antimicrobial Properties of Midkine - New Roles for an old Protein

Sara Svensson

Department of Respiratory Medicine and Allergology, Lund University, Lund, Sweden

Our bodies are constantly exposed to potentially harmful microbes which could cause disease unless prevented by host defense mechanisms. Antimicrobial polypeptides (AMPs) constitute an important part of the innate immune system and have a direct antimicrobial effect, and often additional roles as growth factors and in inflammation. Midkine (MK) is a heparin-binding growth factor with a structure similar to β -defensins, a known AMP. Could MK work as an AMP? Using viable count assay we've shown MK to display strong antibacterial activity against both gram-positive and gram-negative bacteria. Electronmicroscopy and experiments using artificial lipid-bilayers, suggest that MK exert the antibacterial action via membrane disruptive mechanisms. MK consists of two domains and the antibacterial activity was mapped to the unordered COOH-terminal tail and the last β -sheets of the NH₂-terminal. Analysis of highly conserved MK orthologues suggests antibacterial activity in corresponding domains. In support of an evolutionary conserved function, the most distant orthologue, Miple2 from the insect *Drosophila melanogaster*, also displayed strong antibacterial activity. MK is expressed by keratinocytes of the skin and is also present at the site of infection during fungal dermatitis. In addition to the antibacterial role of MK we investigated possible antifungal activity. MK had high antifungal activity against both *Candida albicans* and *Candida parapsilosis* at physiological salt-concentrations. MK affected liposomes with ergosterol more greatly than liposomes with cholesterol, meaning that MK preferentially disrupt fungal rather than host membranes. These findings suggest that MK, in addition to earlier described activities, may have important roles as an innate antibiotic.

Keywords: Midkine, innate immunity, AMP, antibacterial, antifungal

Midkine, hypoxia and Inflammation: Signalling Pathways

Nuray Yazihan¹, Mehtap Kacar Kocak², Ethem Akcil¹

¹Ankara University, Faculty of Medicine, Pathophysiology Department, Ankara, Turkey

²Yeditepe University, Faculty of Medicine, Pathophysiology Department, Istanbul, Turkey

Inflammatory processes in lung alveoli, such as in pneumonia, acute respiratory distress syndrome, hyperoxia, ischemia-reperfusion, sepsis, COPD, cause the release of pro-inflammatory factors and reactive oxygen species from activated macrophages or pulmonary epithelial and endothelial cells. They damage the lungs and initiate inflammation causing dysfunction of the alveolar barrier and edema. Midkine is one of the molecules playing a significant role in the control of inflammatory processes. It promotes cell adhesion, migration, proliferation and survival. The signaling pathways mediating the effects of midkine in the lung tissue are only partially understood. The receptor mediating midkine responses is not well defined. Receptor tyrosine phosphatase cell-surface proteoglycan PTPzeta/RPTPbeta, low density lipoprotein receptor related protein and anaplastic lymphoma kinase (ALK), have been described to mediate the mitogenic activity of midkine. A heparin-independent high affinity binding site (p200+/MKR) causes midkine-dependent, heparin-independent phosphorylation and activation of the JAK/STAT pathway. Midkine interacts with HIF-1 α - and hypoxia-dependent processes. LPS induced midkine secretion in monocytic cells depends on KATP channel and protein kinase C activation. In midkine deficient mice leukocyte infiltration into the blood vessels and kidney after ischemic injury is suppressed. However, the signaling pathways mediating the effects of midkine and mechanisms of action are only poorly understood. Protein kinase C, MAP kinase and G protein activation, as well as PTP, Erk1 and 2 and PI3-kinase are involved midkine induced migration of these cells. In this section signalling pathways take part in midkine related immune cell functions in normoxia and hypoxia will be discussed.

Keywords: Midkine, inflammation, intra cellular signal pathways, hypoxia, ATP dependent K channels, protein kinase C

Induction of Cultured Human Monocytic THP-1 Leukemia Cells by Phorbol-12 –myristate-13-acetate (pma): Midkine may be a new marker of differentiation

Derya Biriken¹, Nuray Yazihan², Nurhan Albayrak³, Sukran Yilmaz⁴, Hatice Ozenci¹

¹Ankara University Faculty of Medicine, Department of Medical Microbiology, Ankara, Turkey

²Ankara University Faculty of Medicine, Pathophysiology Department, Ankara, Turkey

³Refik Saydam National Public Health Agency, Ankara, Turkey

⁴Foot and Mouth Diseases Institute, Ankara, Turkey

Monocytes and macrophages play important roles in many inflammatory reactions. Phorbol-12-myristate-13-acetate (PMA) is stimuli commonly used to induce macrophage differentiation in monocytic cell lines, but the extent of differentiation in comparison to primary tissue macrophages is unclear. Major functions of macrophages include maintaining tissue homeostasis and responding to microorganisms. They can mediate immune and inflammation processes via the production and release of a variety of soluble mediators such as radicals like superoxide anions, cytokines and eicosanoids. These biologically active agents are also known to modulate cell differentiation and proliferation. Midkine is a fibrinolytic, anti-apoptotic, mitogenic, transforming, angiogenic, and chemotactic molecule which takes part in inflammatory conditions. THP-1 cell line were exposed to different dosage and time duration protocols of PMA. Cells are compared as morphology. Midkine, TNF- α , IL-10, IFN- γ secretion functions are measured by ELISA. PMA stimuli induced changes in cell morphology indicative of differentiation. PMA differentiation induced midkine, TNF- α , IL-10, IFN- γ secretions in monocytic cells even in lowest dosage (10 ng/ml). Moreover, with increasing dosage PMA (>20 ng/ml) induced cytotoxicity in cells. There was no difference in cytokine profile and midkine secretion between different dosages of PMA induced cells. These findings suggest a modified PMA differentiation protocol (20 ng/ml and 48 h incubation) can enhance macrophage differentiation of THP-1 cells without induced cell death (viability 92.2 %) and cytokine secretion and midkine responses as important discriminators of the level of macrophage differentiation for transformed cells. This study was supported by TUBITAK-BMBF (SBAG108S262 and SBAG110S242).

Keywords: Monocyte, macrophage differentiation, midkine, Phorbol-12 –myristate-13-acetate (pma), TNF- α , IFN- γ , IL-10

Dexamethasone Suppresses *Klebsiella Pneumoniae* Induced Cytokine Production and Midkine Expression in Rat Lungs

Nuray Yazihan¹, Mine Erguven², Ahmet Levent Bas³, Ezgi Ermis⁴, Ayhan Bilir⁵, Mehtap Kacar Kocak⁶, Ethem Akcil¹, Emel Baloglu⁷, Heimo Mairbaurl⁷

¹Ankara University, Faculty of Medicine, Pathophysiology Department, Ankara, Turkey

²Yeni Yuzyil University, Biochemistry Department Istanbul, Turkey

³Selcuk University, Veterinary Faculty, Pharmacological Toxicology Department, Konya, Turkey

⁴Ankara University, Faculty of Medicine, Molecular Biology R&D Unit, Ankara, Turkey

⁵Istanbul University, Department of Histology and Embryology, Istanbul, Turkey

⁶Yeditepe University, Faculty of Medicine, Pathophysiology Department Istanbul, Turkey

⁷Heidelberg University, Sport Medicine Division, Heidelberg, Germany

Midkine is a retinoic acid-responsive, heparin binding growth factor expressed in various cell types including lung during embryogenesis. In vitro and in vivo studies demonstrated that midkine promotes angiogenesis, cell growth, cell migration and growth of the pulmonary mesenchyme during branching morphogenesis of embryonic lung but its role and expression pattern in lung in general is still unknown. In this study; cytokine and midkine expression responses of rat lung in a pneumonia model that is produced by intratracheally instilled with *Klebsiella pneumoniae* suspension was evaluated. Rats were exposed to 10 % O₂. Lung tissue midkine, TNF- α , IL-6, IL-10 levels were measured by ELISA. Midkine expression was evaluated immunohistochemically. Midkine expression was more prominent in bronchiole epithelial nucleus in healthy rats. TNF- α , IL-6 and midkine levels increased in *K.pneumoniae* infected rat lungs, whereas IL-10 levels were found decreased. Hypoxia and dexamethasone suppressed proinflammatory cytokine and midkine secretion responses in infected rat lungs. Midkine levels correlated with TNF- α levels. Our results showed that adult rat lung has basal midkine secretion, which was induced more by bacterial infection. This study was supported by TUBITAK-BMBF (SBAG108S262).

Keywords: Midkine, pneumonia, hypoxia, dexamethasone, TNF- α , IL-6, IL-10

Visualizing hypoxia and cellular oxygen sensing

Joachim Fandrey

Institut für Physiologie, Universität Duisburg-Essen, Germany

The activation of hypoxia-inducible factor-1 (HIF-1) has been recognized as the key event in adaptation to hypoxia. HIF-1 is composed of the O₂-labile alpha- and the constitutive beta-subunit. From here the whole cascade of cellular O₂ sensing was followed back ultimately leading to the elucidation of cellular O₂ sensors, the prolyl and asparaginyl hydroxylases that regulate abundance and activity of oxygen-regulated alpha-subunit of HIF-1. Assembly of the HIF-1 complex then requires dimerization with constitutive nuclear beta-subunit. To localize HIF-1 subunits within the nucleus of hypoxic cells we applied 2-photon-laser microscopy (2PLM). Mobility studies of fluorescently labelled HIF-1 subunits by fluorescence recovery after photo bleaching (FRAP) revealed that HIF-1alpha migrates more slowly than HIF-1beta within the nucleus indicating that both subunits do not immediately “find” each other but may be prone to modification prior to dimerization. HIF-1 assembly was then studied in living cells in a specialized hypoxic chamber mounted on the microscopic stage which allows the in vivo analysis under a well defined oxygen tension. Finally, in vivo imaging was extended to preparations of intact mouse carotid body ex vivo. Using multifocal Nipkow disk-based imaging with oxygen-, calcium- and potential-sensitive cellular dyes we mapped oxygen-sensing properties of carotid bodies. Additionally in vivo FRET measurement revealed activation of the HIF-1 complex in vivo in carotid body preparations coinciding with changes in cellular potential. Collectively, we have successfully visualized in vitro and ex vivo oxygen sensing to further characterize cellular responses to hypoxia.

Keywords: Hypoxia, cellular oxygen sensing and HIF-1

Gene/Protein Expression Analysis: Proteome, Microarrays

Duygu Ozel Demiralp¹, Hilal Ozdag², Fikret Sahin³, Nuray Yazihan⁴

¹Ankara University, Biotechnology Institute, The Proteomics Unit of the Central Laboratory Ankara, Turkey

²Ankara University, Biotechnology Institute, The Genomics & Functional Genomics Unit of the Central Laboratory Ankara, Turkey

³Ankara University, Faculty of Medicine, Microbiology Department, Ankara, Turkey

⁴Ankara University, Faculty of Medicine, Pathophysiology Department, Ankara, Turkey

The complete sequence of the genome of many organisms including the human is available today. However, our knowledge on the proteins, functional products of the genes, is very limited. Proteins give more accurate information about the dynamic condition of the cells, tissues or organs. For this reason, a concept called “proteome” has emerged and became important. The word proteome can be defined as “all the proteins synthesized by the cell or whole organism” or “all the proteins synthesized by a particular cell at particular time”. Parallely, the discipline of “proteomics” includes all the methods to investigate proteomes and can be defined as “the qualitative and quantitative comparison of proteomes under different conditions”. Proteomics workflow can be grouped main titles: Protein isolation, protein separation and expression analyses, protein identification, protein interactions and structures. Two dimensional gel electrophoresis (2D-PAGE) has an important role in protein separation and fractionation. Mass spectrometry (MS) (i.e. MALDI-TOF, ESI Q-TOF) is one of the most informative methods for studying proteomics. Protein identification is achieved by matching the cleaved peptides to the database masses with this approach. Proteomics also allow for identification of the protein changes caused by the disease process in a relatively high-throughput manner, because it permits an analysis of thousands of modified or unmodified proteins simultaneously. Thus, together with microarray analysis it is increasingly becoming popular in identifying biomarkers for cancer diagnosis, progression, as well as therapeutic targets. In this section microarray and proteome results of midkine and HIF-1 silenced/overexpressed alveolar macrophage cells will be discussed. This study was supported by TUBITAK-BMBF (SBAG108S262)

Keywords: Proteome, microarray, mass spectrometry, 2D protein separation, gene transcriptional analysis

High Throughput Experimentation for Hypothesis Generation and Testing

Ting Chan¹, Jie Ming¹, Pang Chao¹, Duygu Ozel Demiralp², Bertal Huseyin Aktas¹

¹Harvard Medical School, Boston, MA, USA

²Ankara University, Biotechnology Institute, Proteomic Unit of the Central Laboratory Ankara, Turkey

Classical cell/molecular biology approach of studying one or a few molecules at a time for their role in normal/patho-biology limits the speed of scientific discovery and often lead to conclusions with narrow applicability. The alternative of studying all genes/proteins for their role in normal- and patho-biology circumvents these limitations and allows for identification of dominant and secondary functional genetic networks and generation of novel hypothesis that may be tested using similar system-wide approaches. The experimental system should have high throughput, be highly accurate, reproducible, and cost effective. An example of high-throughput hypothesis generation is our studies of the molecular and chemical genetics of the integrated endoplasmic reticulum (ER) stress response (IERSR). The ER is the site of protein folding and processing in the cells. Our knowledge of how ER homeostasis is maintained, and if/how disturbances in ER homeostasis contribute to patho-biology of human disorders is poorly understood. To ameliorate this, we developed a high throughput experimental system capable of high-throughput genetic and chemical interrogation of ER-physiology. We generated two dual-luciferase assay systems to interrogate two different arms of IERSR, the PERK/eIF2 α arm that reduced protein synthesis, and Ire1/Xbp-1 arm that increases size and folding capacity of the ER to resolve the ER-stress. We will show how we developed these assay systems, how we screen siRNA, micro-RNA, morpholino and small chemical libraries, how we integrate data from two reporters in two different assay systems and genetic and chemical screens to generate and test new hypotheses. Finally we will demonstrate how this new approach is changing the face of the scientific inquiry.

Keywords: endoplasmic reticulum (ER), stress response, PERK/eIF2 α , siRNA, micro-RNA, molecular biology assay systems

Teaching Workshop: Integration of Physiology Learning in Clinical Settings

Mehmet Ali Gülpınar¹, Şeref Erdoğan³, Özgür Çakır Kasımay¹, Ersin Oguz Koylu², Levent Öztürk⁴, Berrak Ç. Yeğen¹

¹Marmara University, School of Medicine, Istanbul Turkey

²Ege University, School of Medicine, Istanbul, Turkey

³Çukurova University, School of Medicine, Istanbul, Turkey

⁴Trakya University, School of Medicine, Istanbul, Turkey

Recent reports on medical education research emphasize the importance of two issues: (1) to introduce elements of practice in the early years, while revisiting basic sciences later on, rather than having a traditionally rigid barrier between the preclinical and clinical settings and (2) to increase the level of integration. Regarding that physiology is an important core component of any medical curriculum, while its learning at medical schools relies mostly on didactic lectures during the preclinical years, the aim of the workshop is to practice the integration of a physiology curriculum into a clerkship or a residency program based on the appropriate learning and assessment methods for this integration. Thus, the participants will discuss how to integrate physiology education in the preclinical/clinical phases of graduate/post-graduate medical education by integrating physiology education with other basic sciences as well as clinical and social sciences in the clinical context. Following a short instructive presentation and a brief group discussion, the groups of 4-5 people will work on different organ system-based programs (e.g. “cardiovascular” or “nervous system and behavioral sciences”) or on different themes (e.g. “nutrition and healthy life” or “aging”). Based on these themes or organ-system-programs, groups will discuss on the integrated content and learning methods and suggest possible templates of physiology learning in the clinical settings.

Keywords: physiology learning, contextual, assessment, integration

Oral Communications

OC01(EYPS)

Role of estrogen receptor beta in pancreatic beta-cell function

Paloma Alonso Magdalena¹, Sergi Soriano¹, Marta García Arévalo¹, Ivan Quesada¹, Esther Fuentes¹, Jan Ake Gustafsson², Angel Nadal¹

¹CIBER of Diabetes and Associated Metabolic Diseases, CIBERDEM and Institute of Bioengineering, University Miguel Hernandez of Elche, Elche, Spain

²Department of Cell Biology and Biochemistry, Center fo Nuclear Receptors and Cell Signaling, University of Houston, SA

OBJECTIVE: Type 2 diabetes is characterized by the emergence of fasting hyperglycemia. This hyperglycemia is associated with an inadequate insulin secretion from pancreatic β -cells to compensate for insulin-resistance in peripheral tissues. The ATP-potassium channel (KATP channel) is the key molecule involved in glucose-stimulated insulin secretion (GSIS). We have previously demonstrated that physiological concentrations of 17 β -estradiol (E2) decrease KATP channel activity thus enhancing insulin secretion. Here we evaluate the new role of estrogen receptor beta (ER β) as an insulinotropic molecule.

METHODS: C57BL/6 and ER β $-/-$ mice were used. Electrical activity and calcium records were analyzed in the whole islet of Langerhans. Plasma insulin, leptin, tryglicerides and glycerol levels were measured by ELISA

RESULTS: In vitro studies demonstrate that low doses of WAY 200070, an ER β specific agonist, decrease KATP channel activity and enhance GSIS in the presence of stimulatory (7 and 16 mM glucose) but not low glucose concentrations. In a set of in vivo experiments we showed that a single-administration of WAY 200070 leads to an improvement in the plasma glucose response to a glucose challenge with a concomitant increase in insulin levels. Thus, we next studied the potential clinical use of ER β agonists by using streptozotocin-nicotinamide-induced mildly diabetic mice. These animals exhibit moderate hyperglycaemia and impaired glucose tolerance because of the loss of early-phase insulin secretion. One-week treatment with the ER β agonist caused a significant improvement in glucose tolerance, with a significant increase in the plasma insulin and a decrease of glycerol levels in the diabetic treated group.

CONCLUSIONS: We conclude that ER β agonists should be considered as new antidiabetic drugs.

Keywords: Pancreatic beta-cell function, islet of Langerhans, estrogen receptor beta, 17 b-estradiol, diabetes

HN1 contributes to cellular morphology via regulating GSK3B phosphorylations, and also regulates cell cycle through cyclin B-Cdk1 association

Kemal Sami Korkmaz

Cancer Biology Lab. Dept. of Bioeng, Faculty of Eng., Ege University, Bornova, Izmir, Turkey

OBJECTIVE: EGF and androgen signaling pathways are crucial for cell cycle regulation, differentiation and survival in prostate epithelium. Since, AKT is a major survival pathway and is influenced via EGFR signaling, determines the androgen receptor transactivation in prostate cells. Therefore, many researches have shown that the deregulation of AKT signaling is a decisive step for prostate carcinogenesis. HN1 is a ubiquitously expressed gene and encodes an evolutionary conserved, but an uncharacterized small protein.

METHODS: In this study, how HN1 influenced by EGF and androgen treatments were investigated using androgen responsive cell line LNCaP. The effects of HN1 on AKT-GSK3B phosphorylations and consequently B-Catenin stabilization were investigated using silencing and overexpression approaches involving flow cytometry, western blotting and immunofluorescein techniques.

RESULTS: As a result, we have shown that EGF and androgen regulate HN1 in AKT dependent manner, and HN1 knockdown results an increase in AKT activation. Further, subsequent GSK3B phospho-inhibition results with partial stabilization of B-catenin, which was evidenced as increases in c-myc and cyclin D1 expressions, and nuclear accumulation of cyclin D1. Moreover, HN1 overexpression also influences androgen signaling and alters the cell morphology by E-cadherin association of B-catenin. Thus, HN1 is a ubiquitously expressed gene; its expression is tightly regulated with multiple signaling pathways and it is required for cellular morphogenesis.

CONCLUSIONS: HN1 is a hormone/growth factor regulated gene and it has important roles in AKT dependent GSK3B signaling, regulates cell division, nucleus morphology and cytoskeletal protein expressions.

Keywords: HN1, EGF, Androgen Receptor, AKT signaling

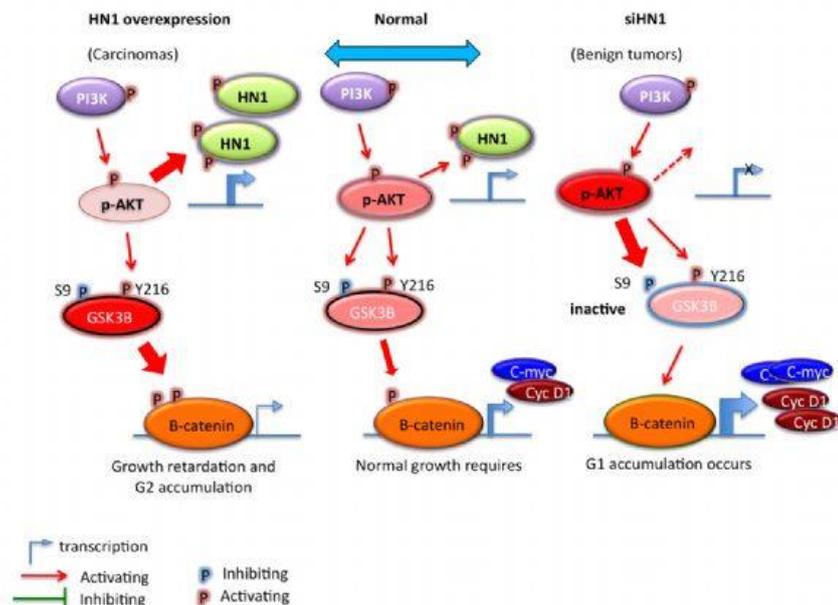


Figure. EGF responding up-regulation of HN1 expression and phosphorylation are regulated via AKT pathway and HN1 knockdown results an increase in pAKT(S473) and pGSK3β as a novel feedback mechanism. These alterations consistently occur in PC-3 and MCF-7 cell lines and implicates a global mechanism of cell cycle regulation. G1/S transition is regulated tightly by cyclin D1 accumulations and HN1 knockdown results a clear G1 accumulation. However, G2/M transition is also regulated by HN1 knockdown through GSK3B phosphorylations, and subsequently stabilized β-catenin increases cyclin D1 and c-myc expressions. The balance in between G1/S and G2 transitions could be dependent on HN1 expression levels in cell, and its deregulation might contribute to developing aggressive cancer phenotype, which is summarized in schema (DNA and Cell Biol. June 2011).

Dual vasodilator mechanism of the AMP-activated protein kinase (AMPK) in arterial microvessels

Kai Michael Schubert¹, Claus Kreutz¹, Michael Mederos Y Schnitzler², Thomas Gudermann², Ulrich Pohl¹

¹Walter Brendel Centre of Experimental Medicine, Ludwig-Maximilians-University, Munich, Germany

²Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-University, Munich, Germany

OBJECTIVE: Adaptation of blood flow to tissue metabolic demands occurs through changes of microvascular arterial tone. The energy-sensing enzyme AMPK may be a potential regulator of smooth muscle tone in response to metabolic challenges. Thus we studied the potential vasomotor function of vascular smooth muscle AMPK in isolated small arteries.

METHODS: Studies in intact segments as well as smooth muscle patch clamp studies were conducted in mesenteric and skeletal muscle resistance arteries (hamster and mouse; n=110). The isolated vessels, were pre-treated with indomethacin (30 μ M) and L-NAME (30 μ M). We measured simultaneously smooth muscle intracellular calcium levels [Ca²⁺]_i (FURA2-AM) and vascular diameters.

RESULTS: The AMPK-stimulator A769662 (A76) induced an endothelium independent vasodilation which was associated with a decrease in [Ca²⁺]_i in vessels pre-constricted with norepinephrine (0.3 μ M). Partial knock down of the AMPK by siRNA induced a significant rightward shift in the dose-response-curves of A76. In vessels pre-constricted by high extracellular potassium (60mM) the vasodilation was diminished and the [Ca²⁺]_i decrease abolished completely. The remaining dilator effect could be completely blocked by the myosin-light-chain-phosphatase (MLCP) inhibitor calyculinA (100nM). Other AMPK-activators (metformin 0.1-3mM, 2-deoxyglucose 5mM) qualitatively mimicked the effect of A76. Patch-clamp studies revealed activation of BKchannels by A76, which could be blocked by the specific inhibitor paxilline (500nM) and the AMPK inhibitor compoundC (100 μ m). Accordingly, paxilline (1 μ M) inhibited the A76 dilator effect of norepinephrine pre-constricted microvessels by about 30%.

CONCLUSIONS: AMPK augments BKchannel current and reduces calcium sensitivity of the contractile machinery of arterial microvessels. These two mechanisms may contribute to microvascular vasodilation in response to accelerated tissue metabolism.

Keywords: AMPK, BKCa, MLCP, calcium-desensitization, microvessels, resistance arteries, vascular smooth muscle, A769662, metabolism

Target-specific siRNA to protein kinase C delta isoform gene expression normalize vascular function in SHR

Tetiana Novokhatska¹, Irina Ivanova¹, Sergey Tishkin¹, Victor Dosenko², Anatoly Soloviev¹

¹Institute of Pharmacology and Toxicology, Academy of Medical Sciences, Ukraine

²Bogomoletz Institute of Physiology, National Academy of Sciences, Ukraine

OBJECTIVE: It is known that protein kinase C (PKC) family plays an important role in hypertension development (Soloviev, Bershtein, 1992). The vascular smooth muscle (SM) tone is closely coupled to membrane potential, which, in turn, is determined by K⁺ channels activity. Potassium conductance is altered in both radiation-induced (Soloviev et al., 2009) and essential hypertension (Cox et al., 2001).

METHODS: The goal of this study was to identify the most vulnerable target for pharmacological interventions in arterial hypertension. Experimental design of the study comprised patch-clamp technique, RT-PCR and standard ACh-test.

RESULTS: We have measured the level of δ -PKC gene expression in thoracic aorta from SHR and SHR treated with δ -PKC siRNA relative to control rats. The RT-PCR analysis showed that PKC- δ -isoform mRNA expression is sixfold increased in SMCs from SHR and was significantly higher than seen in SHR treated with δ -PKC siRNA (control, 11.8 ± 1.71 , n=6; SHR, 43.61 ± 6.32 , n=6; SHR treated with δ -PKC siRNA, 33.76 ± 1.28 , n=6; p<0.05). BKCa component of outward current is significantly appears decreased from 48 ± 5 pA/pF in healthy rats to 25 ± 2 pA/pF in SHR while in SHR treated with δ -PKC siRNA it was 35 ± 3 pA/pF, P<0.05, n=18. The target-specific to δ -PKC siRNA administration led to an increment in amplitude of ACh-relaxation and BKCa activity, and promoted arterial blood pressure normalization in SHR.

CONCLUSIONS: In conclusion, δ -PKC gene silencing restores endothelium-dependent relaxation and BKCa channels function in vascular SHR SM cells. It is likely that siRNA is a good approach to inactivate PKC gene encoding function and to normalize vasodilator potential in SHR.

Keywords: hypertension, SHR, δ -PKC, siRNA, BKCa channels.

Does acute or chronic stress affect peripheral blood helper and regulatory T cells?

Murat Dogan, Bilge Pehlivanoglu, Ayşe Meltem Sevgili, İlknur Kozanoğlu
Department of Physiology, Hacettepe University, Ankara, Turkey

OBJECTIVE: An effective immune response protects organism, differentiate self from non-self and terminate rapidly when threat disappears. Smooth functioning of the immune response, controlled by cytokinergic interactions of helper T (Th1, Th2&Th17) and regulatory T (Treg) cells, is crucial and modulated by stressors. Albeit the extensive studies on stress-immune system interactions, Th17 and Treg response to stress has not been investigated, yet. We aimed to determine the effect of acute or chronic stress on peripheral T lymphocyte distribution.

METHODS: Male, Swiss-albino mice (30-40 g) were allocated in 3 groups as; control (n=11), acute stress (n=11) and chronic stress (n=11). Cold-immobilization stress was applied in a restrainer at 4°C once for 6 hours in acute stress group and 5 consecutive days for 6 hours/day for chronic stress group. In the blood samples withdrawn after stress protocols and corresponding times in control groups serum cortisol and cytokine levels (IL-2, IL-4, TNF α , IL-17) were measured and distribution of lymphocyte subgroups were determined flow-cytometrically.

RESULTS: The effect of acute and chronic stress on distribution of T-lymphocyte subgroups was different. Chronic stress attenuated the number of Th17 and Treg cells more.

CONCLUSIONS: Stress is a key modulator of immune response altering the reaction of Th cells, chief executive officers of acquired immune system. Further insights about mutual interaction of Treg and Th17 cells will help better understanding of the pathogenesis and provide a new era in management of especially autoimmune diseases. This study is granted by HUBAB (Project number: 011 D04 101 003)

Keywords: T Cell, Autoimmunity, Th-17, Immune Response

Comparison of melatonin and ozone in the prevention of reperfusion injury following unilateral testicular torsion: an experimental study in rats

Sinan Ekici¹, Işın Doğan Ekici², Güler Öztürk³, Fehime Aksungar⁴, Güven Turan¹, Nurettin Lüleci⁵

¹Department of Urology, Maltepe University, Istanbul, Turkey

²Department of Pathology, Yeditepe University, Istanbul, Turkey

³Department of Physiology, Maltepe University, Istanbul, Turkey

⁴Department of Biochemistry, Maltepe University, Istanbul, Turkey

⁵Department of Algology, Maltepe University, Istanbul, Turkey

OBJECTIVE: Testicular torsion/detorsion (TdT) is an ischemia/reperfusion injury (IRI). Melatonin has been shown as the most powerful antioxidant in attenuation of testicular IRI. We investigated the efficacy of ozone in comparison with melatonin in an experimental rat model of TdT.

METHODS: Twenty four male Wistar rats were divided into four groups designed as sham-operated, TdT, TdT plus melatonin and TdT plus ozone. The intraperitoneal injected doses of melatonin and ozone were 10 mg/kg/day and 4 mg/kg/day, respectively. Melatonin and ozone were injected daily beginning 15 minutes before detorsion for the following 7 days. At seventh day, blood and tissue samples were obtained. Johnsen score (JS), malondialdehyde (MDA), inhibin B (IB), reduced glutathione (GSH) plasma total sulfhydryl group (RSH) levels and nitric oxide (NOx) were studied. This study was approved by the local ethics committee.

RESULTS: TdT caused increases in tissue MDA and NOx along with decreases in JS, tissue and plasma IB and GSH levels. Melatonin prevented the rise in MDA and NOx levels and improved JS, tissue and plasma IB and tissue GSH levels along with decreases in plasma RSH level. Ozone presented similar results except for NOx level. Concomitantly, in contralateral testis, similar but insignificant changes were seen in JS, MDA and IB by melatonin and ozone, along with significant changes in GSH level. Melatonin caused a decrease, but ozone caused an increase in NOx level in both testes.

CONCLUSIONS: On different pathways, ozone was comparable with melatonin in amelioration of IRI. Protective effects of ozone were associated with NO. The potential for ozone as a treatment for TdT, therefore, deserves to be further elucidated.

Keywords: testicular torsion, ischemia-reperfusion injury, melatonin, ozone

Effect Of Sensory Deprivation And Locus Coeruleus (Lc) Electrical Stimulation On Response Properties Of Layer Iv Barrel Cortex Neurons In Male Rats

Ali Siahposht Khachaki¹, Vahid Sheibani²

¹Department of Physiology, Kerman, Iran

²Neuroscience Research Center, Kerman University of Medical Science, Kerman, Iran

OBJECTIVE: Barrel cortex rats is responsible in sensory information processing from facial whiskers. Locus coeruleus (LC) has influence in sensory information processing. In this study, the effect of phasic electrical stimulation of LC on response properties of layer IV barrel cortex neurons following sensory deprivation in male rats was investigated.

METHODS: Response properties of eighteen neurons in non-sensory deprivation group (control, unplugged) and twenty two neurons in sensory deprivation group P4 (the D2 vibrissa were first plucked on postnatal day 4) with extracellular single unit recordings in following controlled displacement of the principal or adjacent whiskers or all whiskers were assessed. LC was stimulated in interval times 0, 50, 100, 200, 400 and 800 ms before principal or adjacent whiskers controlled deflection and its effect on Latency, Response Magnitude, CTR (lateral inhibition index) were assessed.

RESULTS: Sensory deprivation (alone) in comparison to control group increased response magnitude and CTR index and also decreased response latency. In P4 group compared to control group showed that LC electrical stimulation 50 ms before principal whisker deflection caused significant increase of response magnitude neurons and increased CTR value was observed in more times LC stimulation (0-400ms) followed together whisker deflection ($P < 0.05$). Latency in all of the times LC stimulation followed principal whisker deflection was decreased ($P < 0.05$).

CONCLUSIONS: These results showed that electrical stimulation of LC following sensory deprivation was caused by the modulation of response properties of neurons and due to the change in their response patterns.

Keywords: Barrel cortex, Sensory deprivation, Locus coeruleus, rats

Cellular Models for Peripheral Nociception: Use of Cultured Rat Sensory Neurons

Mete Ozcan¹, Ahmet Ayar²

¹Department of Biophysics, Firat University, Elazig, Turkey

²Department of Physiology, Karadeniz Technical University, Trabzon Turkey

OBJECTIVE: Although pain is among the homeostatic mechanisms just as hunger and thirst, chronic pain is a common problem that has a major impact on individual's everyday life. Due to poor knowledge about the mechanism(s) of the pain, the current treatment strategies are far from satisfactory and ethical issues require great reliance on animal and cellular models for deciphering neurobiological bases of nociception and pain. Since the dorsal root ganglion (DRG) contains cell bodies of primary sensory neurons that convey information about a wide variety of sensory signals including noxious stimuli, DRG neurons in culture are well established model for the investigation of peripheral nociception. Cytosolic calcium $[Ca^{2+}]_i$, as a ubiquitous second messenger, involved in a wide range of neuronal functions including control of excitability and neurotransmitter release, and nociceptive signal transmission/transduction.

METHODS: DRGs were removed from 1-2 day old (any gender) Wistar neonatal rats after decapitation and DRG neurons were isolated by enzymatic and mechanical procedures, and grown in the presence of nerve growth factor.

RESULTS: By using whole cell mode of patch clamp and ratiometric fluorescence calcium imaging techniques, we investigated the effects of potential pain relieving agents including some anticonvulsants (levetiracetam, valproic acid), local anesthetics (levobupivacaine, bupivacaine) as well as endogenous agents (melatonin, orexin A and -B) in this nociceptive cellular model.

CONCLUSIONS: In conclusion, in vitro models including DRG's serve important tool for providing information on complex mechanisms of nociception and pain, and thereby provide basic evidence potentially useful for successful targeting and improvement in pain therapy.

Keywords: pain, dorsal root ganglions (DRG), patch clamp, calcium imaging

Effects of Three Months Venlafaxine Treatment on Nesfatin-1, Nitric Oxide and Ghrelin Levels in Patient With Major Depressive Disorders

Sermin Algül¹, Oguz Ozcelik², Fazilet Erman³, Bilge Kara⁴, Murat Kara⁵, Orhan Erman¹

¹Firat University, Faculty of Science, Department of Biology, Elazig, Turkey

²Firat University, Faculty of Medicine Department of Biophysics (and Physiology), Elazig, Turkey

³Firat University, Health High School, Department of Biochemistry, Elazig, Turkey

⁴Harpur State Hospital, Department of Psychiatry, Elazig, Turkey

⁵Firat University, Faculty of Medicine Department of Medical Genetics, Elazig, Turkey

OBJECTIVE: It is known that nesfatin-1, nitric oxide (NO) and ghrelin levels are markedly changed in patients with Major Depressive Disorders (MDD). However, it is not known that whether the changes in hormone levels trigger MDD or MDD cause changes in these hormones. We have investigated changes in nesfatin-1, NO and ghrelin levels in response to the 3-month venlafaxine treatment in patients with MDD.

METHODS: This study was approved by the local ethics committee. Informed consent (signed) was obtained from each subject. A total of 60 (20 MDD patients and 40 healthy control) subjects were enrolled into this study. Venlafaxine (225 mg/day for 3 months) was given to patients. Fasting blood samples were taken at onset of the study and at the end of the 3 months. Biochemical parameters, ghrelin, nesfatin-1 and NO were analyzed. Paired t-test used for pre- ad post-treatment values and unpaired t-test used for control and treatment group values. $P < 0.05$ was accepted as significant.

RESULTS: At basal level, nesfatin-1 was higher in MDD group compared to control: 82.2 ± 2.1 ng/ml vs 11.4 ± 4.7 ng/ml, respectively. However, NO and ghrelin levels were found to be lower in MDD group compared to control group: 2.2 ± 0.5 nmol/L vs 6.2 ± 0.3 nmol/L, and 40.6 ± 7.7 pg/ml vs 316.9 ± 49.7 pg/ml, respectively. Importantly, nesfatin-1 ($p < 0.001$) and ghrelin ($p < 0.001$) were decreased significantly, but NO levels were significantly increased in patients with MDD after venlafaxine treatment ($p < 0.05$).

CONCLUSIONS: Our findings suggest that improvements in nesfatin-1 and NO levels in MDD patients following treatment could be an important criterion to evaluate prognosis of disease and patients' response to treatment.

Keywords: Nesfatin-1, NO, Ghrelin, Major Depressive Patients, Venlafaxine

Alteration of electrical activity in the pacemaker and working myocardium of mammalian heart by exogenous and endogenous carbon monoxide

Denis Valerievich Abramochkin, Maria Viktorovna Porokhnya, Galina Sergeevna Sukhova
Department of Human and Animal Physiology, Moscow State University of MV Lomonosov, Moscow, Russia

OBJECTIVE: Carbon monoxide (CO) is a toxic gas, which also acts in the organism as a neurotransmitter. It is generated as a by-product of heme breakdown catalyzed by heme oxygenase. We have studied influence of exogenous CO and inhibitor of endogenous CO production zinc protoporphyrin IX on contractile activity in Langendorff-perfused rat heart and electrical activity in rat atrial and ventricular working myocardium and mouse sinoatrial node.

METHODS: Standard microelectrode technique was used for intracellular registration of action potentials (APs) in isolated preparations of rat right atrium, rat right ventricular wall and mouse sinoatrial node. In Langendorff-perfused isolated hearts left ventricular pressure was registered directly.

RESULTS: Solution containing dissolved exogenous CO (100 μ M-1mM) caused prominent decrease of AP duration in working rat atrial myocardium accompanied with significant acceleration of sinus rhythm and similar decrease of APD in ventricular myocardium. In the mouse sinoatrial node preparations CO (100 μ M-1mM) produced acceleration of pacemaker activity associated with increase in velocity of slow diastolic depolarization and decrease in AP amplitude. In addition high concentrations of CO (100 μ M – 300 μ M) depressed contractile activity of isolated hearts, while low concentration (50 μ M) enhanced it. Inhibitor of heme oxygenase zinc protoporphyrin IX (10 μ M) produced opposite electrophysiological effects: prolongation of AP in atrial and ventricular working myocardium and reduction of sinus rhythm rate. Therefore, endogenous CO, which may be generated in the heart due to the presence of active heme oxygenase, is likely to exert the same effects as exogenous CO applied to the perfusing medium.

CONCLUSIONS: We conclude that both endogenous and exogenous CO may act as important regulators of electrical and contractile cardiac activity.

Keywords: carbon monoxide, heart, action potential, sinoatrial node, atrium

Nesfatin-1 ameliorates sepsis-induced remote organ injury: the role of oxidant- antioxidant status and neutrophils

Zarife Nigâr Özdemir¹, Ahmet Cumhur², Ali İhsan Oluk², Aykut Hoşcan², İdris Önem², Gazi Contuk³, Feriha Ercan³, Berrak Ç. Yeğen¹

¹Marmara University School of Medicine, Department of Physiology

²Marmara University School of Medicine, Student

³Marmara University School of Medicine Department of Histology & Embryology

OBJECTIVE: Nesfatin-1, an anorexigenic and anxiogenic peptide, was identified in 2006 in the adipose tissue, gastric mucosa, pancreatic β -cells and several brain areas. Recent studies have shown that nesfatin-1 has antiapoptotic and anti-inflammatory properties. The aim was to investigate the protective effects of nesfatin-1 against oxidative organ injury in sepsis.

METHODS: Under anesthesia, sepsis was induced by cecal ligation-perforation method. Male Sprague-Dawley rats (n=24) were divided into sham-operated control, saline-treated sepsis and nesfatin-1 (10 μ g/kg; i.p.) - treated sepsis groups. Rats were decapitated at the 16 h of surgery and liver, kidney, brain and lung samples were obtained for histological analysis and for the measurement of myeloperoxidase (MPO) activity and malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) levels. Values are compared by ANOVA.

RESULTS: Compared to control group, sepsis resulted in significant decreases in GSH, SOD and CAT levels with increases in MDA levels and MPO activities ($p < 0.05$ – 0.001), showing oxidative damage in all tissues of saline-treated rats. Treatment with nesfatin-1 alleviated sepsis-induced tissue damage, while MDA levels and MPO activities were reduced along with the preservation of the antioxidant GSH, CAT and SOD levels in all tissues ($p < 0.05$). All histological data also support these findings.

CONCLUSIONS: The current results suggest the anti-inflammatory effects of nesfatin-1 in sepsis-induced oxidative damage by the augmentation of endogenous antioxidants and the inhibition of neutrophil recruitment. Thus, nesfatin-1 may be regarded as a potential therapeutic agent in the treatment of septic shock to reduce subsequent remote organ failure.

Keywords: catalase, glutathione, myeloperoxidase, malondialdehyde, nesfatin-1, oxidative stress, sepsis, superoxide dismutase

The effects of Rosuvastatin and Chitosan on Extrahepatic Cholestasis after Bile Duct Ligation in Wistar rats

Elena Diana Olteanu¹, Simona Clichici¹, Adriana Filip¹, Andras Nagy², Marina Ducea¹, Remus Moldovan¹, Adriana Muresan¹

¹Department of Physiology, The University of Medicine and Pharmacy "Iuliu Hațieganu", Cluj-Napoca, Romania,

²Department of Pathology, USAMV, Cluj-Napoca, Romania

OBJECTIVE: Statins exert pleiotropic actions in ex vivo and in vitro studies, having antioxidative and antiinflammatory effects. These are important in preventing liver injury following biliary obstruction. We aimed to evaluate the effects of rosuvastatin and chitosan, a natural compound, on liver damage caused by bile duct ligation in rats.

METHODS: We divided 50 female Wistar rats into these groups: 1 (SHAM) rats underwent laparotomy, 2 (BDL) only bile duct ligation, 3 (RO-5 mg) rosuvastatin 5 mg p.o. after BDL, 4(RO-10 mg) rosuvastatin 5 mg p.o. and group 5 (CS) - chitosan p.o. (daily for for 6 days). The following parameters were measured: malondialdehyde (MDA), carbonylated protein (CP), glutathione, total SH groupings, nitric oxide, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). A histopathological examination was performed (Knodell Histological Activity Index and the number of biliary canals in five portal sites for each section were noted).

RESULTS: The levels of MDA in the liver were significantly lower in the CS group compared to the BDL group ($p < 0.05$). Rosuvastatin increased MDA, CP levels and hepatocytolysis as compared to the control group. The levels of AST and ALT were higher in the groups that received the statin compared to the BDL group ($p < 0.05$). The histological findings were significantly improved by chitosan.

CONCLUSIONS: We confirmed that bile duct ligation and rosuvastatin induced severe oxidative stress damage only six days after the procedure. Chitosan proved very efficient in protecting the hepatic cells against oxidative stress. The histological findings in the CS group were very promising.

Keywords: bile duct ligation, rosuvastatin, chitosan, reactive oxygen species

Endocrine Modulatory Effects of Bisphenol A and Oestradiol on the Brain Kisspeptin/GPR54 System in Immature Rat Uterotrophic Assay Model

Signem Eyuboglu¹, Taha Kelestimur¹, Ertugrul Kilic¹, Ulkan Kilic², Bayram Yilmaz¹

¹Yeditepe University, Medical School, Department of Physiology, Istanbul, Turkey

²Bezmi Alem University, Medical School, Department of Medical Biology, Istanbul, Turkey

OBJECTIVE: Bisphenol A (BPA) is a plasticizer and has been extensively used in consumer products. BPA can leach from food and beverage containers, and some dental sealants and composites under normal conditions of use and enter into the human body. It has been suggested that BPA can act an endocrine disrupter in various experimental models. In the present study, we have evaluated the effects of BPA on female reproductive system by using uterotrophic assay. In addition, possible effects of BPA on the regulation of related factors including, kisspeptin, GPR54 and ERK-1/2 expression were analyzed in the brain.

METHODS: Twenty day-old immature female Sprague-Dawley rats were selected and divided into four groups and treated with (n=6/group) vehicle (0.1 ml corn oil, sc), oestradiol (E2, 100 µg/kg), BPA (1 mg/kg) and BPA (10 mg/kg) for three consecutive days. Twenty-four hours after last treatment, the animals were decapitated and uterus tissue was harvested for the uterotrophic assay. The brains were dissected and processed for the analysis of kisspeptin, GPR54 expressions and ERK-1/2 phosphorylation by Western blotting and immunohistochemistry.

RESULTS: E2 significantly increased the ratio of uterine weight to the body weight compared to the control group. Although this ratio was slightly raised by BPA administration, the changes were not statistically significant. E2 and BPA treatments significantly increased kisspeptin, GPR54 expression and ERK-1/2 phosphorylation in the brain at the level of hypothalamus.

CONCLUSIONS: The present results indicate for the first time that BPA stimulates kisspeptin and GPR54 expression in the hypothalamus of immature female rats. These findings suggest that BPA may exert estrogenic effects on the brain even before its significant impact on the uterine tissue.

Keywords: Bisphenol A, Kisspeptin, GPR54, Brain, Uterotrophic Assay

Response of oxidant/antioxidant systems in different organs and tissues to prolonged repetitive hyperbaric oxygen: collective results of a series of experimental studies

Kemal Simsek¹, Sukru Oter², Mehmet Ozler², Bulent Uysal², Hakan Ay¹, Serdar Sadir², Turgut Topal², Ahmet Korkmaz², Senol Yildiz¹

¹Department of Undersea & Hyperbaric Medicine, Gulhane Military Medical Academy, Ankara, Turkey

²Department of Physiology, Gulhane Military Medical Academy, Ankara, Turkey

OBJECTIVE: Hyperbaric oxygen (HBO) is known to cause oxidative stress. Previous studies with single HBO exposure had shown that HBO-induced oxidative stress is proportional to both its exposure pressure and duration. However, HBO treatments commonly depend on longer periods including repetitive exposures. In a series of studies we investigated the interactions of oxidant/antioxidant system markers in erythrocytes, lung and brain tissues of rats exposed to HBO from 5 up to 40 sessions.

METHODS: Adult male rats were allotted into six study (n=8 for each) plus one control group (n=12). The study groups were exposed to 5, 10, 15, 20, 30, and 40 daily consecutive 2.8 ATA/90 min HBO sessions. Animals were sacrificed 24 h after the final session. Malonyldialdehyde and carbonylated protein levels were determined as measures of oxidative stress; superoxide dismutase and glutathione peroxidase activities were used to reflect the antioxidant status.

RESULTS: In the erythrocytes, oxidative stress markers tended to increase at the early stages of repetitive HBO exposures and declined to their control values at the end of the 40-session procedure. In the lung tissue, lipid and protein oxidation products began to increase after 20 HBO exposures and then continued to increase. The increase of oxidation products was simultaneously accompanied by the antioxidant enzymes' activities. In the brain tissue (cerebral cortex, white matter and cerebellum), no alteration was recorded in any of the measured parameters.

CONCLUSIONS: These findings provided evidence for an oxidative effect of HBO exposure which is limited successfully via the endogenous antioxidant systems. The brain tissue represented a more resistant state against HBO-induced oxidative stress than the lungs and erythrocytes.

Keywords: Antioxidant enzymes; Hyperbaric oxygen; Oxidative Stress; Rat

In vitro and in vivo characteristics of cycled lung surfactant are preserved by fibrinogen

Andrea Calkovska¹, Bim Linderholm², Marie Haegerstrand Bjorkman², Tore Curstedt²

¹Department of Physiology, Comenius University, Jessenius Faculty of Medicine, Martin, Slovakia

²Laboratory for Surfactant Research, Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden

OBJECTIVE: The aim of the study was to prove the hypothesis that fibrinogen protects pulmonary surfactant against inactivation by surface area cycling.

METHODS: Natural modified surfactant Curosurf was mixed with fibrinogen (ratio 2:1) and subjected to long-term surface area cycling at 37°C. Minimum and maximum surface tension (gamamin and gamamax) of samples at 6, 9 and 12 days of cycling were evaluated by captive bubble surfactometer. Immature newborn rabbits were treated intratracheally with Curosurf. Curosurf was cycled for 6 days without (CuroCyc) or with fibrinogen (CuroCycFib). In controls, no material was instilled. Animals were ventilated with 100% O₂. Tidal volume (VT) and lung-thorax compliance (CLT) were recorded. Post mortem lung gas volumes and alveolar expansion were evaluated.

RESULTS: Surface properties of surfactant cycled at 10 mg/ml were not influenced by fibrinogen. At 80 mg/ml, surfactant cycled without fibrinogen for 6 days did not reach gamamin < 5 mN/m. CuroCycFib had low gamamin comparable with non-cycled Curosurf. Moreover, addition of fibrinogen prevented lipid peroxidation of Curosurf. In vivo, values of VT and CLT in CuroCyc animals did not differ from non-treated controls. Animals receiving Curosurf and CuroCycFib had significantly higher VT and CLT than controls. Lung gas volumes in CuroCyc, but not in CuroCycFib group, were significantly lower than in Curosurf group.

CONCLUSIONS: Effect of fibrinogen on pulmonary surfactant cycled at 37 °C depends on phospholipids (PLs) concentration. At high PLs concentration used in clinical practice fibrinogen has protective effect on natural modified surfactant subjected to long-term surface area cycling. First author is supported by Center of Excellence for Perinatal Research No.26220120036 co-financed from EU-sources.

Keywords: lung surfactant, fibrinogen, surface area cycling, newborn rabbits model

Effect of estrogens and phytoestrogens on age-induced inhibition of surfactant synthesis by rat type II pneumocytes

Lisa Rancan¹, Cruz Garcia¹, Carmen Castillo², Sara Cuesta², Roman Kireev², Jesus A. F. Tresguerres², Elena Vara¹

¹Department of Biochemistry and Molecular Biology Medical School, University Complutense of Madrid, Spain

²Department of Physiology, Medical School, University Complutense of Madrid, Spain

OBJECTIVE: Alterations in surfactant production may play a role in the pathogenesis of lung dysfunction secondary to aging. Phosphatidylcholine (PC) and phosphatidylglycerol (PGL) are main surfactant components. The aim of the present study was to investigate the lung PC and PGL synthesis in old female rats with or without treatment with estrogens or phytoestrogens. We also investigated the possible involvement of nitric oxide and lipid peroxidation.

METHODS: Rats were randomly assigned to 2 groups A: intact and B: ovariectomized at 12 months. Rats were killed by decapitation at 14, 18, 22 months of age. Ovariectomized 24-months-old rats were divided into 3 groups: treated with estrogens (estradiol 125µg/week s.c.); treated with soy extract (phytsoya®, 312mg/kg/day in drinking water); non-treated animals. Two-months-old rats were used as young controls. Type II pneumocytes were isolated by enzymatic digestion, adherence separation of macrophages, and gradient purification. Cells were precultured for 24 h and then cultured for another 24 h. NO and CO release to the medium and LPO, cGMP and the incorporation of D-(U-14C) glucose into PC and PGL (cells) were determined.

RESULTS: Age-reduced glucose incorporation into PC and PGL, while it increases LPO and cGMP content and NO and CO release. These effects were more apparent on ovariectomized rats. Both estrogens and phytoestrogens treatments were able to reduce age-induced effects.

CONCLUSIONS: Our results suggest that age may alter lung function by reducing its hydrophobic phospholipid content. Also they suggest that both Eo and Phyt could exert a protective effect on age-induced lung damage.

Keywords: aging, inflammation, pulmonary surfactant, type II pneumocytes, estrogens

Regulation by hypoxia of AQP1 gene by a mechanism that partially depends on Hif-1-alpha

Miriam Echevarria Irusta¹, Irene Abreu Rodríguez¹, Rocío Sánchez Silva¹, Ana Paula Martins², Graça Soveral³, José López Barneo¹, Juan José Toledo Aral¹

¹Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla (Departamento de Fisiología Médica y Biofísica), Seville, Spain

²REQUIMTE, Dep. Química, FCT-UNL, 2829-516 Caparica, Portugal

³Faculdade de Farmácia, Universidade de Lisboa, 1649-003 Lisboa, Portugal.

OBJECTIVE: Contribution of Aquaporin-1 (AQP1) to gas permeation through the membrane has been demonstrated. Recent experiments indicated that expression of this protein is upregulated by hypoxic conditions. In the present work we want to confirm the regulation by hypoxia of AQP1 and to explore the molecular mechanism underlying this regulatory process.

METHODS: Levels of Aqp1 mRNA and protein were measured by RT-qPCR in mouse brain and lung and in a culture cell line (rat gliosarcoma, 9L). Stopped-flow light-scattering experiments were used for water permeability (Pf) measurements and transcriptional activity of AQP1 promoter was evaluated in vitro by transient transfections into mouse endothelial cells with a 1297 bp 5' proximal Aqp1 promoter-luciferase construct.

RESULTS: An increment on AQP1 mRNA and protein expression, as well as increase on the Pf of cells exposed to hypoxia was confirmed. Incubation at low oxygen tension produced a dose and time dependent induction on the luciferase activity-AQP1 promoter derived that was obtained also after treatments with hypoxia mimetics (DMOG and CoCl₂) and by overexpressing mutated forms of HIF-1 α . Single mutations or full deletions of the three putative HIF binding domains present in the promoter significantly reduced its hypoxia response, and transfection with siRNA of Hif-1 α decreased the in vivo hypoxic induction on the Aqp1 mRNA and protein levels.

CONCLUSIONS: HIF-1 α participates in the hypoxic induction of AQP1 expression, although other transcription factors might also contribute on it. Physiological relevance of this regulation in different human pathologies can be presumed but further studies are necessary.

Keywords: mouse AQP1-promoter, hypoxia, Luciferase expression, immunohistochemistry, HIF-siRNA, water permeability

Altitude Training Induced Alterations in Erythrocyte Rheological Properties and Oxidative Stress

Melek Bor Küçükataş¹, Rıdvan Colak², Gülten Erken³, Emine Kılıç Toprak¹, Vural Küçükataş¹

¹Department of Physiology, Pamukkale University, School of Medicine, Denizli, TURKEY

²Üsküp Primary School Bağcılar, İstanbul, TURKEY

³Department of Physiology, Balıkesir University, School of Medicine, Balıkesir, TURKEY

OBJECTIVE: Altitude training is frequently used by athletes to improve sea-level performance. However, the objective benefits of altitude training are controversial. This study aimed to investigate the possible alterations in hemorheological parameters and oxidative stress in response to altitude training.

METHODS: 37 Sprague Dawley male rats, were divided into 6 groups: live low-train low (LLTL), live high-train high (LHTH), live high-train low (LHTL) and their controls live high and low (LHALC), live high (LHC), live low (LLC). LHC and LHTH groups were exposed to hypoxia (15% O₂) for 4 weeks. LHALC and LHTL were exposed to 12 hours hypoxia / normoxia per day, 4 weeks. Hypoxia was maintained by hypoxic tent. The training protocol corresponded to 60-70 % of maximal exercise capacity. Rats of training groups ran on treadmill for 20-30 min a day, 4 days per week, 4 weeks. At the end of the experiment, blood was collected at low altitude. Erythrocyte deformability and aggregation were determined by an ektacytometer, oxidant (TOS) and antioxidant (TAS) status by kits. “Kruskal Wallis Variance Analysis” and “Mann Whitney U test” were used for statistics.

RESULTS: Erythrocyte deformability of LHC group was increased compared to LHALC and LLC. Deformability of LHTH group was higher than LHTL and LLTL groups. No statistically significant alterations were observed in aggregation parameters. TOS of LHTH was higher than LLTL whereas TAS of the same group was increased compared to LHTL and LLTL groups.

CONCLUSIONS: Living (LHC) and training at altitude (LHTH) seem more advantageous in hemorheological point of view. This study was supported by Pamukkale University Research Fund.

Keywords: Exercise, altitude training, erythrocyte rheology, oxidative stress

Inhibitory effect of μ -opioids on P2X3 receptors in DRG neurons of rat

Oleg Krishtal¹, Igor Chizhnikov², Vyachslav Kulyk¹, Donald Simone³, Georgy Bakalkin⁴

¹Department of Cellular Membranology, Bogomoletz Institute of Physiology, Kiev Ukraine

²State Key Laboratory for Molecular and Cellular Biology, Kiev, Ukraine

³School of Dentistry, Univ. Minnesota, Minneapolis, USA

⁴Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden

OBJECTIVE: According to existing paradigm, homomeric P2X3 receptors (expressed almost exclusively in the sensory neurons) play especially important role in the nociception. In this study we have examined the effect of μ -opioids on the currents generated by P2X3 receptors in the neurons from DRG of rat.

METHODS: Acutely isolated or cultured for 12-24 hours neurons from dorsal root ganglia (DRG) of rat were studied using conventional patch clamp techniques and rapid application method.

RESULTS: In approximately every fifth of the examined small sensory neurons demonstrating functional P2X3 receptors the inward currents elicited by their activation was effectively blocked by μ -opioid agonist leu-enkephalin (IC₅₀ close to 10 nM). We have found that pretreatment of the neuron with naloxon causes approximately ten-fold increase in the sensitivity of P2X3 receptor-induced current to the blocking action of opioids (IC₅₀ decreases to 1 nM). Cross reactions of P2X3 receptors to rapid successive applications of opioids and naloxone indicate at the possibility of multiple states of opioid receptors.

CONCLUSIONS: P2X3 receptors in a fraction of small (putatively nociceptive) sensory neurons of rat are under inhibitory control of opioid receptors. The effectiveness of this control is strongly modulated by pre-treatment with naloxone. This effect can serve as at least partial explanation for the well-known phenomenon of functional naloxone-induced supersensitivity to opioids.

Keywords: sensory neurons, P2X3 receptors, μ -opioids, naloxone, nociception

Effect of Endogenous Opioids on Injured Neurons

Gurkan Ozturk¹, Nurettin Cengiz², Elif Kaval Oguz³, Elcin Yenidunya Yardim³, Aydin Him⁴

¹Istanbul Medipol University, Medical School, Physiology Dept., Istanbul, Turkey

²Yuzuncu Yil University, Medical School, Histology and Embryology Dept., Van, Turkey

³Yuzuncu Yil University, Medical School, Neuroscience Research Unit, Van, Turkey

⁴Yuzuncu Yil University, Medical School, Physiology Dept., Van, Turkey

OBJECTIVE: Investigation of the effect of endogenous opioids on the survival of primary sensory neurons following an axonal injury.

METHODS: Local ethical committee approval was obtained prior to experiments. Primary sensory neurons were isolated from dorsal root ganglia of young adult mice and cultured in glass-bottom culture dishes. After 24 hours in culture, outgrown axons were precisely cut with a UV laser at 100 micrometer distance from the soma. The axotomies were performed under physiological conditions and in the presence of beta endorphin (5 uM), morphine (5uM) and / or other agents.

RESULTS: Under control conditions, 24 hours after axotomy death rate was 70 %, which was reduced to 46 % by beta-endorphin ($p<0.05$). This effect was much more pronounced with selective mu agonist morphine (9 %). Since beta endorphin has affinity also to delta opioid receptors (DORs) we tested the possibility that DORs may mediate a death signal in our experimental model. Indeed, when we blocked DOR activity with naltrindole (10 uM) or naltriben (10 uM) the death rate was reduced to 47 and 38 % respectively ($p<0.05$). We have obtained many hints from other experiments that we performed to explain the mechanism underlying this dual role of opioid receptors on the survival of injured neurons, regarding opioid receptor trafficking between cytosole and cell membrane and receptor dimerization.

CONCLUSIONS: This study has shown that depending on the receptor subtype, endogenous opioids may be either a survival or a death factor for injured neurons. This novel finding may have implications in physiopathological processes as well as for pharmacological interventions.

Keywords: Neuronal injury, neuronal death, axotomy, endogenous opioids.

CXCL16 orchestrates adenosine A3 receptor and MCP-1/CCL2 activity to protect neurons from excitotoxic cell death in the CNS

Flavia Trettel, Maria Rosito, Cristina Deflorio, Cristina Limatola
Department Physiology and Pharmacology, Sapienza University of Roma, Italy

OBJECTIVE: Glutamate (Glu) induced excitotoxicity likely is a major cause of neuronal cell death upon different brain injuries and in several pathological conditions such as AD, PD, ischemia and ALS. A role for chemokines as important molecules that mediate neuron-glia cross-talk has emerged in recent years, both in physiological and pathological conditions where they might drive brain repair and recovery. For the first time we demonstrate that in the CNS soluble CXCL16 is able to prevent neuronal cell death upon excitotoxic damage.

METHODS: Primary hippocampal cell cultures of wt animals, CXCR6GFP/GFP, or AR3^{-/-} mice alone or co-cultured with pure wt glia cells were used for excitotoxic experiments (Glu exposure and oxygen glucose deprivation, OGD). Specific ARs antagonists, adenosine deaminase and ELISA were used to identify mediators of CXCL16 neuroprotection.

RESULTS: We found that CXCL16, acting on astrocytes, prevents hippocampal neuronal cell death upon excitotoxic damage due to excessive Glu exposure and OGD, Fig.1. CXCL16 neuroprotection requires the presence of extracellular adenosine and pharmacological or genetic inactivation of the adenosine A3 receptor (A3R) prevents CXCL16 effect, Fig.2. Moreover we found that upon stimulation with CXCL16, astrocytes release soluble factor/s, that are essential to mediate neuroprotection. In particular: i) CXCL16 induces astrocytes release of monocyte chemoattractant protein-1 MCP-1/CCL2; ii) CCL2 neutralizing antibody significantly reduces CXCL16 neuroprotection, Fig.3.

CONCLUSIONS: For the first time we demonstrate that chemokine CXCL16 is able to mediate cross-talk between astrocytes and neighboring neurons and, in pathological conditions such as excessive Glu or OGD exposure, is able to counteract neuronal cell death through an adenosine-dependent chemokine-induced chemokine-release mechanism.

Keywords: Excitotoxicity, neuroprotection, CXCL16, adenosine, A3R, MCP-1/CCL2

Persistent organic pollutant exposure results in changes in serum lipid profiles

Zafar Aminov, David O. Carpenter

Institute for Health and the Environment, University at Albany, New York

OBJECTIVE: The main objective of the current study was to investigate relations of different groups of organochlorines with serum lipid fractions.

METHODS: Blood samples, obtained from 760 Caucasian and African-American residents of Anniston, AL, were analyzed for 35 polychlorinated biphenyl (PCB) congeners, 9 pesticides and serum levels of cholesterol, triglycerides, high density lipoproteins (HDL), and low density lipoproteins (LDL). Serum level of total lipids was estimated by mathematical equation. Multiple linear regressions and Student's t-test were used to analyze the data.

RESULTS: Serum levels of total PCBs and pesticides each showed positive correlation with the serum level of total lipids, total cholesterol and triglycerides, but not LDL nor HDL. For those on lipid lowering medication, the relation with cholesterol and organochlorines disappeared but that for triglycerides did not. Analyses with smaller grouping of the organochlorines showed that 1) serum levels of total lipids were positively correlated with serum levels of tri- and tetra-ortho PCBs, total chlordane, mirex, and hexachlorbenzene (HCB); 2) serum levels of total cholesterol were positively correlated with serum levels of mirex and HCB; 3) serum levels of triglycerides were positively correlated with the serum level of total chlordane and HCB; 4) elevation of the serum level of HDL was associated only with elevated serum levels of mono-ortho PCBs; and 5) serum levels of LDL were positively correlated with the serum level of HCB.

CONCLUSIONS: These results indicate that serum levels of total lipids, cholesterol, and triglycerides are elevated in relation to serum concentration of PCBs and pesticides.

Keywords: PCBs and lipid profiles

Determination of the Relationships Between Anaerobic Threshold, Respiratory Compensation Point and Critical Power Output During Muscular Exercise in Sedentary Subjects

Ihsan Serhatlioglu¹, Bayram Yilmaz², Oguz Ozcelik¹

¹Firat University Faculty of Medicine Department of Biophysics, Elazig, Turkey

²Yeditepe University Faculty of Medicine, Department of Physiology, Istanbul, Turkey

OBJECTIVE: We have comparatively investigated the relationships between anaerobic threshold (AT), respiratory compensation point (RCP) and critical power (CP) to determine an optimum exercise training protocol for clinical and sports science.

METHODS: A total of 25 male subjects (22.8 \pm 3.6 yr, 76 \pm 8.8 kg) performed an incremental exercise test and 7 different constant load exercise tests using an electromagnetically-braked cycle ergometer. AT and RCP were estimated from ventilation and respiratory gas exchange parameters. The data were evaluated breath-by-breath. The constant load exercise protocols were designed using AT and the mean duration of performed exercise tests were recorded. CP was estimated with linear (power-(1/time)) mathematical model using the data obtained from these tests. The Linear Regression Analysis and the paired t-test were used to compare values between the dependent groups. This study was approved by the local ethics committee, and informed (signed) consent was obtained from all subjects.

RESULTS: The work rate was found to be 117 \pm 22 W at AT and 132 \pm 27 W at RCP. CP values were found to be 141 \pm 31 W in 3 test model analysis and 133 \pm 27 W in 4 test model analysis. There was a significant correlation between the values estimated from linear regression analysis power-(1/time) models (4 test model) and CP estimated using RCP (R=0.986 p<0.0001).

CONCLUSIONS: The present findings have shown for the first time that CP reflects the RCP and CP can be estimated easily using pulmonary gas exchange variables. The number of tests used for mathematical modelling to estimate CP and the relation between the work rate used in the constant load exercise test and work rate at RCP are important factors affecting CP estimation.

Keywords: Anaerobic threshold, critical power output, respiratory compensation point, exercise test, pulmonary gas exchange

Nordic walking decreased circulating chemerin and leptin concentrations in prediabetic middle-aged men

Mika Eerik Venojärvi¹, Sirpa Manderöos², Niko Wasenius², Miika Hernelahti³, Jukka Surakka⁴, Harri Lindholm⁵, Olli Heinonen³, Jaana Lindström⁶, Sirkka Aunola⁷, Mustafa Atalay⁸, Johan Eriksson²

¹Turku University of Applied Sciences, Turku, Finland

²University of Helsinki, Helsinki, Finland

³University of Turku, Turku, Finland

⁴Arcada, University of Applied Sciences, Helsinki, Finland

⁵Finnish Institute of Occupational Health, Helsinki, Finland

⁶National Institute for Health and Welfare, Helsinki, Finland

⁷National Institute for Health and Welfare, Turku, Finland

⁸University of Eastern Finland

OBJECTIVE: An elevated secretion of various adipokines and cytokines, including chemerin, interleukin 6 (IL-6), leptin, retinol binding protein-4 (RBP-4) and tumor necrosis factor alpha (TNF- α) and decreased secretion of adiponectin are considered to play an important role in the development of diseases like the metabolic syndrome and type 2 diabetes. In the present study, the effects of a 12 weeks exercise intervention on serum levels of adipokines and cytokines in obese pre-diabetic men were investigated.

METHODS: Prediabetic obese men (n = 144) aged 40–65 years were studied at baseline and at 12 weeks in a randomized controlled multi-centre intervention study. Their BMI varied from 25.1 to 34.9. The subjects were randomised to one of three groups: (1) a control group (C, n = 47) that had no supervised exercise during the intervention period, (2) a Nordic walking group (NW, n = 48) that trained aerobically 60 minutes with Nordic walking exercises three times per week, or (3) a resistance training group (RT, n = 49) that trained 60 minutes with strength and power type exercises three times per week.

RESULTS: Both types of exercise decreased significantly serum chemerin concentrations compared to control group. In addition, plasma leptin concentration decreased in the NW group. There were no significant changes in the circulating concentrations of adiponectin, IL-6, RBP-4 and TNF- α and in energy intake during the intervention period between study groups.

CONCLUSIONS: Nordic walking decreased circulating leptin concentrations after 12 weeks of intervention in prediabetic obese middle-aged men and both types of exercise had regulatory effects on circulating chemerin.

Keywords: chemerin, leptin, prediabetes, Nordic walking

Is Glu27 allele a determinant of association among bone mineral density and fat mass, weight and % body fat?

Figen Dağ¹, Mehmet Emin Erdal², Özlem Bölgen Çimen³, Tuba Gökdoğan Edgünlü⁴, Resa Altun³, Hüseyin Beydağı¹

¹Department of Physiology, Medical Faculty, Mersin University, Mersin, Turkey

²Department of Medical Biology and Genetics, Medical Faculty, Mersin University, Mersin, Turkey

³Department of Physical Medicine and Rehabilitation, Medical Faculty, Mersin University, Mersin, Turkey

⁴Healthy Science School, Muğla University, Muğla, Turkey

OBJECTIVE: Bone mineral density (BMD) is the most crucial determinant of bone strength. While some studies suggested that lean body mass (LBM), but not fat mass (FM), was related with BMD; other studies have demonstrated that FM, but not LBM, is an important determinant of BMD. Individual genetic specifications may be the reason of these controversial findings. The polymorphism on codon 27 was more frequent in overweight people and Glu27 allele was associated with weight gain, fat mass and obesity. The purpose of this study was to address the relationship between the anthropometric parameters and lumbar BMD in postmenopausal women who carry Glu27 allele.

METHODS: Forty-four postmenopausal women (55.45±5.55 years) participated in our study. BMD was measured for anteroposterior lumbar spine (L1-L4) by DXA. FM, body fat % (%BF), LBM and weight were determined. The ADRB2 polymorphism (Gln27Glu) was identified by PCR-RFLP.

RESULTS: There were no significant differences between Glu27 allele carriers and non-carriers in mean values of FM, %BF, LBM and weight. While positive significant correlations were found between lumbar BMD and FM ($r=0.43$), % BF ($r=0.41$) and weight ($r=0.40$) in Glu27 allele carriers, there was no significant relationship in Glu27 non-carriers. Yet, the relationship between lumbar BMD and LBM was significant neither in Glu27 allele carriers nor in non-carriers.

CONCLUSIONS: Our results suggest that the presence of the Glu27 allele may be the determinant factor of the association among BMD and FM, and weight. It can be speculated that FM, %BF and weight may not have protective effects on every individual, as claimed, but may be protective only for individuals who carry Glu27 allele.

Keywords: BMD, BF, Glu27 allele, LBM

Effect of 900 MHz Radiofrequency Radiation On Oxidative Stress Parameters of Some Internal Organs

Suleyman Dasdag¹, Mehmet Zulkuf Akdag¹, Ozcan Erel²

¹Department of Biophysics, Medical Faculty Of Dicle University, Diyarbakir, Turkey

²Department of Biochemistry, Medical School of Yildirim Beyazit University, Ankara, Turkey

OBJECTIVE: Most of the studies on biological effects of mobile phone exposure are focused on brain. However, nobody knows whether mobile phone exposure affects other internal organs during the talking. Therefore, the aim of this study was to investigate the effects of 900 MHz radiofrequency radiation exposure on oxidative stress level of some internal organs of rats, which primarily have their heads exposed.

METHODS: The study was carried out on 21 Wistar Albino adult male rats. The rat heads in a carousel exposed to 900 MHz radiofrequency radiation emitted from a generator, which simulate mobile phones. For the study group (n: 14), rats were exposed to the radiation for 2 h per day for 10 months. For the sham group (n: 7), the same procedure was applied to rats except that the generator was turned off. At the end of the study some of the oxidative stress parameters such as catalase, myeloperoxidase, malondialdehyde (MDA), total oxidant status (TOS), total antioxidant capacity (TAC) and oxidative stress index (OSI) were measured in lung, kidney and heart of rats.

RESULTS: The results showed that the parameters such as TOS and OSI increased in the lung of radiation-exposed rats ($p<0.05$). However, parameters such as catalase, myeloperoxidase, TOS and TAC were also increased in the kidney of exposed rats ($p<0.05$). On the other hand, only OSI increased in the heart of exposed rats ($p<0.05$).

CONCLUSIONS: Some of the oxidative stress parameters in the lung, kidney and heart of the rats, which primarily had their brains exposed, can be altered by 900 MHz mobile phone exposure.

Keywords: Mobile phones, oxidative stress parameters, lung, kidney and hearth

Does the magnetic field of a magnetic stirrer in an optical aggregometer interfere with concurrent platelet aggregation?

Engin Sađdilek¹, Ođuz Sebik², Gurbüz Ćelebi²

¹Department of Biophysics, Uludag University, Faculty of Medicine, Bursa, Turkey

²Department of Biophysics, Ege University, Faculty of Medicine, Izmir, Turkey

OBJECTIVE: It is known that magnetic fields have effects on the cellular level on many biological processes through membranes, ions, oxygen radicals and enzymes. Some effects are observed while the magnetic field is applied, for some processes the effects are observed after the exposure. This study evaluates the effects of magnetic field exposure on platelets during the process of aggregation, using a modified aggregometer.

METHODS: Blood samples from healthy volunteers were anticoagulated using citrate (n=27) or heparin (n=26). Platelet-rich plasma samples were prepared. A mechanical stirring device was attached to the aggregometer instead of the magnetic stir bar system in the aggregometer. The platelet-rich plasma samples were stirred using a stirring rod that did not produce any magnetic fields (neutral) in one channel of the aggregometer, and in the other channel a stirring rod that produced a similar magnetic field as the magnetic stir bars used in a standard aggregometer were used. A magnetic field with a frequency range of 0-15Hz and an amplitude range of 1.9-65 mT was applied to the platelets assigned to the channel with the magnetic stirring rod. Aggregation was induced using ADP, collagen and epinephrine. The slopes, maximum aggregation and area under the curve values of the aggregation curves were compared for the neutral and magnetic stirring rod groups.

RESULTS: For samples exposed to a magnetic field, for both citrate and heparin groups, a significant decrease was observed for all the parameters evaluated for aggregations induced using ADP and collagen.

CONCLUSIONS: This observation gives rise to the thought that magnetic field exposure inhibits an active process during platelet aggregation.

Keywords: Magnetic fields, optical aggregometer, platelets.

Effect of calorie restriction on certain plasma coagulation factors and mitotic index in different organs of male Wistar rats

Muharrem Balkaya¹, Cengiz Ünsal¹, Hümeysra Ünsal¹, Aykut Göktürk Üner¹, Ece Koç¹, Mümin Alper Erdoğan¹, Nezihe Tülin Boylu²

¹Department of Physiology, Faculty of Veterinary Medicine of Adnan Menderes University, Aydın, Türkiye

²Department of Histology and Embriology, Faculty of Medicine of Adnan Menderes University, Aydın, Türkiye

OBJECTIVE: To investigate whether calorie restriction affects plasma coagulation factors and cell division.

METHODS: Nineteen male Wistar rats were randomized into three groups and kept individually in metabolic cages. Rats in first group (control) were given normal rat chow ad libitum, while other two groups were given either 80% or 70% of the same chow diet consumed by the controls for 30 days. All groups received tap water ad libitum. Food and water intake and weight changes were recorded throughout the experiment. Blood samples were obtained from tail vein to determine fibrinogen, prothrombine time (PTT), activated partial thromboplastin time (APTT), factor-II (FII), factor-X (FX), and factor-XII (FXII) values or activities at baseline (day 0), 10th, 20th, and 30th days. Duodenum, liver and testes were collected. Coagulation factors were measured using coagulometer. Mitotic index was determined in organs morphometrically. Data were evaluated with analysis of variance for repeated measures in factor time.

RESULTS: Body weights and water consumption of animals are decreased in both food restriction groups, but the decreases were confirmed only for 30% restriction group compared to controls ($P<.001$). Dietary interventions had no effect on coagulation factors. However; APTT, FX, FXII and fibrinogen were affected by time. Compared to their baselines APTT were prolonged at days 20 and 30, fibrinogens were increased at day 30, FX were decreased during all experimental period, and FXII levels were increased at the 10th and 30th days of experiment. Mitotic activity was increased in restricted groups only in duodenum.

CONCLUSIONS: The findings did not confirm any effect of dietary restriction on coagulation factors studied, but the effect of time was significant.

Keywords: Male Wistar rats, calorie restriction, coagulation factors, mitotic index

The effects of experimental epilepsy and environmental enrichment on the mechanical properties of erythrocytes in rats

Aise Seda Artis, Seval Keloglan, Erdem Başaran, Nazan Dolu, Sami Aydoğan
Department of Physiology, Erciyes University Medical Faculty, Kayseri, Turkey

OBJECTIVE: Enriched environment is reported to have a positive impact on brain function in epilepsy. Erythrocytes like brain may be vulnerable to oxidative stress induced by epilepsy. This is the first study aimed to investigate the effects of epilepsy induced by multiple injections of pentylentetrazol and environmental enrichment on the rheological properties of erythrocytes.

METHODS: Experimental procedures were approved by the Local Ethical Committee of Erciyes University. Rats were divided into four groups: (i) control (C), (ii) enriched environment (En), (iii) epileptic (Ep) and (iv) epileptic rats kept in enriched environment (Ep-En). Epilepsy was induced by intraperitoneal pentylentetrazol injections every second day for 38 days. At the end of this period, elongation indexes for erythrocyte deformability, aggregation and % hemolysis for osmotic fragility were determined from blood samples.

RESULTS: Elongation indices were found to be highest and equal in C and Ep-En rats; and lowest in En rats ($p < 0.05$). % hemolysis were observed to be equal in En and Ep rats and then increasing respectively in Ep-En and C rats ($p < 0.05$). Aggregation indices were measured to be equal in Ep and En rats and then decreasing respectively in C and Ep-En rats ($p > 0.05$).

CONCLUSIONS: Decrease in the deformability and increase in the aggregation indices reflect a negative effect of the epileptic seizures on erythrocytes. We think that the changes observed in our study might be explained in the light of previous reports by the balance of oxidant-antioxidant systems in epilepsy and increased adrenocortical function in environmental enrichment. So it seems that environmental enrichment helps to improve the impairment in mechanical properties of erythrocytes in epilepsy.

Keywords: epilepsy, pentylentetrazol, erythrocyte deformability, erythrocyte aggregation, osmotic fragility, enriched environment

Regulation of neurotransmitter release by new botulinum molecules

Bazbek Davletov

MRC Cambridge, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK

OBJECTIVE: A promising therapeutic potential has been reported in a number of clinical studies for botulinum neurotoxin (BOTOX) which reversibly inhibits release of neurotransmitters at synapses, especially at neuromuscular junctions. Importantly, this neurotoxin does not compromise neuronal well-being and neurotransmission resumes after four months. Recently, botulinum neurotoxin has been approved for treatment of migraine; however, the fear of muscle paralysis restricts the use of native neurotoxin for pain management and other hypersecretory conditions. We recently developed new botulinum molecules which have a more selective action. Specifically, our molecules target CNS neurons but do not cause muscle paralysis. This feature makes our molecules attractive for treatment of chronic secretory disorders with a promise of targeted neuronal silencing.

METHODS:

RESULTS:

CONCLUSIONS:

Keywords: Neurotransmitter and botulinum neurotoxin

Physiology curricula in the Czech Republic

Richard Rokyta

Department of Normal, Pathological and Clinical Physiology, 3rd Faculty of Medicine, Charles University in Prague, Czech Republic

OBJECTIVE: The comparison of the physiological curricula (Physiology and Pathological Physiology) in the Czech Republic in seven Medical Faculties. In the Charles University in Prague, there are five Medical Faculties.

METHODS: Especially the experience with teaching of physiological disciplines in the curriculum of 3 Faculty of Medicine, Charles University in Prague is investigated. The results are compared by statistical methods.

RESULTS: The physiological disciplines are taught from the 1st year of medical study up to the end of the 4th year, it means 8 semesters altogether. The curriculum of the 3rd Faculty of Medicine, Charles University in Prague used a new curriculum. The duration of medical study is 6 years, i.e. 12 semesters. This is composed from three cycles:

I. Basic biomedical sciences - first two years represent first cycle, which is based on the integrative principle.

II. Principles of clinical medicine - the second cycle is concerned on the problem based learning.

III. Clinical preparation - third cycle represents clinical applications.

Physiology - The number of lectures and seminars are 90 – 120 hours. The numbers of hours of practical exercises are 120 - 150.

CONCLUSIONS: “Structure and function of the human body” is taught during the first two years, it means four semesters and is ended by a common examination, which is at the end of the fourth semester. The system is based on the successive teaching starting always with the embryology, then anatomy, histology, biochemistry and finally physiology. As a govern discipline, physiology is taught together with all other disciplines, but always at the end. The students are trained in the practical applications of all disciplines.

Keywords: Physiology, teaching, new curriculum, evidence based medicine, experiences

Hearing duration and auditory reaction time in the blind

Şenol Dane¹, Serap Yildirim², Songül Doğanay², Mustafa Gül², Fatih Bingöl³

¹Fatih Üniversitesi, Tıp Fakültesi, Fizyoloji Anabilim Dalı, Ankara

²Atatürk Üniversitesi, Tıp Fakültesi, Fizyoloji Anabilim Dalı, Erzurum

³Atatürk Üniversitesi, Tıp Fakültesi, Kulak Burun Boğaz Anabilim Dalı, Erzurum

OBJECTIVE: Blindness is a physical disability, causing the lower physical work, and leading to posture problems, orientation difficulties, depression and balance problems. We investigated the hearing duration, an index of auditory acuity, and auditory reaction time in the blind compared to controls.

METHODS: Participants were 40 blind and 30 control children, 10 to 22 years old. To measure the duration of hearing, a device used first by Dane and Bayirli (1998) with tuning fork of 256 Hz and a digital chronometer were used. To measure auditory reaction time, New Test 2000 device and the protocol were applied. The statistical software SPSS 11.0 for Windows was used for statistical analysis.

RESULTS: Auditory reaction time was higher in the blind children compared to controls, or, auditory reaction speed was lower in the blind. Also, hearing duration was lower in the blind children compared to controls. Handedness was not important.

CONCLUSIONS: Blindness may cause some deteriorations in perception and processing of hearing.

Reference:

Dane, Ş., & Bayirli, M. (1998) Correlations between hand preference and durations of hearing for right and left ears in young healthy subjects. *Perceptual and Motor Skills*, 86, 667-672.

Keywords: hearing, reaction time, blindness

Means and standard deviations of auditory reaction time (msec.) and hearing duration (sec.) in sighted and blind children.

	Control		Blind			
Auditory Reaction Time	Mean	SD	Mean	SD	t	p
Right hand	221.6	63.97	333.4	144.47	3.95	0.00
Left Hand	241.07	61.14	328.4	119.39	3.66	0.00
Hearing Duration						
Right ear	87.79	18.86	74.61	26.24	2.33	0.02
Left ear	81.23	18.17	69.12	27.31	2.11	0.03

Handedness, eyedness, and hand-eye crossed dominance in patients with different addictions

Ramazan Yüksel¹, Tijen Şengezer², Nesrin Dilbaz³, Şenol Dane¹

¹Department of Physiology, Medical Faculty, Fatih University, Ankara, Turkey

²Department of Family Medicine, Ankara Numune Training and Research Hospital, Ankara, Turkey

³Department of Psychiatry, Ankara Numune Training and Research Hospital, Ankara, Turkey

OBJECTIVE: Some neurologic and psychiatric disorders such as schizophrenia, depression, autism and migraine are referred to as cerebral lateralization abnormalities. Therefore, cigarette, alcohol, heroin, hashish and drug addictions may be associated with left-handedness, left-eyedness and crossed hand-eye dominance. In this study the possible relationships among handedness, eye dominance, and crossed hand-eye dominance in patients with different addictions mentioned above are investigated.

METHODS: Thirty three patients with cigarette, 35 patients with alcohol, 133 patients with heroin, 117 patients with hashish, 13 patients with drug addictions and 102 age matched controls were included in the study. The patient group included 307 men and 24 women who ranged in age from 15 to 70 years. Handedness was ascertained by using the Edinburgh Handedness Inventory. Eye dominance was measured only by the near-far alignment test. Diagnoses were made on the basis of information provided from clinical interviews and Structured Clinical Interview for DSM-IV.

RESULTS: Patients with heroin and hashish had a significantly increased frequency of left-handedness in comparison with the other patients and controls (chi square=29.36, $p<0.001$). Patients with cigarette, alcohol, heroin and hashish addictions had a significantly increased frequency of left-eyedness in comparison with controls (chi square=25.24, $p<0.01$). Also, patients with cigarette, alcohol, heroin and hashish addictions had a significantly increased frequency of the crossed hand-eye dominance in comparison with controls (chi square=19.11, $p<0.01$).

CONCLUSIONS: Different addictions such as cigarette, alcohol, heroin and hashish may be associated with abnormal handedness distribution and accepted as cerebral lateralization abnormalities.

Keywords: Left-handedness, addiction, hashish, heroin, alcohol

Long-term reactions of glia and neurones after spinal cord lesions in triple-transgenic mice with fluorescent proteins

Fabien Nadrigny¹, Payam Dibaj³, Heinz Steffens², Frank Kirchhoff⁴, Eike D. Schomburg²

¹Inserm U862, Université Victor Segalen, Bordeaux, France

²Institute of Physiology, University of Göttingen, Göttingen, Germany

³Max-Planck-Institute of Experimental Medicine, Neurogenetics, Göttingen, Germany

⁴School of Medicine, Department of Physiology, University of Saarland, Homburg/Saar, Germany

OBJECTIVE: Neuronal regeneration after spinal cord injury may be impeded by a glial scar, but its character and temporal development are still vague.

METHODS: Triple-transgenic mice with labeled neurons and glial cells with different fluorescent proteins (axons/YFP, microglia [MG]/GFP, astrocytes/CFP) were imaged with two photon microscopy before and after laser-induced spinal cord lesions. The lesions were regularly re-inspected for up to one year. The initial laminectomy at spinal cord L4 and re-opening for re-inspections were performed under full volatile anaesthesia. The days after operation, the mice received buprenorphin.

RESULTS: Within minutes after the lesion, MG sent their processes towards the lesion. During the next days, nearby MG cells migrated toward the lesion accumulating and staying there for about a week, then slowly diminishing during the next five months. Astrocytes started to extend processes to the lesion after two days. An astroglial reaction surrounding the lesion site was fully developed after a week and subsequently decreased within five months. Within hours after the lesion, dissected axons formed bulbous debris which was partly engulfed by MG processes. Occasionally, axonal sprouting was detected about three months after injury; they could cross the site of injury, when the glial accumulation had almost vanished.

CONCLUSIONS: Detailed knowledge of the temporal behavior of the main cell types involved in the reaction to spinal cord injury may provide valuable hints for a therapeutical approach to improve axonal survival and regeneration.

Keywords: in-vivo-imaging, microglia, astrocytes, spinal cord lesion, transgenic mice.

Electrophysiological and behavioral demonstration of the restorative action of oxytocin on streptozocin-induced diabetic neuropathy in the rat

Oytun Erbaş, Serdar Demirgören, Gönül Ö. Peker
Ege University Faculty of Medicine, Dept. of Physiology, Izmir

OBJECTIVE: Diabetes mellitus (DM), particularly due to its neurologic complications, is among the major public health problems globally. Oxytocin (OX) has been on the agenda recently, owing to its non-endocrine features, implicating neuroprotection and behavioral serenity. We aimed to identify the development of diabetic peripheral neuropathy (DPN) in the streptozocin (STZ)-induced DPN rat, and then test the possibility of reversing it by OX.

METHODS: Having received Ege-University-Animal-Ethics-Committee's consent, 6-8 w.o. 30 male Sprague Dawley rats were used and 24 were administered a single dose of 60 mg/kg STZ(Sigma-Aldrich), i.p for inducing DM. After 2 days, tail vein blood was drawn to determine plasma glucose levels (PGL). Rats presenting $PGL \geq 250$ mg/dL were included as DM and allowed 20 days for development of DPN;no insulin was applied.At day 0, 20 and 50,all rats were anesthetized(40mg/kg ketamine+ 4mg/kg xylazine,ip), their right hind leg sciatic nerve was stimulated (30-45V; 0.03s) at popliteal muscle and Achilles tendons for submaximal response, and EMG was recorded from 2.-3.interdigital muscles. The latency of M response (compound muscle action potentials=CMAP) and nerve conduction velocity (NCV) were calculated promptly. Inclined plane (IP) and tail flick (TF) tests were also performed.Five groups(n=6/g) were categorized accordingly: G-I) naive control (n=6);G-II) DPN+vehicle/placebo/sham(saline); and G-III),G-IV) and G-V that were administered OX(Synpitan, i.p) 20, 40 and 80 U/kg/day, respectively for 28 days.

RESULTS: NCV; A) %25 decrease in the DPN rats (36.57 ± 3.2 m/sn), compared to naives (50.12 ± 1.36 m/s); B) 47.7 ± 2.7 m/sn in the OX-treated DPN groups, indicating very significant ($p < 0.0001$;G-V) improvement in dose dependent manner. Behavioral assessment: A) IP; profound dysfunction in DPN rats ($69.75 \pm 2.5^\circ$) ($p < 0.005$) compared to naives($75.25 \pm 2.37^\circ$); significant ($p < 0.005$; G-V) reversal; and B) TF: significant deficiency in DPN rats (11.6 ± 0.81 s) compared to naives (7.83 ± 0.75 s), and significant ($p < 0.001$) improvement in the G-V(8.2 ± 0.83 s).

CONCLUSIONS: Our results proved the nerve protecting/restoring effect of OX despite post-deterioration application. Thus, OX may be a potent candidate to restore the sensory and motor features of peripheral nerves following neurodegenerative processes. Histopathological evaluation will also be correlated.

Keywords: EMG, inclined plane, tail flick

Short-Duration Swimming Exercise Decreases Penicillin-Induced Epileptiform Ecog Activity in Rats

Erkut Tutkun¹, Mustafa Ayyıldız², Erdal Agar²

¹Department of Yasar Dogu Physical Education and Sport, University of Ondokuz Mayıs, Samsun, Turkey

²Department of Physiology, Faculty of Medicine, University of Ondokuz Mayıs, Samsun, Turkey

OBJECTIVE: The aim of this study was to determine the impact of different intensities of swimming exercise on penicillin-induced epileptic activity.

METHODS: Adult male Wistar rats weighing 180–220 g composed the material of this study after at least 1 week of acclimatization. Each animal group included seven rats. All animals were adapted to water before the beginning of the experiment. Exercise performed by swimming in two training glass tanks filled with tap water. Animals were swim-exercised for 90 days with 15, 30 and 60 minutes/day. Thereafter, the epileptiform activity was induced by a single microinjection of penicillin (500 units) into the left somatomotor cortex. The electrocorticography activity was continuously monitored on a four-channel recorder.

RESULTS: Short-duration swimming exercise (15 min/day for 90 days) decreased the mean frequency and amplitude of penicillin-induced epileptiform activity in the 70 and 90 minutes after penicillin injection compared to penicillin administered group, respectively. Moderate-duration (30 min/day for 90 days) and long-duration (60 min/day for 90 days) swimming exercise did not alter either the frequency or amplitude of epileptiform activity.

CONCLUSIONS: The data obtained from our experiments showed that moderate duration and long-duration, long-term swimming exercise did not affect either the frequency or amplitude of penicillin-induced epileptiform activity, whereas short-duration, swimming exercise decreased the mean frequency and amplitude of penicillin-induced epileptiform activity in rats. This may suggest that swimming exercise does not increase either severity or duration of epileptic activity in the experimental model epilepsy in rats.

Keywords: ECoG, epilepsy, epileptiform activity, swimming exercise

Age-related alteration in apoptosis processes in hippocampus of male rats. Melatonin and growth hormone could prevent those changes?

Roman Kireev¹, Sara Cuesta², Elena Vara², Jesus A. F. Tresguerres¹

¹Department Physiology, Medical School, University Complutense of Madrid, Spain

²Department Biochemistry and Molecular Biology, Medical School, University Complutense of Madrid, Spain

OBJECTIVE: It has been suggested that the age-related decrease in the number of neurons in the hippocampus that leads to learning and memory alterations may be associated with apoptosis.

METHODS: Twenty-four male Wistar rats of 22 months of age were divided into three groups. One group remained untreated and acted as the control group. The second was treated with growth hormone (GH) for 10 weeks (2 mg/kg/d sc) and the third was submitted to melatonin treatment (1 mg/kg/d) in the drinking water for the same time. A group of 2-months-old male rats was used as young controls. All rats were killed by decapitation at 24.5 month of age. Levels of HSP70 were analysed by ELISA. The expression of pro-apoptotic markers like Bax, Bad, AIF and anti-apoptotic Bcl2, NIAP, XIAP, SIRT 1 and 2 genes were determined by RT-PCR. The protein expressions of Bax, Bad, Bcl-2 and MCL-1 were also studied by Western blot.

RESULTS: Aging was associated with an increase in apoptosis promoting markers and with the reduction of some anti-apoptotic ones. Expressions of SIRT1 and SIRT2 as well as levels of HSP70 were decreased in hippocampus of old rats. GH treatment was able to reduce the pro/anti-apoptotic ratio to levels observed in young animals and to increase SIRT2. Melatonin reduced also expression of pro-apoptotic genes and proteins, and increased levels of MCL-1 proteins and SIRT1.

CONCLUSIONS: We have demonstrated that melatonin and growth hormone can modulate the pro-antiapoptotic ratio and increase sirtuins expression. Our data demonstrated that melatonin and growth hormone could exert a protective effect on hippocampal damage induced by aging.

Keywords: ageing, hippocampus, apoptosis, sirtuins, growth hormone, melatonin

Chronic Central Infusion of Apelin-13 Suppresses Testosterone Levels by Suppressing LH Release in Rats

Suat Tekin¹, Süleyman Sandal¹, Ali Beytur², Fatma Burcu Seker³, Sedat Yildiz¹, Bayram Yilmaz³

¹Department of Physiology, Faculty of Medicine, Inonu University, Malatya, TURKEY

²Department of Urology, Faculty of Medicine, Inonu University, Malatya, TURKEY

³Department of Physiology, Faculty of Medicine, Yeditepe University, Istanbul, TURKEY

OBJECTIVE: Expression of apelin and its receptor, APJ, both in the hypothalamus and testes implicates a role apelin in reproduction. Therefore, the present study was designed to investigate the effects of chronic central infusion of apelin-13 on luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone levels.

METHODS: Male Wistar rats (n=21) were divided into three groups and received either artificial cerebrospinal fluid (vehicle) or apelin-13 at concentrations of 1 or 10 nmol for seven days (via ALZET osmotic mini pumps, 10 µl/h). At the last 90 min of the infusion period, blood samples were collected at 15 min intervals (0, 15, 30, 45, 60, 75 and 90 min.) for LH and FSH analysis. At the last sampling point (90 min), blood samples were analysed for testosterone levels.

RESULTS: Infusion of high dose apelin-13 significantly suppressed LH release compared with the vehicle values at 30, 60 and 75 min. (p<0.05). However, FSH levels did not significantly differ among the groups. Plasma testosterone levels in high dose apelin-13 group were statistically lower than the control group (p<0.05).

CONCLUSIONS: In conclusion, central administration of apelin-13 reduced testosterone release by suppressing LH secretion. Thus, it is suggested that use of apelin receptor antagonists may bring new approaches for the treatment of infertility.

Acknowledgement: This study was supported by Inonu University BAP (Project # 2010/140).

Keywords: Apelin, osmotic icv infusion, LH release, testosteron, rat

Evaluation of acetylcholine esterase activity in the blood of workers exposed to organophosphate and carbamate insecticides by electronic method

Aydin Siddiq Ahmed

Medical Laboratory, Sciences College, Kirkuk - Iraq

OBJECTIVE: The purpose of the present study was to use the modified electrometric technique for measuring blood ChE in workers exposed to the organophosphate and carbamate insecticides in Kirkuk, Iraq.

METHODS: ChE activities in whole blood of male workers (n = 40) who were exposed to organophosphate and carbamate insecticides, for a duration of more than five years and non-exposed healthy male volunteers (n = 12) were measured by an electrometric method; The pH (pH1) of the mixture was measured, and then 0.1 ml of 7.5% of acetylcholine iodide, as a substrate, was added. The reaction mixture was incubated at 37°C for 20 minutes. The pH (pH2) of the reaction mixture was measured after the end of the incubation period. Enzyme activity was expressed as $\Delta\text{pH}/20 \text{ min} = [\text{pH1} - \text{pH2} - (\Delta\text{pH of the blank})]$. The blank was without the blood sample.

RESULTS: Mean values of ChE activities ($\Delta\text{pH}/20 \text{ min}$) in the whole blood of healthy non-exposed subjects and workers were 1.41 and 1.2, respectively. Ten minutes after in vitro addition of quinidine sulfate to inhibit pseudo cholinesterase activity in the blood, the estimated true cholinesterase activities in exposed workers and non-exposed individuals were 0.08 and 0.07 $\Delta\text{pH}/20 \text{ min}$, respectively. Whole blood ChE activities of the exposed workers were significantly lower than those of healthy individuals.

CONCLUSIONS: These findings are the first collective report of human and whole blood cholinesterase activities as determined by the modified electrometric method and extend the usefulness of this method by detecting ChE inhibition after exposing to the organophosphate and carbamate insecticides.

Keywords: Cholinesterase, organophosphate, workers, electrometric method

Vagal response to the Deep Breathing test at young footballers

Souad Aboudrar¹, Hanane Rkain¹, Laila Errguig¹, Youssef Radjab¹, Latefa Oukerraj⁵, Mohamed Azzaoui³, Leslie Coghlan⁴, Mohamed Cherti⁵, Taoufiq Dakka¹, Halima Benjelloun²

¹Université Mohammed 5 Souissi, Laboratoire de Physiologie, Faculté de Médecine et de Pharmacie de Rabat, Maroc.

²Unité d'exploration du Système Nerveux Autonome, Service Cardiologie A, CHU Ibn Sina de Rabat, Maroc

³Institut National des Sports Moulay Rachid – Salé, Maroc

⁴Al Akhawayn University, School of Science and Engineering, Ifrane, Morocco.

⁵Université Mohammed 5 Souissi, Service de Cardiologie B Faculté de Médecine et de Pharmacie de Rabat, Maroc.

OBJECTIVE: Deep breathing test is a simple and reproducible cardiovascular reflex test to assess parasympathetic function. The aim of this study was to compare the vagal response of a group of young footballers with that of a group of age-matched untrained normal subjects, using this test.

METHODS: Deep breathing test was performed in 2 groups: one group of 20 adult young footballers (average 19.3 ± 0.6 years), and a second age-matched group of 20 untrained subjects (average 19.6 ± 0.6 years). Subjects underwent the test after 30 min resting in supine position, and responses were expressed as a percentage of variation of the heart rate during the stimulation. Student's t-test was used for each of the parameters, in order to evaluate statistical differences among the two groups. $p < 0.05$ was considered as significant

RESULTS: Vagal response to deep breathing test was significantly higher in the young footballers when compared to the untrained controls (72.6 ± 16.2 % vs 55.0 ± 12.8 %, respectively, $p = 0.03$). The basal heart rate was significantly lower in footballers than in the controls group (52.1 ± 7.4 beats/min vs 69.8 ± 14.3 beats/min, $p < 0.01$).

CONCLUSIONS: Our data point to a significant increase in basal parasympathetic response and a significant decrease of basal heart rate in adult young footballers compared to controls.

Keywords: Deep breathing test; parasympathetic response; autonomic nervous system; sudden death; young footballers

Effect of KATP channel blockade on the electrophysiological stability of the myocardium submitted to acute regional ischemia. Study in isolated rabbit heart

Luis Such Belenguer¹, Carlos Soler¹, Antonio Manuel Alberola¹, Germán Parra¹, Laia Brines², Nathalia Gallego¹, Manuel Zarzoso¹, Irene Del Canto², Juan Guerrero³, Francisco Javier Chorro², Carmiña Rams¹, Luis Such Miquel⁴

¹Department of Physiology, Universitat de València, Valencia. Spain

²Hospital Clínico Universitario-Incliva, Valencia, Spain

³Department of Electronic Engineering, Universitat de València, Valencia. Spain

⁴Department of Physiotherapy, Universitat de València, Valencia. Spain

OBJECTIVE: It has been reported that blockade of IKATP current exerts a protective effect against arrhythmias in ischemic myocardium. Nevertheless, authors suggest that the opening of this channel protects ischemic myocardium. We have investigated the effect of glibenclamide, an IKATP blocker, on electrophysiological stability by analyzing the signal regularity of induced ventricular fibrillation (VF), in isolated hearts subjected to regional ischemia.

METHODS: Eighteen NZW rabbits were anaesthetized (ketamine, 10 mg/kg i.v.), killed and the hearts excised, isolated and perfused in a Langendorff system. A pacing electrode and a plaque with 256 recording electrodes were positioned on the left ventricle. VF was induced by pacing, maintaining the perfusion. The dominant frequency of VF was obtained by a spectral analysis. The spectrum concentration (percentage of the total power spectrum, contained in a range of ± 1 hertz around the dominant frequency) as an index of the signal regularity was determined. Five minutes after VF induction, the circumflex coronary artery occlusion (CAO) was performed. Measurements were obtained before and five minutes after CAO. Glibenclamide (10 μ M) was infused (treated group), through the aortic root. A two-factor ANOVA with repeated measures on one factor was used for comparisons.

RESULTS: Spectral concentration (SC) decreased ($p < 0,05$) after CAO, in control ($18 \pm 3\%$ vs. $24 \pm 5\%$; $n=9$) but not in treated group. SC was higher ($p < 0,05$) in treated than control group after CAO ($25 \pm 4\%$ vs. $18 \pm 3\%$; $n=9$).

CONCLUSIONS: KATP channel blockade prevents against fibrillatory signal deterioration due to ischemia. This observation reinforces the idea that the blockade of this channel has a beneficial electrophysiological effect.

Keywords: IKATP current, myocardial electrophysiology, acute ischemia, rabbit heart

Effects of folate supplementation on hyperhomocysteinemia and lipid profile in ovariectomized rats

Hanan Fathy Alsaaid, Mona Gamal El Din Anan, Taysier Mohammed Mandour, Soad Abd El Hamid Kandil, May Mohammed Farrag
Department of Physiology, Faculty of Medicine, AlAzhar University, Egypt

OBJECTIVE: The present study aims to clarify the importance of folate and its effect on the level of plasma lipid, lipoproteins and homocysteine (Hcy) in ovariectomized rats, since adequate folate supplementation may result in reduction of hyperhomocysteinemia, which is a significant risk factor for atherosclerotic vascular disease

METHODS: The present study conducted on thirty sexually mature female rats. Bilateral ovariectomy was done to 20 rats, to ensure surgical menopause. Two weeks later, the rats were divided into 3 equal groups, a group of intact rats (control A), a group of ovariectomized rats (control B) and a group of ovariectomized rats that was treated with folic acid (0.0019 %, orally) for four weeks. Blood samples were then collected for plasma separation. Plasma total homocysteine and lipid profile were determined.

RESULTS: In ovariectomized rats, folic acid significantly reduced total homocysteine and induced significant improvement in the disturbances resulted from ovariectomy on the plasma lipid and lipoproteins. This was manifested by significant decrease in plasma triglyceride (TG), total cholesterol (TC) and low density lipoprotein cholesterol (LDLc) levels, but it has a non significant increasing effect on plasma levels of high density lipoprotein cholesterol (HDLc), in comparison to ovariectomized rats. Moreover, the total plasma homocysteine levels were significantly correlated to plasma TC and LDLc.

CONCLUSIONS: This study favors the view that after menopause, homocysteine level increases significantly and a simple, non toxic and relatively inexpensive folic acid intervention might be useful in primary cardiovascular prevention in postmenopausal women.

Keywords: Hyperhomocysteinemia – Folate – Lipids – Lipoproteins - Ovariectomy – Rats

Both mitochondrial K(ATP) channel opening and sarcolemmal K(ATP) channel blockage confer protection against ischemia/reperfusion-induced arrhythmias in anesthetized male rats

Gonca Ersoz¹, Ömer Bozdoğan²

¹Biology Department, Faculty of Arts and Sciences, Zonguldak Karaelmas University, Incivez/Zonguldak, Turkey

²Biology Department, Faculty of Arts and Sciences, Abant İzzet Baysal University, Gököy/Bolu, Turkey

OBJECTIVE: This study was performed to assess the relative effect of sarcolemmal and mitochondrial ATP-dependent K⁺ channel (KATP) modulation on ischemia reperfusion (I/R)-induced arrhythmias in different gender of rats. We compared the effect of a selective sarcolemmal KATP blocker, HMR 1098; a selective mitochondrial KATP opener, diazoxide; a nonselective KATP opener, pinacidil and the combination of pinacidil with HMR 1098 on the incidence and duration of ventricular arrhythmias in 2 groups: anesthetized males (n = 31) and females (n = 31).

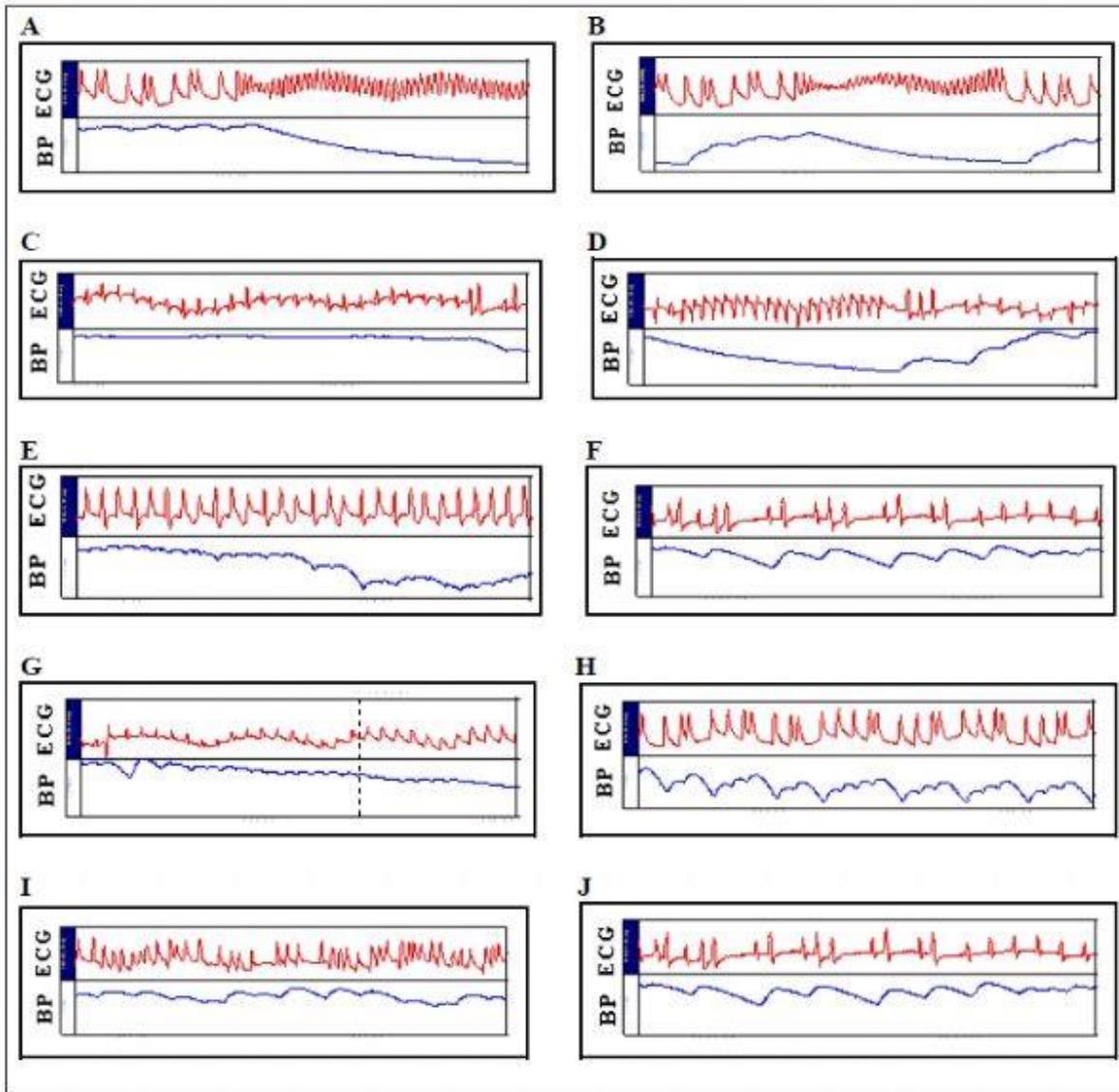
METHODS: Ischemia and reperfusion was produced by occluding the left main coronary artery of anesthetized Sprague-Dawley rats for 6 minutes followed by re-opening of the artery for 6 minutes.

RESULTS: The arrhythmia score and the duration of arrhythmias were significantly reduced by HMR 1098, diazoxide, and pinacidil in male rats. The combination of pinacidil with HMR 1098 did not change the antiarrhythmic effect of pinacidil (Arrhythmia scores: HMR 1098, 1.5±0.2; diazoxide, 1.6±0.4; pinacidil, 1.1±0.3; and pinacidil+HMR 1098, 1.5±0.2 vs control, 3.1±0.6; P<0.05). The duration of arrhythmias was shorter in females. Drug treatments were not effective in decreasing arrhythmias in female groups to the same extent as in the male group.

CONCLUSIONS: Results of the current study indicate that both mitochondrial KATP activation and sarcolemmal KATP inhibition exert antiarrhythmic action in male rats. The antiarrhythmic effect of pinacidil does not depend on the sarcolemmal KATP opening. These results also indicate that KATP modulators show no discernable effect in female rats due to the already low incidence of arrhythmias in females.

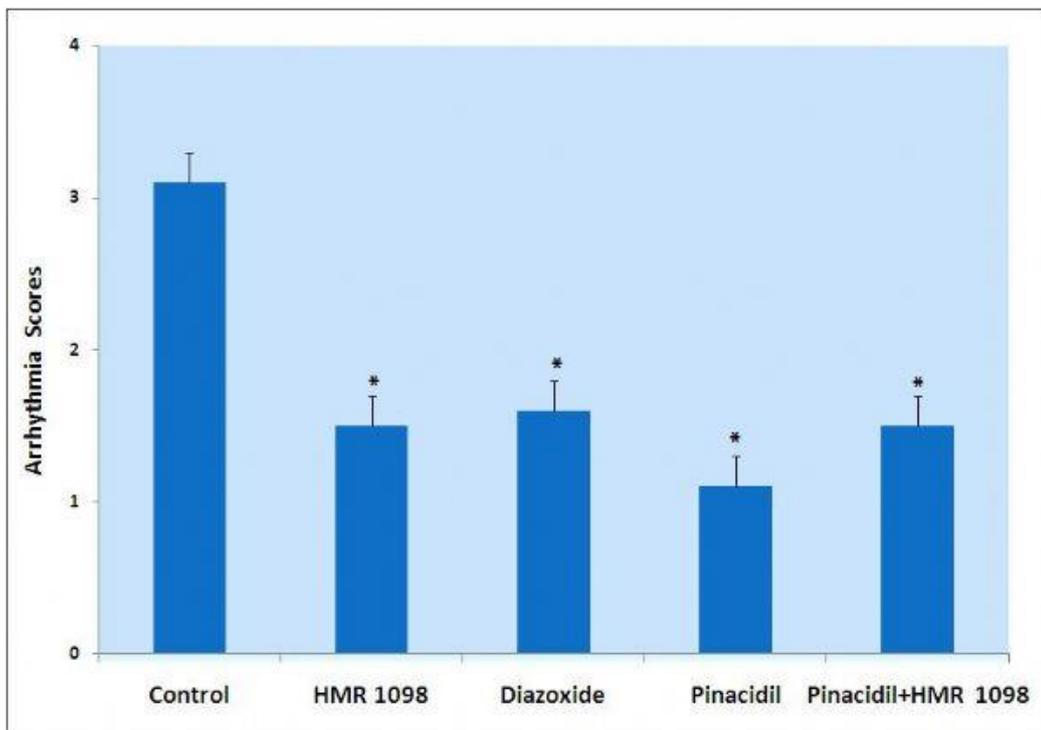
Keywords: HMR 1098, diazoxide, pinacidil, K(ATP) channel, ischemia reperfusion arrhythmia

Figure 1. Original electrocardiogram (ECG) and blood pressure (BP) recordings.



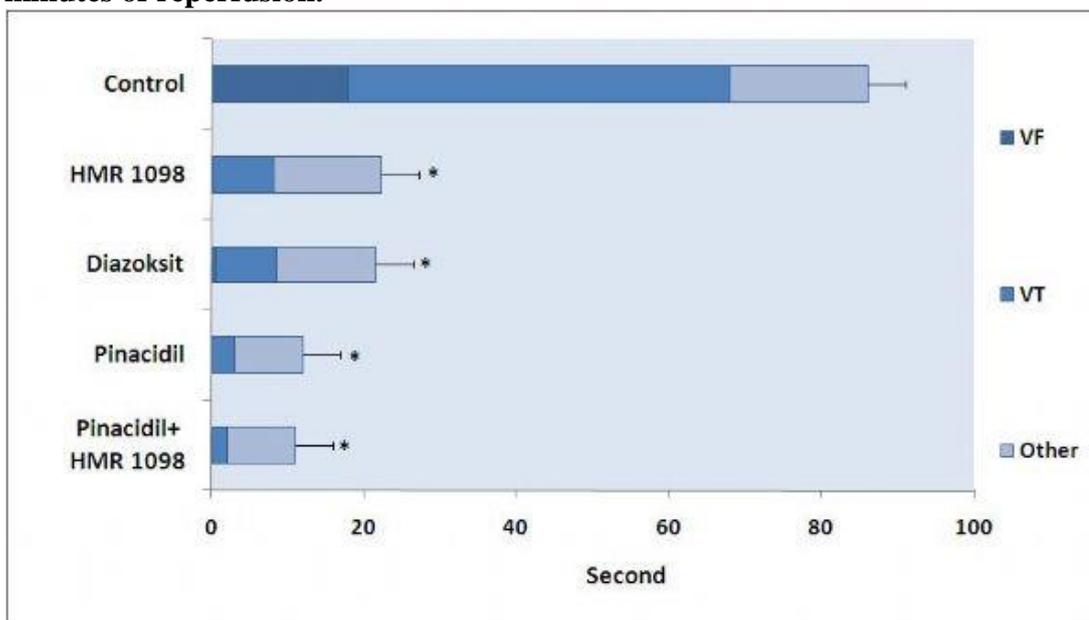
(A) a control anesthetized male rat, (B) a control female rat, (C) an HMR-1098-treated male rat, (D) an HMR-1098-treated female rat, (E) a diazoxide-treated male rat, (F) a diazoxide-treated female rat, (G) a pinacidil-treated male rat, (H) a pinacidil-treated female rat, (I) a pinacidil- and HMR-1098-treated male rat, (J) a pinacidil- and HMR-1098- treated female rat.

Figure 2. The effect of drug treatments on the arrhythmia score in male group during 6 minutes of reperfusion.



Values represent means + standard deviation. * $P < .05$ compared to control.

Figure 3. The effect of drug treatments on the total length of arrhythmias in male group during 6 minutes of reperfusion.



Values represent means + standard deviation. * $P < .05$ compared to control.

Table 1. The heart rate (HR) and mean arterial blood pressure (MBP) during 6 min. of ischemia and reperfusion in male rats

Subgroup	n	Basal HR	Basal MBP	Occlusion n 1 min. HR	Occlusion n 1 min. MBP	Occlusion n 5 min. HR	Occlusion n 5 min. MBP	Reperfusion n 5 min. HR	Reperfusion n 5 min. MBP
I.Control	7	387±17	108±5	388±16	97±4	375±26	104±5	422±43	94±6
II.HMR 1098	6	412±10	123±10	408±11	83±13	381±25	76±15	396±8	104±10

III. Diazoxide	6	405±15	98±7	408±17	73±10	424±23	91±13	380±27	111±6
IV. Pinacidil	6	420±22	108±10	410±17	83±15	415±20	94±15	417±17	99±10
V. Pinacidil+HMR 1098	6	408±15	114±10	415±19	95±6	400±16	109±3	406±16	118±2

N: The number of survived animals after 6 min. of reperfusion.

Table 2. The heart rate (HR) and mean arterial blood pressure (MBP) during 6 min. of ischemia and reperfusion in female rats

Subgroup	N	Basal HR	Basal MBP	Occlusion 1 min. HR	Occlusion 1 min. MBP	Occlusion 5 min. HR	Occlusion 5 min. MBP	Reperfusion 5 min. HR	Reperfusion 5 min. MBP
I. Control	7	407±17	116±8	420±14	107±27	419±18	117±9	419±16	117±7
II. HMR 1098	6	425±35	127±6	416±14	98±25	376±29	95±14	376±31	103±16
III. Diazoxide	6	416±35	112±7	398±16	86±26	404±19	98±13	400±16	95±10
IV. Pinacidil	6	398±38	109±11	404±17	95±27	409±22	108±10	406±14	111±11
V. Pinacidil+HMR 1098	6	444±42	117±7	438±12	83±44	441±15	110±12	472±40	96±18

N: The number of the survived animals after 6 min. of reperfusion.

Table 3. The effect of sex on the duration of arrhythmias during 6 min of ischemia and reperfusion (Results: mean ± SE).

Group	n	Are at Risk (% of total)	Occlusion VF	Occlusion VT	Occlusion Other	Occlusion Total	Reperfusion arrhythmic period (s)	Reperfusion VF	Reperfusion VT	Reperfusion Other	Reperfusion Total	Reperfusion arrhythmia scores
Male Control	7	45±1	0	0	3.4±1.8	3.4±1.8	300±31	18±14	50±22	18±2	86±34	3.1±0.5
Female Control	7	48±2	0	0	0.4±0.1*	0.4±0.1*	149±36*	0*	16±3*	19±4	35±6*	2.0±0.2*

*N: The number of survived animals after 6 min of reperfusion. VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: extrasystoles, salvos, and/or bigeminy, Total: the total length of VF, VT and other type of arrhythmias, *P<0.05: Compared to male.*

Table 4. The effect of drug treatments on the duration of arrhythmias during 6 min of ischemia and reperfusion in male rats

Subgroup	N	Zone at risk (% of total)	Occlusion VF	Occlusion VT	Occlusion Other	Occlusion Total	Rep arrhythmic period	Reperfusion VF	Reperfusion VT	Reperfusion Other	Reperfusion Total	Reperfusion Arrhythmia Score
I.Control	7	45±1	0	0	3.4±1.8	3.4±1.8	300±31	18±14	50±22	18±2	86±34	3.1 ± 0.6
II.HMR 1098	6	49±1	0	0	0	0	150±36*	0.3±0.3*	8±3**	14±4	22.3±7*	1.5 ± 0.2*
III.Diazoxide	6	47±3	0	0	2.7±1.7	2.7±1.7	157±35*	0.5±0.5*	8±3**	13±2	21.5±6*	1.6 ± 0.4*
IV.Pinacidil	6	46±3	0	0	0	0	125±55**	0*	3±2**	9±4	12±4**	1.1 ± 0.3*
V.Pinacidil +HMR 1098	6	48±4	0	0	0.3±0.3	0.3±0.3	115±27**	0*	2±1**	9±2	11±2**	1.5 ± 0.2*

N: The number of the survived animals after 6 min of reperfusion. *P<0.05; **P<0.01: Compared to control.

Table 5. The effect of drug treatments on the duration of arrhythmias during 6 min of ischemia and reperfusion in female rats

Subgroup	N	Zone at risk (% of total)	Occlusion VF	Occlusion VT	Occlusion Other	Occlusion total	Rep arrhythmic period	Rep VF	Rep VT	Rep Other	Rep Total	Rep arrhythmia score
I.Control	7	48±2	0	0	0.4±0.1	0.4±0.1	149±36	0	16±3	19±4	35±6	2.0 ± 0.2
II.HMR 1098	6	49±6	0	0	1.0±0.4	1.0±0.4	181±50	0	9±4	16±6	24±8	1.7 ± 0.4
III.Diazoxide	6	48±3	0	0	1.5±0.8	1.5±0.8	125±81	0	4±1	11±3	15±4	1.3 ± 0.3
IV.Pinacidil	6	50±4	0	0	2.4±1.4	2.4±1.4	166±42	0	10±4	21±9	31±9	2.0 ± 0.4
V.Pinacidil+HMR 1098	6	49±3	0	0	0.8±0.4	0.8±0.4	96±34	0	5±2	7±3	12±4*	1.3 ± 0.3

N: The number of the survived animals after 6 min of reperfusion. *P<0.05; Compared to control.

Contrasting ventilatory actions of centrally administered tachykinins, pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal peptide in trout

Jean Claude Le Mével¹, Frédéric Lancien¹, Nagi Mimassi¹, John Michael Conlon²

¹Université Européenne de Bretagne. Université de Brest; INSERM U650, Laboratoire de Neurophysiologie, IFR 148 ScInBioS, CS 93837, 29238 Brest Cedex 3; CHU de Brest, France.

²Department of Biochemistry, Faculty of Medicine and Health Sciences, United Arab Emirates University, 17666 Al Ain, United Arab Emirates.

OBJECTIVE: Little is known regarding the central action of tachykinins, pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) on ventilation in fishes. Therefore, this study was carried out to investigate in our experimental model, the unanesthetized rainbow trout *Oncorhynchus mykiss* whether these neuropeptides are involved centrally in the control of ventilation.

METHODS: In group 1 trout, we investigated the effects of intracerebroventricular (ICV) administration of vehicle (0.5 μ l, = 16) or 25-100 pmol (in 0.5 μ l) of trout neuropeptide gamma (NP γ , n=8-9), substance P (SP, n=8-10), neurokinin A (NKA, n=7-10). Group 2 trout were injected ICV with vehicle (n=16), trout PACAP (n=8-11) and also VIP (n=8-11). Buccal ventilatory pressure was used to calculate ventilation frequency (VF) and ventilation amplitude (VA).

RESULTS: ICV vehicle, SP and NKA were without effect on the ventilatory parameters but NP gamma evoked a significant ($P < 0.05$) and dose-dependent elevation of VF but a reduction of VA. The net effect of NP gamma was to produce an hypoventilatory response since the total ventilation ($VTOT=VF*VA$) was significantly reduced by 60% at a dose of 100 pmol. In contrast, only PACAP significantly elevated VF and VA, but PACAP and VIP significantly increased VTOT. At a dose of 100 pmol, PACAP and VIP increased VTOT by 300 % and 100 % respectively. Intra-arterial injections of NP gamma, SP, NKA, PACAP or VIP were without any effect on the ventilatory variables.

CONCLUSIONS: Our results suggest that endogenous central NP gamma, PACAP and VIP are components of a cocktail of brain neuropeptides that are differentially implicated in the neuroregulatory control of ventilation in fish.

Keywords: brain, tachykinins, PACAP, VIP, ventilation, fish.

The effects of curcumin treatment to responses of rat aorta: Role of nitric oxide

Kısmet Esra Nurulloğlu Atalık¹, Hakkı Gökbel², Mehmet Öz², Nilsel Okudan², Muaz Belviranlı², Hasan Esen³

¹Department of Pharmacology Meram Faculty of Medicine, Selçuk University, Konya, Turkey

²Department of Physiology Meram Faculty of Medicine, Selçuk University, Konya, Turkey

³Department of Pathology Meram Faculty of Medicine, Selçuk University, Konya, Turkey

OBJECTIVE: The aim of the study was to evaluate the effects of curcumin treatment on the vascular responses to 5-hydroxytryptamine (5-HT) and potassium chloride (KCl), both of which cause receptor-dependent and receptor-independent contractions, respectively and also to examine the role of nitric oxide in these responses.

METHODS: The study protocol was approved by the Ethics Committee of the Selçuk University Experimental Medicine Research and Application Center. The rats were randomly divided into two groups: Control and curcumin-treated (200 mg/kg/day, p.o., for 4 weeks). After four weeks rats were sacrificed by cervical dislocation. The descending thoracic aorta was quickly isolated, cleaned, and sectioned into 3- to 4-mm-long rings. The rings were then placed in organ baths. Changes in isometric tension were recorded by a force-displacement transducer. Starting from each aorta, a single ring was sent for pathological research.

RESULTS: The concentration response curves to 5-HT (10^{-9} - 3×10^{-4} M) and KCl (5-100 mM) were shifted to the right and the maximal response was significantly decreased in curcumin-treated rat aortas. Pretreatment of rings with L-NAME (a NOS inhibitor, 10^{-4} M) was increased both in the sensitivity and maximal response to only 5-HT. No apparent histological changes were demonstrated in smooth muscle and connective tissue layers in the aortas of control and curcumin-treated rat preparations.

CONCLUSIONS: The results of the present study suggest that pretreatment with curcumin decreases the sensitivity and maximal response to both 5-HT and KCl, and in curcumin-treated rat aorta NO release from endothelial cells modulates contraction induced by 5-HT, but not by KCl.

Keywords: Aorta, contractions, curcumin, KCl, nitric oxide, serotonin

Relationships among PPAR γ agonists, β 1 blockers, oxidative stress and endothelial dysfunction in diabetes mellitus-experimental study

Joanta Adela Elena, Batar Sergiu, Remus Moldovan, Nicoleta Decea, Adriana Muresan
Department of Physiology, University of Medicine and Pharmacy, Cluj-Napoca, Romania

OBJECTIVE: Oxidative stress may occur as a result of increased free – radical generation and/or to a decrease in antioxidant defense mechanism. For instance, in diabetes mellitus endothelium-dependent relaxation may be impaired by an excess generation of superoxide, which destroys nitric oxide. The aim of our research was to establish a possible relationship among peroxisome proliferator- activated receptor (PPAR) gamma agonist, β 1 blockers, oxidative stress and endothelial dysfunction in diabetic animals.

METHODS: Experiments were performed on adult male Wistar rats divided in five groups (n=10 / per group) as follows: group I: control group, group II: streptozotocin -induced diabetic rats, group III: diabetic rats treated with β 1 blocker (Nevibolol), group IV: diabetic rats treated with PPAR gamma agonist (Pioglitazone) and group V: diabetic rats treated with PPAR gamma agonist and β 1 blocker. Animals were sacrificed under ether anesthesia after 30 days and aortic segments and blood were taken in order to assess the oxidative stress markers (malondialdehyde, protein carbonyl groups), antioxidant defense (thiol groups, superoxide dismutase) and endothelial dysfunction (nitrates).

RESULTS: There was a significant decrease ($p < 0.001$) in malondialdehyde levels due to both Nevibolol and Pioglitazone administration (0.6 ± 0.2 nmol/mg protein) compared to the diabetic animals (3.85 ± 1.8 nmol/mg protein), diabetic and Nevibolol treated animals (1.9 ± 0.5 nmol/mg protein), diabetic and Pioglitazone treated animals (1.4 ± 0.4 nmol/mg protein). A significant decrease ($p < 0.001$) in protein carbonyl groups levels in animals treated with both Nevibolol and Pioglitazone (1.6 ± 0.4 nmol/mg protein) was seen compared to the diabetic animals (9.3 ± 3.1 nmol/mg protein), diabetic and Nevibolol treated animals (4.1 ± 2.4 nmol/mg protein), diabetic and Pioglitazone treated animals (3.9 ± 1.6 nmol/mg protein). Blood antioxidant defense assessment revealed a significant increase ($p < 0.001$) in thiol groups levels in animals treated with both Nevibolol and Pioglitazone (4 ± 2.6 μ mol/ml) as compared to the: diabetic animals (1.4 ± 0.3 μ mol/ml), diabetic and Nevibolol treated animals (2.4 ± 1.2 μ mol/ml), diabetic and Pioglitazone treated animals (2.8 ± 1.1 μ mol/ml). Superoxide dismutase activities significantly increased ($p < 0.005$) in animals treated with both Nevibolol and Pioglitazone (10.10 ± 3.6 U/mg protein) compared to the diabetic animals (4.66 ± 2.4 U/mg protein), diabetic and Nevibolol treated animals (7.8 ± 3.4 U/ mg protein), diabetic and Pioglitazone treated animals (8.3 ± 3.1 U/mg protein). Blood nitrates levels significantly increased ($p < 0.001$) in Nevibolol + Pioglitazone (0.6 ± 0.2 nmol/ml) treated animals compared to the: diabetic animals (0.1 ± 0.04 nmol/ml), diabetic and Nevibolol treated animals (0.2 ± 0.02 nmol/ml), diabetic and Pioglitazone treated animals (0.4 ± 0.1 nmol/ml).

CONCLUSIONS: Our findings suggested that PPAR gamma agonist and β 1 blocker improved antioxidant defense decreased oxidative stress markers and normalized endothelium – dependent relaxation by increasing nitric oxide bioavailability via a reduction in the level of vascular reactive oxygen species.

Keywords: PPAR γ , β 1 blockers, oxidative stress, endothelial dysfunction, diabetes mellitus

Ghrelin ameliorates acute stress induced oxidative damage: role of atosiban

Özgür Çakır Kasımay¹, Dilek Özbeyli³, Gazi Contuk², Şule Çetinel², Berrak Ç. Yeğen¹

¹Marmara University School of Medicine, Departments of Physiology, Istanbul, Turkey

²Marmara University School of Medicine, Histology and Embryology, Istanbul, Turkey

³Marmara University Animal Center, Istanbul, Turkey

OBJECTIVE: Ghrelin, a peptide released from stomach with potent effects on appetite, also mediates behavioral responses to stress. The aim was to investigate the effect of ghrelin on stress-induced oxidative injury.

METHODS: Female Sprague-Dawley rats were exposed to an acute psychological 30-min water avoidance stress (WAS). Ten minutes before WAS, either glucocorticoid-receptor antagonist (RU-486, 10 mg/kg, ip) or oxytocin-receptor antagonist (atosiban, 1 mg/kg, ip) was injected, and 5 min later rats were injected with either ghrelin (10 ng/kg) or saline. After cessation of WAS, rats were decapitated to obtain gastric and colonic samples for the measurement of lipid peroxidation (LP), glutathione (GSH) levels, myeloperoxidase (MPO) activity, and for histological analysis. For statistical analysis Student's t test or ANOVA was used.

RESULTS: Compared to control group, LP levels and MPO activities were increased in both tissues of saline-treated stress group ($p < 0.05-0.001$), which were reversed in the ghrelin-treated stress group ($p < 0.05-0.001$). Colonic and gastric LP and MPO activities were increased in atosiban pre-treated groups as compared to ghrelin-injected stress group ($p < 0.05-0.001$). Gastric GSH levels, which were depleted in stressed and atosiban-pre-treated groups, were replenished in ghrelin-injected stress group ($p < 0.05$). Histological analysis verified stress-induced damage in both tissues with significant reductions in damage scores of ghrelin-treated stress group and exaggerated damage in atosiban- and RU-486-treated stress groups.

CONCLUSIONS: Ghrelin ameliorated stress-induced gastric and colonic oxidant damage, which was worsened by blocking oxytocin receptors. Thus, it appears that the protective effect of ghrelin against stress-induced oxidative damage involves the activation of oxytocin receptors.

Keywords: stress, ghrelin

Protective Effect of Carvacrol on Cyclophosphamide-Induced Hematotoxicity in Rats

Adnan Ayhanci¹, Ruhi Uyar², Husnu Can Baser³, Betül Peker Cengiz⁴, Yılmaz Altuner⁵

¹Department of Biology, Faculty of Arts and Science, Eskisehir Osmangazi University, Eskisehir, Turkey

²Department of Physiology, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey

³Department of Pharmacognosy University of Anadolu Faculty of Pharmacy, Eskisehir, Turkey

⁴Department of Pathology, Yunusemre State Hospital, Eskisehir, Turkey.

⁵Department of Mudwifery, Faculty of Karabuk University, School of Health, Eskisehir, Turkey

OBJECTIVE: Cyclophosphamide (CP) is a widely used antineoplastic drug that causes toxicity in the normal cell due to its metabolites. The major drawback of this drug is an undesirable myelosuppression. The possible protective effects of Carvacrol (Car) against CP-related toxicity of blood cells and bone marrow of rats were investigated in this study.

METHODS: Male Sprague Dawley were divided into ten groups. The groups that had CP treatment alone were killed 3 days after the CP injection. For the groups having CP+Car, Car (5 or 10 mg/kg i.p) administration was started 3 days earlier than the CP administration and continued to the end of the experiment (6 days). On day 4, the animals were weighed again, relative doses of CP were estimated, and CP+Car were administered together. A vehicle-treated control group was also included. On day 7, blood samples were collected and bone marrows of animals were resected under anesthesia.

RESULTS: Intraperitoneal (i.p) administration of 50, 100, or 150 mg/kg of CP caused, in a dose-dependent manner, reductions in the number of leukocytes, thrombocytes, and bone marrow-nucleated cells. Car protects the animals from the toxic effect of CP. The best recovery was obtained when 5 mg/kg of Car was given in combination with 50 mg/kg of CP.

CONCLUSIONS: CP is toxic to bone marrow, leukocytes, and platelets. However, the toxicity depends on the dose of the drug. Our findings suggest that at the appropriate concentration, Car could be a potentially effective drug in the treatment of CP-induced damage and could be used in the prevention and treatment of CP toxicity.

Keywords: Cyclophosphamide, Carvacrol, Hematotoxicity, Cytoprotectivity, Rat

Tissue Differentiation using Surface-Enhanced Raman Scattering

Mustafa Culha¹, Omer Aydin¹, Mehmet Kahraman¹, Murat Altas¹, Omer Faruk Bayrak¹, Ertugrul Kilic²

¹Yeditepe University, Faculty of Engineering and Architecture, Department of Genetics and Bioengineering, Istanbul, Turkey

²Yeditepe University, Faculty of Medicine, Department of Physiology, Istanbul, Turkey

OBJECTIVE: Surface-enhanced Raman scattering (SERS) is powerful spectroscopic technique which is routinely used for material characterization. The technique has advanced to a point where it can now be used in medicine for clinical decision making as well. Its power can be attributed to its fingerprinting property, low sensitivity to water, minimum sample preparation step and increased sensitivity with the recent developments in instrumentation. In this study, we demonstrate the capability of SERS for tissue differentiation for clinical applications.

METHODS: Tissue samples obtained from model animals and human subjects were quickly frozen by submerging in liquid nitrogen and crashed to homogenize. Then, these samples were mixed with concentrated colloidal silver nanoparticles (AgNPs). A 2 μ L of this mixture was spotted on CaF₂ substrate and let it dry at room temperature. The SERS spectra were acquired from a Raman spectrometer (Renishaw InVia Flex, England) equipped with an 830 nm laser. The spectra obtained from tissues were comparatively evaluated to distinguish their disease status.

RESULTS: First, tissue samples from several different rat organs such as brain, kidney and heart were analyzed. It was found that the technique can provide valuable molecular level information about the physiological function of the organ. Second, the tissue and tumor samples from patients undergoing brain surgery were evaluated for possible differentiation without going through conventional diagnosis methods. It was again found that the technique can identify the healthy tissue and tumor, even the peripheral tissue surrounding the tumor.

CONCLUSIONS: In this study, we first time in literature demonstrated the applicability of SERS for tissue differentiation. Further, brain tumors and tissue surrounding the tumor through the healthy tissue can be identified quickly. This suggests that the technique can be used as decision making tool even during the surgery without going through lengthy procedures.

Keywords: SERS, tissue, brain tumor, differentiation

Increased acetylcholine sensitivity in abdominal subcutaneous arteries from obese patients

Emil Toft Brøndum¹, Morten Borg¹, Peter Funch Jensen², Per Ivarsen³, Christian Aalkjaer¹

¹NeuroSearch A/S and Inst. of Biomedicine, Aarhus University, Denmark

²Clinical Inst. Aarhus University, Denmark

³Dept of Renal Medicine, Universityhospital, Skejby, Denmark

OBJECTIVE: Patients with obesity have an increased risk for cardiovascular disease, possibly related to endothelial-dysfunction. This study investigates the acetylcholine mediated relaxation in subcutaneous resistance arteries from obese patients (BMI > 40) undergoing gastric-bypass operation or undergoing surgery for non-cardiovascular related reasons (i.e. kidney transplant donors).

METHODS: Arteries dissected from biopsies taken from 12 control and 12 obese matched for age and sex were mounted in an isometric myograph. Endothelium-dependent relaxation was investigated by increasing concentrations of acetylcholine on noradrenaline pre-contracted vessels. The EDHF-like response was investigated in the presence of eNOS and COX inhibitors and pharmacological modulators of the pathways involved in the EDHF response.

RESULTS: There was no difference between the groups in maximal relaxation under control conditions (86±6 and 89±3 %, obese and control, respectively). However, the sensitivity to acetylcholine was significantly higher in obese (logEC50 was -7.4±0.1 and -7.1±0.1, obese and control, respectively (P=0.02)). Also after blocking eNOS and COX the sensitivity to acetylcholine was higher in obese (logEC50 -6.6±0.1 and -6.2±0.1 (P=0.02)) while maximal relaxation was not different (61±9 and 70±4 % (P>0.5), obese and control, respectively). Treatment with the IK and SK channel opener NS309 (1µM) abolished the difference in sensitivity (logEC50 -7.3±0.1 and -7.2±0.2) while increasing maximal relaxation (77±4 and 79±14 %, n= 11 and 3) obese and control, respectively.

CONCLUSIONS: Subcutaneous resistance arteries from obese patients seem to have increased sensitivity to the endothelial specific vasodilator acetylcholine, also after blockade of the eNOS and COX dependable pathways.

Keywords: EDHF, obesity, resistance arteries

Histopathologic Evaluation of the Effect of Low-energy Laser Therapy for Subacute Healing of Surgical Wounds Primarily Closed with Different Suture Material

Onur Uzun¹, Ünal Uslu², Alev Cumbul², Bahar Gürsoy¹

¹Department of Oral and Maxillofacial Surgery, Marmara University, Faculty of Dentistry, İstanbul, Turkey

²Department of Histology and Embryology, Yeditepe University, Faculty of Medicine, İstanbul, Turkey

OBJECTIVE: The aim of this research is the histological evaluation of the effectiveness of 7-day treatment with Low-energy Laser at different power levels on the healing of surgically induced surgical wounds on rats that were closed with three different suture materials.

METHODS: Eighteen Wistar Albino rats were randomly allocated to 3 groups. Under sterile and proper surgical conditions, rats were subdermally incised 1-cm on their necks, backs and tail region. Then they were respectively closed with silk, vicryl, and prolene. Each group was treated with laser at 50mw, 4 j for 120 seconds while the control group received no laser treatment. On the 7th day, after decapitation, the wound regions were collected for histopathologic evaluation. The skin samples were immersed in 10% neutral formaldehyde in 0.1 M phosphate buffered saline. Paraffin-embedded skin tissues were sectioned to 5 µm. Samples were stained by using hematoxylin-eosin and Masson Trichrome techniques. The specimens obtained were evaluated and scored for the epithelial damage, inflammation, congestion, collagen synthesis, and tissue regeneration.

RESULTS: In comparison with the control group, the experimental groups showed no effect of laser treatment with respect to epithelial damage, congestion and immigration of inflammatory cells. However, an improved healing of wounds and an increase in new collagen synthesis have been observed at low power doses laser treated wounds sutured with silk and prolene. Similar results have been observed on high power doses laser treated wounds sutured with vicryl.

CONCLUSIONS: It was found that, the silk and prolene suture material was effective at low dose laser, whereas the vicryl was effective at high dose laser treatment.

Keywords: Low-energy Laser; Subacute Wound Healing; Suture Material; Histopathology

Relation between 3435C> T Multidrug Resistance 1 gene polymorphism with High Dose Methylprednisolone Treatment of Children Acute Idiopathic Thrombocytopenic Purpura

Mehmet Akin¹, Sebahat Turgut², Ceylan Ayada², Yusuf Polat³, Yasemin Isik Balci⁴, Firat Erdoğan⁵

¹Denizli State Hospital, Department of Pediatric Hematology

²Pamukkale University, Faculty of Medicine, Department of Physiology

³Denizli State Hospital, Department of Microbiology

⁴Pamukkale University, Faculty of Medicine, Department of Pediatric Hematology

⁵Medipol University, Department of Pediatric

OBJECTIVE: The present study was conducted to assess 3435C>T Multidrug Resistance 1 gene polymorphism and the efficacy of high dose methylprednisolone(HDMP) in childhood acute Idiopathic thrombocytopenic purpura.

METHODS: A total of 31 childhood acute Idiopathic thrombocytopenic purpura (17 females, 14 males) between the ages of 2 years to 16 years were included in the study. High-dose methyl prednisolone was given at a dose of 30 mg/kg/day for 3 days and 20 mg/kg/ day for 4 days, consecutively and intravenously. Fragments obtained were 238 bp to T/T genotype, 172 bp and 60 bp fragments to the C/C genotype, and 238 pb, 170 bp and 60 bp to the C/T genotype.

RESULTS: The distribution of CC, CT and TT genotypes were 19%, 61.3%,19.4 %, respectively. There was no significant difference in genotype and allele distribution between the patients with ITP and the controls ($\chi^2 = 0,84$ $p=0.65$, $\chi^2 = 0,2$ $p=0.63$, respectively. There was no significance in age, gender, pre-treatment and post-treatment platelet count between CC, CT and TT genotypes of MDR gene. After 1 year follow-up, becoming chronic course rates in CC, CT and TT genotypes were 33,3%, 47,3%, 50%, respectively. The distribution of genotype, age and gender weren't significant risk factors in logistic regression analysis of becoming chronicity. The odd ratio in individuals with CC/CT genotype was 1.25 ($p>0.5$).

CONCLUSIONS: In our study, there was no difference in HDMP treatment response between MDR1 gene genotypes. We have a small group of patients, our data should be considered preliminary, awaiting further confirmatory studies.

Keywords: MDR1, Idiopathic thrombocytopenic purpura, High dose metilprednizolon

Transgelin identified in seminal vesicles of sand rat *Psammomys obesus* during the breeding season

Kaci Ouchfoun Naima¹, Boudrissa Abdelkrim², Hadj Bekkouche Fatima³, Gernigon Spychalowicz Thérèse¹

¹Laboratoire des Zones Arides, Faculté des Sciences Biologiques, USTHB, BP 132 El Alia, 16100 Alger, Algeria

²Service de Parasitologie, Institut Pasteur d'Algerie, 01 Rue du Docteur Laveran, El Hamma, Alger, Algeria

³Laboratoire de Biologie et Physiologie des Organismes, FSB/USTHB, BP 132 El Alia, 16100 Alger, Algeria

OBJECTIVE: The aim of this study was to investigate immunolocalization and identification of POSVP21 in seminal vesicles of sand rat *Psammomys obesus*.

METHODS: Using a rabbit polyclonal antibodies preparation against POSVP21, we demonstrated by the avidin-biotin peroxidase procedure and the whole RNA from seminal vesicles translation the site synthesis localization of POSVP21.

RESULTS: POSVP21 is localized in the cytoplasm of epithelial cells and in secretory products in the lumen. To identify POSVP21, two dimensional gel analysis and tandem mass spectrometry (nanoLC-MS/MS) was performed. POSVP21 is separated on pH-gradient electrophoresis into at least 4 variants a, b, c and d with similar apparent Mw and pHi values varying from 4 to 7. Peptides sequences analysis of POSVP21 showed that it has a similar amino acid composition to the "Transgelin" peptide sequence from the rat *Rattus norvegicus*.

CONCLUSIONS: We conclude that POSVP21 is a similar protein to the transgelin from the rat *Rattus norvegicus*. "Transgelin" is identified for the first time within sand rat *Psammomys obesus*.

Keywords: *Psammomys obesus* - Immunohistochemistry - nano LC-MS/MS - Transgelin - seminal vesicle

Purinergic signaling in astrocytes: Induction of iodothyronine deiodinases enzyme activities

Stanislav Pavelka¹

¹Central-European Technology Institute, Masaryk University, Brno and

²Department of Radiometry, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

OBJECTIVE: The aim was to characterize in more details our earlier finding that purinergic agonists can induce the enzyme activities of iodothyronine deiodinases (ID) of types 2 (D2) and 3 (D3) in glial cells.

METHODS: Astroglial cells obtained from cerebrocortical hemispheres of 2-day-old rats were grown to confluence in DMEM medium containing 10 % fetal calf serum. The changes in ID activities, caused by short-term incubation (for 2-12 h) of the confluent cells in a chemically defined medium with different concentrations of purinergic effectors, without and with retinoic acid (RA, 1 micro M) pre-incubation (for 2-3 days), were quantified with the aid of our newly developed radiometric enzyme assays.

RESULTS: Physiological concentrations of ATP, ADP, AMP or adenosine and of a series of their analogues caused a very marked (up to 30-fold) increase in D2 activity. D3 activity was also induced, but to a much lesser extent (3 to 7-fold increase). For the first time, the induction of iodothyronine deiodinase of type 1 (D1) by endogenous purines in these cells was also shown. Induction of ID activities was time- and concentration-dependent. Pre-incubation of cells with RA had a crucial influence on the degree of induction of D1 and D2 activities by the action of purinergic agonists (up to 42-fold increase in D2).

CONCLUSIONS: A new signaling pathway in the multiregulation of induction of ID activities in astroglial cells has been demonstrated. Support from the Ministry of Education of the Czech Republic (Project No. MSM0021622413), Academy of Sciences of CR (Project No. AV0Z50110509) and from the Czech Science Foundation GA CR (Grant No. 304/08/0256) is acknowledged.

Keywords: Astrocyte, Enzyme, Iodothyronine deiodinase, Purinergic agonist

The Role Of Resistin And IL-6 In The Metabolic Syndrome

Arif Ata¹, Ahmet Ergün², S Kenan Köse³

¹Family Health Central Öveçler, Ankara Turkey

²Department of Physiology Medical Faculty of Ankara University, Ankara Turkey

³Department of Biyoistatistik Medical Faculty of Ankara University, Ankara Turkey

OBJECTIVE: The metabolic syndrome (MS) is a complex of symptoms that resulted from as a reply to inner and outer effects that influence the organism. Main components of MS are the obesity related to insulin resistance, dyslipidemia, hypertension, and hyperinsulinemia. It is considered that resistin and interleukin (IL)-6 are as effective on developing of insulin resistance and inflammation processes and there are a little studies on this subject.

METHODS: In our study, WC \leq 88 cm 22 women; \leq 102 cm 22 men; WC $>$ 88 cm 20 women and $>$ 102 cm 22 men were chosen from 86 subjects totally. According to ATP III criteria, results of mean systolic and diastolic blood pressure, levels of fasting plasma glucose, fasting plasma insulin, total cholesterol, very low density lipoprotein cholesterol, triglyceride, plasma uric acid, resistin and IL-6 were compared each other statistically.

RESULTS: In the group MS (+) mean systolic blood pressures ($p < 0,001$), mean diastolic blood pressures ($p < 0,001$), levels of mean fasting plasma glucose ($p < 0,05$), mean fasting plasma insulin ($p < 0,05$), total cholesterol ($p < 0,05$), low density lipoprotein-cholesterol ($p < 0,05$), very low density lipoprotein-cholesterol ($p < 0,001$), triglyceride ($p < 0,001$) acid were found high ($p < 0,001$) compared with MS (-) group, and values of high density lipoprotein-cholesterol were found less than those.

CONCLUSIONS: In MS (+) group, hypertension, insulin resistance progress, dyslipidemia were found high by comparing with MS (-) group. Levels of plasma resistin and IL-6 were not statistically significant ($p > 0,05$), compared with each others; however levels of IL-6 were found correlated with WC.

Keywords: metabolic syndrome, waist circumference, insulin, lipid profile, resistin, IL-6

Licofelone, a dual inhibitor of COX/LOX pathways, kills glioma cells in vitro

Gokhan Kus¹, Selda Kabadere¹, Pinar Oztopcu Vatan², Ruhi Uyar¹

¹Department of Physiology, Faculty of Medicine, Eskişehir Osmangazi University, Eskişehir, Turkey

²Department of Biology, Faculty of Art and Sciences, Eskişehir Osmangazi University, Eskişehir, Turkey

OBJECTIVE: Through cyclooxygenase (COX) and lipoxygenase (LOX) pathways arachidonic acid generates a number of compounds including prostaglandins, thromboxanes and leukotrienes. Beside wide range of clinical effects, these compounds also inhibit tumor cell apoptosis or induce tumor-cell proliferation. Interestingly, the dual COX/LOX inhibition is expected to possess clinical advantages over the selective inhibitors of only COX or only LOX enzymes, and could reduce the incidence or the progression of many cancers. One of the most promising compounds belonging to this dual inhibitor category is licofelone. We questioned whether licofelone effects the survival of rat glioma cell line (C6) in vitro.

METHODS: The cells were preincubated for 24 hours in 96 well plates containing 1×10^4 cells/well, Dulbecco's modified Eagle's medium, 10 % fetal bovine serum and 1 % antibiotics. Then these cells were treated with only medium (control) or licofelone doses (10, 50, 100, 150, 200 or 250 μM) for 24 or 48 hours ($n=24$). For viability test, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was applied.

RESULTS: Treatment of the cells with 10, 50 and 100 μM licofelone for 24 or 48 hr did not show any action on cell viability. However, 150, 200 and 250 μM licofelone for 24 hr reduced the number of living cells by 20, 45 and 74 % as compared to the control, respectively. Incubation of the cells for 48 hr with 150, 200 and 250 μM licofelone decreased further the percentage of surviving cells down to 42, 12 and 7 %, respectively.

CONCLUSIONS: The present study reveals the possibility that licofelone possesses a strong (upto 93 %) dose and time dependent anticancer property.

Keywords: Licofelone, COX, LOX, glioma, arachidonic acid.

Impact of Acute Stress on Parasympathetic System of Rat Heart

Magdalena Chottova Dvorakova¹, Eliska Mistrova¹, Jana Slavikova¹, Sixtus Hynie², Pavel Sida², Vera Klenerova²

¹Department of Physiology, Faculty of Medicine, Pilsen, Charles University in Prague, Czech Republic

²Institute of Medical Biochemistry, 1st Faculty of Medicine, Prague, Charles University in Prague, Czech Republic

OBJECTIVE: Alteration of cardiovascular activity caused by stress is induced by activation of sympathetic nervous system (NS). Whether parasympathetic NS participates on stress-induced responses has not been extensively studied. Principal neurotransmitter of parasympathetic NS is acetylcholine, which exerts its function by activation of muscarinic receptors.

METHODS: In the rat heart atria we studied the expression of mRNAs for muscarinic M2 receptors (M2R) and for choline acetyltransferase (CHAT), enzyme forming acetylcholine. We used two rat strains, Sprague-Dawley (SD) and Lewis (LEW), the latter being known to have a blunted hypothalamic-pituitary-adrenal response. A restraint stressor (immobilization, IMO) and IMO combined with partial immersion of rats into water (IMO+C) were applied to rats for one hour. Rats were killed three hours after stress termination. Relative expression of mRNA for both genes, estimated with Real-Time PCR, was expressed as a ratio of target gene Cq value to Cq value of reference gene.

RESULTS: IMO+C caused increased expression of mRNA for CHAT; this effect was more pronounced in SD than in LEW rats. Expression of mRNA for M2 receptors was decreased by IMO in both rat strains. Exposure to two types of restraint stressors caused similar changes in CHAT and M2R mRNA production in the heart atria of both SD and LEW rats.

CONCLUSIONS: In the heart atria we demonstrated the expression of mRNA both for enzyme forming acetylcholine and its M2 receptor. Present studies did not show the differences in response to two restraint stressors. Supported by grants MSM 0021620806 and MSM 0021620819.

Keywords: acute stress, heart, parasympathetic

Preproglucagon (PPG) neurons innervate neurochemically identified autonomic neurons in the mouse brainstem

Ida J Llewellyn Smith¹, Greta J E Gnanamanickama¹, Frank Reimann², Fiona M Gribble², Stefan Trapp³

¹Cardiovascular Medicine, Physiology and Centre for Neuroscience, Flinders University, Bedford Park, Australia

²Cambridge Institute for Medical Research, Addenbrooke's Hospital, Cambridge, United Kingdom

³Department of Surgery and Cancer & Biophysics Section, Imperial College, London, United Kingdom

OBJECTIVE: PPG neurons produce GLP-1, a satiety peptide, and occur primarily in the nucleus tractus solitarius. Our immunohistochemical studies in transgenic mice expressing YFP under PPG promoter control showed that PPG neurons project widely to central autonomic regions, including brainstem nuclei. Functional studies have highlighted the importance of hindbrain receptors for GLP-1's anorexic effects.

METHODS: Here, we assessed YFP innervation of neurochemically-identified brainstem neurons in transgenic YFP-PPG mice. Immunoreactivity for YFP plus choline acetyltransferase (ChAT), tyrosine hydroxylase (TH) and/or serotonin (5-HT) were visualised with two- or three-colour immunoperoxidase labelling using black (YFP), brown and blue-grey reaction products.

RESULTS: In the dorsal vagal nucleus (DMV), YFP-containing axons closely apposed only a few ChAT neurons, mostly lying rostral to area postrema (AP) and in dorsal and lateral DMV beneath the AP. Within the nucleus ambiguus, some ChAT neurons in the loose formation received appositions, but innervation was mostly absent from the compact formation. In the vagal complex, few TH neurons were closely apposed by YFP axons. In the A1/C1 column in the ventrolateral medulla, close appositions on TH neurons were more common. A single YFP-immunoreactive axon usually provided 1-3 close appositions on individual ChAT- or TH-positive neurons. Serotonin neurons were most heavily innervated, with many raphé pallidus, raphé obscurus and parapyramidal neurons receiving several close appositions from YFP axons.

CONCLUSIONS: These results indicate that GLP-1 neurons innervate brainstem autonomic neurons, including some vagal efferent neurons. Our data also demonstrate a link between GLP-1 neurons and 5-HT neurons, which are involved in appetite regulation.

Keywords: GLP-1; NTS; brainstem; incretins

Expression of Voltage-Gated Sodium Channel Protein in Primary Tumour and Corresponding Metastasis in an Rat Model of Prostate Cancer

Senay Yildirim¹, Seyhan Altun¹, Mustafa B A Djamgoz², Handan Kaya³

¹Department of Biology, Faculty of Science, Istanbul University, Vezneciler, Istanbul 34134, Turkey.

²Division of Cell and Molecular Biology, Neuroscience Solutions to Cancer Research Group, Imperial College London, Sir Alexander Fleming Building, South Kensington Campus, London SW7 2AZ, United Kingdom.

³Department of Pathology, Marmara University Hospital, Pendik, Istanbul, Turkey.

OBJECTIVE: Voltage-gated sodium channels (VGSCs) are present in excitable cells, where they are responsible for the upstroke of the action potentials. Interestingly, presence of VGSCs in non-excitable cells including metastatic cancer cells has been demonstrated using electrophysiological and molecular methods. Although the physiological basis of cancer is not known it is beginning to emerge that expression of VGSC contributes to the metastatic progression of various types of cancer, such as prostate, breast and lung. The purpose of this study was to evaluate the expression of VGSC protein in Mat-LyLu tumour, an established rat model developed to study prostate cancer (PCa) progression.

METHODS: PCa model was induced in male Copenhagen rats by subcutaneous implantation of strongly metastatic Mat-LyLu rat PCa cell. Subcutaneous implantation of Mat-LyLu cells resulted in the development of lung metastases. The primary tumours and the corresponding lung metastases were then examined by immunohistochemistry using anti-sodium channel (III-IV linker region) antibody.

RESULTS: Expression of VGSCs protein was confirmed by immunocytochemistry in cultured Mat-LyLu cells, which was consistent with the patch-clamp experiments in literature. In immunohistochemical studies, all tumour samples showed a positive VGSC expression. The same VGSC protein expression pattern between primary tumour and related metastasis was observed. No expression of VGSCs was detected in negative controls without the antibody.

CONCLUSIONS: Our results suggest that the Mat-LyLu tumour is a reliable model system for evaluating the role of VGSCs in PCa pathobiology/physiology. Further studies regarding the modulation of VGSCs activity in this model will provide valuable information with regard to the role of these channels in the development and metastatic progression of PCa.

Keywords: Voltage-gated sodium channel, Prostate cancer, Metastasis, In vivo

The role of N-methyl-D-aspartate type glutamate receptors in chondrogenesis

Janos Fodor¹, Tamás Juhász², Csaba Matta², Krisztina Holló², Balázs Pál¹, Áron Köszeghy¹, Tamás Oláh¹, Pál Gergely³, László Csernoch¹, Róza Zákány²

¹Department of Physiology, University of Debrecen, Hungary

²Department of Anatomy, Histology and Embryology, University of Debrecen, Hungary

³Department of Medical Chemistry, University of Debrecen, Hungary

OBJECTIVE: N-methyl-D-aspartate type glutamate receptors (NMDARs) are non-selective cationic channels with high Ca²⁺-permeability and require glycine coagonist to become activated. Functional NMDAR is a heterotetramer and NR1 subunit is essential to the receptor assembly and trafficking to the plasmamembrane. The presence of glycine-binding NR3 subunit decreases the Ca²⁺-permeability. Previously, we had reported characteristic changes in the basal cytosolic Ca²⁺-concentration [Ca²⁺]_i in chondrogenic cells of chondrifying high density cell cultures (HDC). Here we investigated the role of the NMDAR in chondrogenesis.

METHODS: We detected expression of NR1, NR2A, NR2B, NR3A and NR3B subunits in HDC, but only NR1, NR2B and NR3B were present on Western blots of plasma-membrane fraction, with the highest expression on day 2 of culturing, when final commitment of chondrogenic cells occurs.

RESULTS: We recorded rapid spontaneous Ca²⁺ -oscillations responding to NMDA with longer duration. NMDA also generated a pronounced elevation of basal [Ca²⁺]_i. Pre-incubation with ifenprodil (an NR2B-specific NMDAR inhibitor) suppressed the calcium transients. On whole cell patch clamp records weak inward current became visible in the presence of NMDA with NMDG based internal solution. These responses to NMDA were the most pronounced on day 2. The agonist glutamate and NMDA both failed to influence cartilage formation, but both glycine and ifenprodil promoted chondrogenesis. Transient gene silencing via introduction of NR1 siRNA, blocked cartilage formation and almost completely abolished spontaneous Ca²⁺ oscillations.

CONCLUSIONS: Our results prove the profound role of NMDARs in the regulation of chondrogenesis via influencing [Ca²⁺]_i of differentiating cells.

Keywords: glutamate receptor, chondrogenesis, Ca²⁺ oscillation.

The Role Of Actin Cytoskeleton And Eukaryotic Elongasyon Factor 2 In The Intracellular Traffic Of Diphtheria Toxin

Bilge Özerman, Başak Varol, Ebru Hacıosmanoğlu, Muhammet Bektaş, Rüstem Nurten
Department of Biophysics, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

OBJECTIVE: Actin cytoskeleton supports the endosomal traffic in the cell. The actin also regulates signaling pathways by interacting with actin-binding proteins. It has been observed in our previous studies that the depolymerization of the filamentous actin occurs right after the interaction with fragment A (FA) of diphtheria toxin (DT) which is a well defined bacterial pathogen. The intracellular trajectory of DT has not been fully elucidated comparing to its inhibitory effect of protein synthesis which occurs following the ADP-ribosylation of eukaryotic elongasyon factor 2 by FA. In this study it is aimed to visualize the traffic of DT loaded endosomes and to show cytosolic and endosomal FA activity.

METHODS: Human umbilical vein endothelial cells (HUVEC) were cultured and treated with DT. Filamentous actin, early endosomes and FA were detected by immunofluorescence microscopy. FA release into the cytosol from early endosomes was detected by Western blotting of affinity isolated protein complex. The activity of FA in fractions of cell lysates was checked by ADP-ribosylation assay.

RESULTS: Immunostaining revealed that FA loaded endosomes were overlapped with actin and were accumulated around the nucleus. FA activity in nuclear cell fraction was found elevated.

CONCLUSIONS: FA release from toxin loaded endosomes was considered to be occurring due to the protein-protein interactions. Preliminary data presented here form the background for a new study with actin skeleton stabilizing drugs.

Keywords: Cytoskeleton, actin, endosome, diphtheria toxin, HUVEC

Apoptotic Effects of Corticosteroids on CD34+ Hematopoietic Stem Cells and Modulation of This Effect with Several Cytokines

Muzaffer Beyza Cetin¹, Serap Erdem Kuruca¹, Kadriye Ak Gün Dar², Ebru Gürel², Sabriye Karadenizli¹, Gülyüz Öztürk³

¹Department of Physiology, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey

²Department of Biology, Istanbul University Faculty of Science, Istanbul, Turkey

³Department of Pediatrics, Istanbul University Faculty of Medicine Division of Hematology/Oncology, Istanbul, Turkey

OBJECTIVE: Stem cells that can self-renew, proliferate and differentiate are indispensable component for regeneration of hematopoietic system. In chemotherapy applications, malign and normal cells are together damaged. Corticosteroids, essential drug for all chemotherapy protocols, efficiently induce apoptosis not only in malign cells also in normal hematopoietic cells. In several studies, it has been showed that cytokines can suppress apoptosis induced by chemotherapy. In this study, for the first time we investigated apoptotic effects of corticosteroids on pure CD34+ hematopoietic stem cell population collected from human peripheral blood buffy coats by using immunomagnetic positive selection method and protective effects of cytokines.

METHODS: Control groups were incubated with serum-free medium (SFC) and induced with cytokine cocktail including interleukin3, thrombopoietin, stem cell factor and flt3/flk2ligand (CC). Test groups were dexamethasone group (D), prednisolone group (P) and groups induced with cytokines before drug application (CD, CP). To determine apoptotic mechanisms, immunohistochemical staining was carried out with monoclonal fas, caspase3, cytochromeC, bax and bcl-2 antibodies, degree of peroxidase reactions were quantified using H-score. For statistical analysis paired t-test and one sample t-test were used.

RESULTS: As a result, both dexamethasone and prednisolone induced apoptosis. Compared with control groups, corticosteroids application caused a significant increase for all apoptotic markers ($p<0.05$), and a significant decrease for anti-apoptotic bcl-2 ($p<0.05$). Also, cytokines caused a significant decrease for all apoptotic markers ($p<0.05$) and a significant increase for bcl-2 ($p<0.05$).

CONCLUSIONS: So that, corticosteroids induced apoptosis in human CD34+ hematopoietic stem cells and cytokines decreased apoptotic effects of these agents and prevented apoptosis.

Keywords: Apoptosis, corticosteroid, cytokine, hematopoietic stem cells

Effect of STZ diabetes on CGRP/ADM signalling system in the rat heart

Eliska Mistrova¹, Magdalena Chottova Dvorakova¹, Jana Slavikova¹, Wolfgang Kummer²

¹Department of Physiology, Medical Faculty in Plzen, Charles University, Czech Republic

²Institute for Anatomy and Cell Biology, Justus-Liebig-University, Giessen, Germany

OBJECTIVE: Both calcitonin gene-related peptide (CGRP) and adrenomedullin (AM) regulates vascular tone in the heart, being cardioprotective in hypoxia. Additionally CGRP exerts positive inotropic and chronotropic effect, while AM exhibit antiproliferative and antiapoptotic functions in the myocardium. Their actions are mediated through specific G protein-coupled receptors, calcitonin receptor-like receptor (CRLR), respectively. Ligand affinity of CRLR is determined by receptor activity modifying proteins (RAMP1-3). CGRP binds to the complex formed by CRLR/RAMP1, whereas CRLR/RAMP2 and CRLR/RAMP3 serve as receptors for ADM. Here, we investigated this signalling systems in the control rat heart and compared it to that in diabetic rats.

METHODS: The separated rat heart compartments from animals 8 weeks after administration of streptozotocin (STZ; 65 mg/kg i.v) and in the age-matched controls (n=6 per group) were analyzed by real-time RT-PCR. Relative expression of AM, CGRP, CRLR, RAMP1-3 mRNA was expressed as a ratio of target gene Cq value to Cq value of reference gene – beta-actin. The results were considered significantly different when $p < 0.05$.

RESULTS: We have observed some changes in relative expression of the genes just in right heart compartments. In the right atrium AM expression declined to 50% of controls, while in the right ventricle RAMP1 was strongly upregulated. Relative expressions of other tested genes were not significantly altered.

CONCLUSIONS: In summary, the shifts observed in short-term diabetes may favour a trend of a pronounced CGRP signalling. These observations may provide a new possible therapeutic strategy for diabetic cardiomyopathy. Granted by GAUK 99510 and DFG, 436 TSE 113/51/0-1.

Keywords: heart, CGRP, adrenomedullin, CRLR, STZ diabetes, rat

Investigation of the trophic support and the restorative effect of oxytocine in glial culture with and without rotenone-induced toxicity

Gonca Dalkurt Mola, Oytun Erbaş, Gönül Ö. Peker, Dilek Taşkiran, Özlem A. Yılmaz
Ege University Faculty of Medicine Dept. Physiology, Izmir, TURKEY

OBJECTIVE: Rotenone (R), an organic and lipophilic insecticide inhibits complex-1 in electron transfer chain, upregulates superoxide, and oxidative stress-induced mitochondrial damage and cell death. Its selectivity in damaging dopaminergic neurons and potency of developing Parkinsonism like symptoms in the rat have been reported. We identified and reported the toxic effects of R in glia culture previously. Recent research has attributed neuroprotective and behavioral equilibrating serenity effects to oxytocin (ox) in addition to its well-known endocrine functions. We reported previously that ox attenuates / restores neural damage induced by diabetic neuropathy and experimental convulsions. Here, we aimed to investigate in glial culture, the trophically supportive, and neurorestorative effects of ox in general and following R toxicity, respectively.

METHODS: Primary astroglial culture was prepared using newborn rat cortical cells. Following passagings, cells were processed in 6 groups accordingly (n=15/group; Control (C), 1 μ M R, 10 nmol/L ox (ox1), 100 nmol/L ox (ox2), ox1+R and ox2+R; 104 cells/well x 96 wells/group). After 24 hours, cell viability was assessed using MTT method.

RESULTS: Cell viability - compared to controls - decreased significantly ($P<0.005$) in R group, and increased significantly ($P<0.05$) and dose-dependently in the ox1 and ox2 groups. Moreover, cell viability – compared to R group – increased significantly ($P<0.05$) and dose-dependently in ox1+R and ox2+R groups.

CONCLUSIONS: Ox definitely provided trophic support for qualitative and quantitative survival of the glial cells in culture, and robustly attenuated cell damage and death rate following R toxicity. We foresee identifying and defining the site(s) and mechanism(s) of action of in-vivo and in-vitro neuroprotection induced by ox.

Keywords: Oxytocine, Rotenone, Astroglia, Neurotoxicity, Viability

Are Membrane-Bound G Protein-Coupled Melatonin Receptors MT1 and MT2 Really Necessary for The Neuroprotective Effects of Melatonin in Focal Cerebral Ischemia?

Ulkan Kilic¹, Adnan Yüksel², Bayram Yilmaz³, Sigenem Eyuboglu³, Milas Ugur³, Ertugrul Kilic³

¹Bezmialem Vakif University, Faculty of Medicine, Department of Medical Biology, Istanbul, Turkey

²Bezmialem Vakif University, Faculty of Medicine, Department of Medical Genetic, Istanbul, Turkey

³Yeditepe University, Faculty of Medicine, Department of Physiology, Istanbul, Turkey

OBJECTIVE: Melatonin is synthesized by the pineal gland in a circadian rhythm and predominantly acts through its MT1 and MT2 receptors. The roles of MT1 and MT2 in the neuroprotective effects of melatonin and cell signaling after cerebral ischemia were still unknown. In this study, we investigated the roles of MT1 and MT2 in the neuroprotective effects of melatonin in melatonin receptors 1-2 knockout mice.

METHODS: Adult male melatonin receptors 1-2 knockout mice (mt1/2^{-/-}), with a C3H/HeN strain background and their wt littermates were used. The animals were divided into four groups and submitted to 90 minutes of intraluminal middle cerebral artery (MCA) occlusion, followed by 24 hours of reperfusion and treated with melatonin (4 mg/ kg; i.p. day) or vehicle just after 90 min of ischemia. In these animals, infarct volume, edema formation, iNOS accumulation and signaling pathways, including CREB, ATF-1, p21, JNK1/2, p38 phosphorylation were analyzed.

RESULTS: Here, we show that the infarct volume and brain edema do not differ between mt1/2^{-/-} and WT animals but melatonin treatment decreases infarct volume in both groups and brain edema in WT animals after MCAo. Notably, melatonin's neuroprotective effect was even more pronounced in mt1/2^{-/-} animals compared to WT animals. We also demonstrate that melatonin treatment decreases CREB, ATF-1 and p38 phosphorylation in both groups, while p21 and JNK1/2 were reduced only in melatonin-treated WT animals in the ischemic hemisphere. Furthermore, melatonin treatment decreased iNOS accumulation only in WT animals.

CONCLUSIONS: We provide evidence that neuroprotective efficacy of melatonin after ischemic injury in WT and MT1-2 knockout animals is independent from its membrane receptors.

Keywords: Cerebral ischemia, Melatonin, Melatonin receptor 1-2, Neuroprotection

Antiproliferative Activities Of Homalothecium Sericeum Extracts On Glioma

Pınar Oztopcu Vatan¹, Selda Kabadere², Ruhi Uyar², Filiz Savaroglu¹, Gokhan Kus²

¹Department of Biology, Faculty of Art and Sciences, Eskisehir Osmangazi University, Eskisehir, Turkey

²Department of Physiology, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey

OBJECTIVE: Bryophytes have been used as medicinal plants for more than 400 years in China, Europe and North America. There is also evidence confirming the antibiotic and anticancer activity of bryophytes against fungi, prokaryotes and different cancer cells. The purpose of the current study was to investigate the possible anticancer property of Homalothecium sericeum (hedw.) schimp., which is a bryophyte, extracts on rat glioma (C6) cells, in vitro.

METHODS: We first collected 2 different (acetone and A) extracts from H. sericeum by two different extraction processes. C6 cells were seeded in 96 well plates and incubated for 24 hr. Following this incubation period, the medium was replaced with only medium (control) or medium with extracts at concentrations of 0.17, 1.7, 17, 85 or 170 µg/mL for 48 hr. Cytotoxicity was determined by using 3-(4,5-dimethylthiazol-2yl)- 2,5-diphenyltetrazolium bromide (MTT) colorimetric assay.

RESULTS: Acetone extract of H. sericeum at 0.17, 1.7 and 17 µg/mL concentrations did not change the survival rate of C6, but 85 and 170 µg/mL inhibited about 16 % and 36 % after 48 hr (p<0.001), respectively. Extract A at concentration of 0.17 µg/mL did not also affect C6 viability, but 1.7, 17 (p<0.01), 85 and 170 (p<0.001) µg/mL decreased C6 cell viability by 6, 8, 24 and 33 % for 48 hr, respectively.

CONCLUSIONS: Acetone and A extracts of H. sericeum showed a moderate but similar dose dependent cytotoxicity on C6. Further studies are needed to clarify the content of these extracts.

Keywords: Antiproliferative, Homalothecium sericeum extracts, glioma, MTT

The modulation of calcium dependent exocytosis in pancreatic beta cells by protein kinases

Masa Skelin, Marjan Slak Rupnik

Institute of Physiology/CIPKEBIP, University of Maribor, Slovenia

OBJECTIVE: Insulin secretion from the pancreatic beta cell is controlled by a variety of modulatory pathways and glucose-induced insulin secretion can be amplified. These amplification pathways may utilise an increase in intracellular cAMP that acts in PKA-dependent or PKA-independent manner as well as activation of PKC pathway. In this study we assessed the role of cAMP and PKC-dependent amplifying pathways in Ca²⁺-dependent exocytosis in pancreatic beta cells.

METHODS: The whole-cell patch-clamp was used simultaneously with slow photo-release of caged Ca²⁺ to elicit membrane capacitance change (C_m).

RESULTS: The control cells exposed to slow Ca²⁺ uncaging the C_m increased in a reproducible Ca²⁺-dependent manner. The Ca²⁺-dependency of the rate of the initial C_m change followed the saturation kinetics with half-maximal value (EC₅₀) of $2.9 \pm 0.2 \mu\text{M}$. After clamping the cytosol at 200 μM cAMP or inclusion of a specific PKA activator the Ca²⁺-dependency of the initial C_m has been shifted to significantly lower EC₅₀. Alternatively the specific activation of Epac2 increased the rate of exocytosis without changing the sensitivity to [Ca²⁺]_i. The activation of PKC significantly increased the calcium sensitivity comparing to the cells where PKC was inhibited.

CONCLUSIONS: Our findings suggest that cAMP modulates the rate of exocytosis in pancreatic beta cells mainly through PKA-dependent pathway by sensitizing the insulin releasing machinery to [Ca²⁺]_i. On the other hand activation of PKC appears to be important mechanism to prevent the fusion of the secretory vesicles at insufficient [Ca²⁺]_i.

Keywords: pancreatic beta cells, calcium dependency, exocytosis

Renin Expression and Renal Development in the Renin Cell specific TGFbetaRII Knockout Mouse

Melanie Gerl, Armin Kurtz, Charlotte Wagner

Department of Physiology, University of Regensburg, Regensburg, Germany

OBJECTIVE: The renin distribution pattern varies during kidney development. Renin appears first at embryonic day 15 within the A. arcuatae, from where it migrates along the developing arterial tree to the proximal vessels, to be finally localized at its classical juxtaglomerular position in adulthood. The factors influencing this shift of renin producing cells are still unknown. Previous experiments indicate that the differentiation factor TGFbeta could be involved in renin expression regulation. It was found that renin cells express TGFbetaRII and that its expression is co-regulated with that of renin starting already during nephrogenesis. With the aid of TGFbetaRII RenCre^{fl} knockout mice we tried to investigate if and to what extent missing TGFbetaRII abundance in renin producing does influence the renin expression pattern in the maturing mouse kidney.

METHODS: - 3d-renal arterial tree reconstructions showing the renin distribution pattern in TGFbetaRII RenCre^{fl} knockout mice of embryonic, postpartal and adult kidneys. - Immunohistochemical stainings - Real time PCR measurements of renin mRNA expression during murine nephrogenesis

RESULTS: The renin expression level is lowered in TGFbetaRII RenCre^{fl} knockout mice, but the expression pattern does not differ from wild type neither in adulthood nor during kidney development. There were no visible differences in the appearance of arterial development.

CONCLUSIONS: In summary, with the previous data from 3d reconstructions of TGFbetaRII RenCre^{fl} kidneys, we conclude that the TGFbetaRII does not seem to be essential for a correct positioning of renin producing cells during murine nephrogenesis and has at the most a stimulatory effect on renin expression.

Keywords: Renin, TGFbetaRII, renal Development, 3d reconstructions

Role of blood pressure to mediate the influence of salt intake on renin expression in AT1a receptor deficient kidneys

Katharina Anna Machura, Dominik Ludwig Steppan, Björn Neubauer, Armin Kurtz
Department of Physiology, University of Regensburg, Regensburg, Germany

OBJECTIVE: The salt balance of the organism controls the number of renin producing cells in the kidney in an inverse fashion by yet undefined mechanisms. This study aimed to assess a possible mediator role of preglomerular blood pressure in the control of renin expression by oral salt intake. For our investigations we used mice lacking angiotensin II (ANGII) type 1a (AT1a) receptors, because these mice display an enhanced salt sensitivity of renin expression.

METHODS: Immunohistochemistry, 3D-Reconstructions, Realtime PCR measurements, Blood pressure measurements

RESULTS: Utilizing 3-dimensional tissue reconstructions of the kidneys we found renin expressing cells along the preglomerular vascular tree in a typical distal to proximal distribution gradient which was most prominent at high salt intake and which was obliterated at low salt intake by the appearance of renin expressing cells in proximal parts of the preglomerular vasculature. This disappearance of the distribution gradient from afferent arterioles to arcuate arteries during low salt intake was accompanied by reductions of systolic blood pressure. Unilateral renal artery stenosis in mice on normal salt intake produced a similar distribution pattern of renin expressing cells as did low salt intake. Conversely, increasing blood pressure by administration of the NOS-inhibitor L-NAME in mice kept on low salt intake produced a similar distribution pattern of renin producing cells as did high salt intake alone.

CONCLUSIONS: These findings suggest that the influence of salt intake on the number and distribution of renin producing cells in AT1a deficient mice may be mediated by changes of preglomerular blood pressure.

Keywords: salt control of renin expression, AT1a Receptor

Ion modified calcium phosphates - influence on viability and proliferation of fibroblasts and bone marrow cells

Boika Andonova¹, Tania Zhivkova¹, Lora Dyakova², Orlin Alexandrov³, Milena Kirilova⁴, George Miloshev⁴, Stefka Tepavitcharova⁵, Radostina Alexandrova¹

¹Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

²Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

³Health Service, Gorna Malina, Bulgaria

⁴Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria

⁵Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

OBJECTIVE: Bone disease is a serious health condition that directly impacts on the quality of life of sufferers, particularly among the aged. Although autogenous bone grafts are still considered the gold standard for bone replacement and allogenic bone grafts are widely used, several biomaterials (metals, calcium phosphate ceramics, bioactive glasses, polymers, composites) have been developed with more or less clinical success. That is why there is a significant need and demand for the development of a bone substitute that is bioactive and exhibits material properties (mechanical and surface) comparable with those of natural, healthy bone.

METHODS: The aim of this study was to evaluate the effect of Mg²⁺ and Zn²⁺ ion different degree substitutions in biomimetic synthesized calcium phosphates (Mg-CPs; Zn-CPs) on viability and proliferation of cultured human (Lep3 embryonic cell line), murine (primary bone marrow, NIH 3T3 fibroblasts) and rat (bone marrow) cells. The cells were cultured in DMEM medium incubated for 4 h in the presence of CPs placed on glass slide (100 mg compound/5 cm² glass slide, 10 ml medium).

RESULTS: The investigations were performed after 72 h and 144 h of treatment periods using MTT test, neutral red uptake assay, trypan blue dye exclusion method, crystal violet staining, single cell gel electrophoresis (Comet assay) and double staining with acridine orange and propidium iodide. A positive correlation between the data obtained by the above-mentioned methods was observed indicating the low toxicity of the CPs tested.

CONCLUSIONS: Additional investigations are underway to clarify better the biocompatibility of the materials examined. Acknowledgement: Supported by Grant DTK-02-70/2009 from National Science Fund, Bulgaria.

Keywords: ion modified calcium phosphates, bone disease, biocompatibility

Effect of Mannan Oligosaccharides on the Immune System: Transport of MOS in to Lamina Propria

Haydar Özpınar¹, Kirk C. Klassing², Ismail Hakkı Tekiner¹

¹Department of Food Engineering, İstanbul Aydın University, İstanbul, Turkey

²Department of Animal Science, University of California Davis, CA USA

OBJECTIVE: MOS, a complex carbohydrate, is derived from the cell wall of the yeast *Saccharomyces cerevisiae*. This study determines if MOS crosses over intestinal epithelium and translocated to lamina propria.

METHODS: MOS labeled with a fluorescently tag was introduced directly into intestinal segments. Its location was determined by fluorescent microscopy (FM) in the fixed sections of the intestine. Pure mannan was isolated from the mannan rich fraction by reacting with 7-methoxycoumarin-3-isocyanate in dimethylsulphoxide. The labeled product was isolated by ethanol precipitation. The fluorescently tag labeled glucan was produced by reacting 97% pure yeast cell wall glucan with the same label. Finally the labeled MOS and glucan was checked with FM to see fluoresces signals. Dextran and albumin were purchased from Sigma.

RESULTS: The intestinal segments removed were preserved in 10% formalin and fixed on the slides using the paraffin method. From each segment, 72 glass slides were prepared. Fluorescent microscopy was used to determine the extent of translocation into the lamina propria and images were captured. Slides were evaluated quantitatively by interrogation of color intensity of foci of translocated macromolecules (foci) using a commercial image analysis program that converts color intensity at specific wavelengths into numerical values of intensity. This data was analyzed by ANOVA. A P value of 0.05 was considered to be significant.

CONCLUSIONS: MOS does not interact specifically with epithelial cells but makes its way to the GALT of the lamina propria via an independent method, which appears to be mediated by dendritic cells. MOS has likely a general adjuvant effect on immune system without causing “danger signals” that are inherent in pathogens.

Keywords: Mannan oligosaccharides, glucan, immune system, lamina propria

Nicotine preference affects BDNF mRNA expression in a region and sex specific manner in rat brain

Lütfiye Kanit¹, Tanseli Nesil², Oguz Gozen¹, Melis Olçum², Ersin Oguz Koylu¹, Şakire Pöğün³

¹School of Medicine Dept of Physiology, Izmir, Turkey

²Inst. of Science Biotechnology Department, Izmir, Turkey

³Ege University Center for Brain Research, Izmir, Turkey

OBJECTIVE: Brain-derived neurotrophic factor (BDNF) is accepted to play a critical role in the maintenance of nicotine (NIC) addiction. Our aim was to study possible changes in brain BDNF mRNA levels following chronic NIC intake in rats, preselected according to NIC preference.

METHODS: Adult Sprague Dawley rats were exposed to a 2-bottle, free choice oral NIC self administration from adolescence to adulthood (23 weeks) and NIC intake was used to determine preference as adults. Control (CONT) rats received water from both bottles. There were 6 groups (n=7-10/group): Female or male MAX or MIN and CONT. Total RNA was extracted from the frontal cortex, striatum and hippocampus; semi-quantitative RT-PCR was performed for BDNF mRNA expression.

RESULTS: ANOVAs showed main effects of regions ($p < 0.001$), sex ($p < 0.005$), NIC preference ($p < 0.05$) and a sex x regions interaction ($p < 0.001$). In the frontal cortex, the MAX male rats was lower than MAX females ($p < 0.05$). Similarly, in the hippocampus, MAX males had the lowest levels of all. Hippocampal BDNF mRNA expression in the MAX male group was significantly lower than females with the same preference ($p < 0.05$) or CONT male rats (0.01). These results suggest sex and region specific regulation of BDNF mRNA expression by NIC in rats; in the frontal cortex and hippocampus, BDNF mRNA levels are lower in MAX male rats than MAX females and CONTs.

CONCLUSIONS: NIC preference and intake did not have an effect on regulating BDNF mRNA levels in the female rats. If similar regulatory mechanisms occur in humans, heavy smoker men may be facing the negative consequences of reduced BDNF levels in brain regions related to cognition and affect.

Keywords: Animal models, Individual Differences, BDNF, nicotine addiction

Molecular Analysis of Smad-1, Bmp-2, Bcl-xL and Caspase-3 Genes In Renal Ischemia-Reperfusion Model in Rats

Ahmed Bulut¹, Tuncer Demir¹, Beyhan Cengiz¹, Ediz Tutar², Serdar Öztuzcu³, Abdulkadir Yasir Bahar², Şeniz Demiryürek¹, Duygu Düşmez¹, Şeyda Nur Dağlı¹, Cahit Bağcı¹

¹Department of Physiology, Gaziantep University, Gaziantep, Turkey

²Department of Pathology, Gaziantep University, Gaziantep, Turkey

³Department of Medical Biology, Gaziantep University, Gaziantep, Turkey

OBJECTIVE: In this study, we aimed to investigate the effects of Smad-1 (TGF super family) and BMP-2 genes in renal Ischemia-Reperfusion (I/R) model in rats. Furthermore, we aimed to investigate the gene levels of Caspase-3 (affected by apoptotic) and Bcl-xL (affected by antiapoptotic) genes to find out the possible apoptotic effects of Smad1 and BMP2 in the kidney.

METHODS: Forty female rats were allocated into sham, ischemia, ischemia+Ca channel blocker and ischemia+Na channel blocker groups. The ischemia group did not receive any treatment. Other groups received Ca channel blocker or Na⁺ channel blocker before 30 minutes of anesthesia. Then, the abdomen was opened with a midline incision and bilateral renal arteries were clamped for 60 minutes. After 48 hour reperfusion, blood samples and kidney tissues were collected. Tissue samples were examined by histopathological and RT-PCR methods for the expression levels of Smad-1, Bmp-2, Bcl-xL and Caspase-3.

RESULTS: There was a significant histopathological difference between the calcium channel blocker group and other groups. On the other hand, Bcl-xL gene was significantly different from Smad-1, Bmp-2 and Caspase-3 genes according to the molecular analyses.

CONCLUSIONS: Calcium channel blockers may ameliorate the renal ischemia-reperfusion injury. These agents may be beneficial after controlled clinical studies.

Keywords: renal ischemia, apoptosis, Smad-1, Bmp-2

Selective targeting of Gp60 receptors on liver cancer cells with albumin bioconjugated carbon nanotubes

Lucian Mocan, Teodora Mocan, Rares Stiufiuc, Florin Zaharie, Bartos Dana, Ariana Stir, Cornel Iancu
Third Surgical Clinic, Cluj-Napoca, Romania- Nanomedicine Department

OBJECTIVE: Hepatocellular carcinoma (HC) represents a leading cause of cancer deaths worldwide. As chemotherapy and radiotherapy show modest results and surgery is possible in 10% to 30% of patients, new methods of therapy offer the best hope for a better outcome.

METHODS: Albumin receptor Gp 60 receptor is a tyrosine kinase receptor (RTK) associated with caveolae, invaginations of the plasma membrane that regulate vesicular transport, endocytosis and intracellular signaling. Because Gp 60 is overexpressed in liver cancer cells its targeting using albumin functionalized nanoparticles provides an opportunity to create a new generation of immunonanoconjugates for in vivo imaging and selective photothermal therapy of liver cancer.

RESULTS: We report a method of functionalization of carbon nanotubes with human serum albumin for selective targeting of liver cancer cells. We have investigated the interaction of Gp60-R with FITC-CNT-Alb in human HepG2 cells. Transmission electron, confocal microscopy combined with immunochemical staining was used to demonstrate the selective internalization of fluorescently labeled CNT-Alb via Gp 60-R. We showed that Gp 60-R internalization triggers Cav-1 and PTRF/Cavin translocation from plasma membrane to cytosol and support the critical role of caveolae in CNT-Alb intracellular traveling.

CONCLUSIONS: The presented results impose albumin as having a good potential for the development of CNTs- based targeting agents in human HC.

Keywords: albumin receptor, gp60, carbon nanotubes

Determination of Anti-Carcinogenic Properties of a Newly Synthesized Thiosemicarbazone Derivate on Prostate Cancer Cell Cultures: An In Vitro Study

Ali Beytur¹, Suat Tekin², Süleyman Sandal², Taha Kelestimur³, Mustafa Karatepe⁴, Bayram Yilmaz³

¹Faculty of Medicine, Department of Urology, Inonu University, Malatya, TURKEY

²Faculty of Medicine, Department of Physiology, Inonu University, Malatya, TURKEY

³Faculty of Medicine, Department of Physiology, Yeditepe University, Istanbul, TURKEY

⁴Faculty of Science, Department of Chemistry, Firat University, Elazig, TURKEY

OBJECTIVE: Prostate cancer is the most common type of cancer in males. Thiosemicarbazone (TSC) and Schiff base and their metal complexes have antitumor activity and are being used as antitumor drugs. It has been revealed with quantitative-structure-activity relationship studies that production of new substances by structural changes in these compounds has different effects. In this study, a new TSC (chemical structure: [(4-(1-phenyl-1-methyl-3-cyclobutyl-3-yl)-2-(hydroxybenzylidene hydrazino)thiazol)]) and its Cu complex synthesized in our laboratory was investigated in terms of their mechanism of action and antitumor properties by using androgen-dependent (LNCaP) and independent (DU145) human prostate cancer cell lines.

METHODS: At the first stage of the study, these cell lines were treated with varying concentrations of TSC and Schiff base and their metal complexes (1, 5 and 50 μ M) for 24h. Antitumor activities of these substances were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Mode of action of these antitumor substances was determined by comet assay (reveals DNA damage).

RESULTS: All tested concentrations of TSC, dose-dependently reduced cell viability of LNCaP ($p < 0.005$) while only 50 μ M concentration of TSC caused a significant decrease in viability of DU145 cells ($p < 0.05$). The high dose of Cu complex of TSC reduced cell viability both in LNCaP and DU145 cell lines ($p < 0.001$). TSC and its Cu complex at 50 μ M concentration increased DNA breakage in LNCaP cells, but not in DU145 cells.

CONCLUSIONS: Our results indicate that the tested agents have antitumor activity on human prostate cell lines, and these cytotoxic effects appear to be due to androgen receptor dependent DNA damage. Acknowledgement: This study was supported by Inonu University BAP (Project # 2010/49).

Keywords: LNCaP, DU145, thiosemicarbazone, DNA damage, prostate cancer

Antineoplastic properties of Monensic acid and its biometal(II) complexes

Radostina Alexandrova¹, Tania Zhivkova¹, Dimitar Ivanov¹, Marin Alexandrov¹, Milena Kirilova², George Miloshev², Ivayla Pantcheva³, Mariana Mitewa³

¹Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

²Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria

³Faculty of Chemistry, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

OBJECTIVE: The polyether ionophore Monensin is a natural antibiotic, applied in veterinary medicine as coccidiostatic and antibacterial agent. Recent data have revealed that Monensin expresses also antitumor activity against cell lines from various malignancies including leukemia, lymphoma, myeloma, renal cell carcinoma and cancers of the colon, breast and cervix. The aim of our study was to evaluate the putative antineoplastic properties of metal complexes of Monensic acid (MonH) - $[M(\text{Mon})_2(\text{H}_2\text{O})_2]$ (M = Mg, Ca, Mn, Co, Ni, Zn).

METHODS: The following model systems were used in our experiments: cultured human permanent cell lines established from glioblastoma multiforme (8MGBA) and cancers of the lung (A549), breast (MCF-7), uterine cervix (HeLa), liver (HepG2) and skin (A431); virus-transformed chicken and rat tumor cells expressing v-myc or v-src oncogene, respectively; primary cultures of chemically induced liver cancer of Zajdela in rat. The investigations were carried out by MTT test, neutral red uptake cytotoxicity assay, crystal violet staining, trypan blue dye exclusion technique, method of Bradford and colony forming method. The ability of the compounds to induce double stranded DNA damages was examined by Comet assay. Double staining with acridine orange and propidium iodide was applied to demonstrate the presence of cytopathological changes.

RESULTS: The results obtained reveal that applied at concentrations of 0.5-25 microg/mL for 24-72 h the examined compounds decreased significantly the viability and proliferation of the treated cells in a time- and concentration-dependent manner.

CONCLUSIONS: These metal(II) complexes have been found to exhibit higher cytotoxic and cytostatic activities as compared to the non-coordinated Monensic acid. Acknowledgement: Supported by Grant DO-02-84/2008, National Science Fund, Bulgaria.

Keywords: Human cancer cell lines, Monensin biometal(II) complexes, cytotoxic activity, antiproliferative effects

On pathophysiological motor reactions of urinary bladder preparations after toxicants

Ulrich Haerlin¹, Eva Neu¹, Alfons Hofstetter², Walter Seidenbusch³, Ursula Welscher¹, Michael Ch. Michailov¹

¹Inst. Umweltmedizin c/o ICSD/IAS e.V. Postfach 340316, 80100 Muenchen, Germany (Int. Council Sci. Develop./Int. Acad. Sci. Berlin-Muenchen-Paris-Sofia-Vienna) & Univ. Erl.-Nuernberg, Botanical Garden & Univ. Innsbruck, Inst. Exp.Physics

²Medical Faculty, Univ. Muenchen, Germany

³Inst. Exp.Physics, Univ. Innsbruck, Austria

OBJECTIVE: Therapeutic/pathological reactions to hormones/drugs, esp. in urogenital tract, are well known, but not to toxicants. Summary of recent and earlier observations is given [1,2].

METHODS: Isolated detrusor preparations of guinea-pig (GP) and rat urinary bladder in organ-bath with McEwen-solution (37°C): Influence of chlorophenols and pyrethroids (hydrophobe substances), i.e. pentachlorophenol/cypermethrin in physiological solution +0.1-1% ethanol, trichlorophenol/deltamethrin in physiological solution +0.1-1% acetone, on motor activity (isotonic recording) was investigated. Spontaneous phasic contractions (SPC-frequency:3.84±0.95/min, n=61) and contractions to neurogenic electrical-stimulation (CES:10&100Hz, 0.3ms-3s-3min) were recorded. Changes are given in % of initial values [2].

RESULTS: 1. GP-Detrusor: (a) SPC-amplitudes decreased stronger after pentachlorophenol (10.1±8.3%) than trichlorophenol (55.4±16.2%). (b) Pentachlorophenol/deltamethrin (100µM) inhibited CES-100Hz stronger (25.1±27.5%/9.7±3.8%) than trichlorophenol/cypermethrin (70.9±13.0%/74.7±3.6%). 2. Rat-detrusor: (a) Pentachlorophenol-effect (100µM) was stronger, i.e. abolished SPC-frequency (0.0±0.0%), than at GP-detrusor (55.7±33.9%), (b) also inhibition of rat CES-10Hz (5.6±5.5%) & 100 Hz (2.4±0.3%) was stronger than this of GP (21.3±10.4% & 25.1±27.5% resp.). 3. Varia: (a) Frequency of slow tonic contractions (STC:0.13±0.55/min, n=26) of GP-trigone decreased upto 50% after cypermethrin (10-100µM). (b) Contrary to this HgCl₂ (1-10µM) had strong positive chrono-/inotropic effects.

CONCLUSIONS: Immediate pathophysiological vesical motor reactions could be high sensitive indicators for prophylaxis of functional disturbances (incontinence, overactive-bladder, etc.) - it is recommendable to proof human relevancy on surgical tissue. These toxicants could be also useful for physiological analysis (Cl.Bernard), e.g. role of mechanosensitive ionic channels (Ca²⁺-activated K⁺) for SPC-STC: Pentachlorophenol & cypermethrin (1-100µM) transformed spikes into bursts/burst-plateaus in detrusor-myocytes, i.e. activates stretch channels. Differentiation of neurogenic and myogenic mechanisms needs further experiments [1-3]

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Keywords: pathophysiology, motor reactions, electrostimulation, xenobiotics

Single walled carbon nanotubes functionalized with single strand DNA effects over redox balance. An in vitro and in vivo study

Teodora Mocan¹, Simona Clichici¹, Adriana Filip¹, Alexandru Biris², Stefania Simona², Doina Daicoviciu¹, Nicoleta Decea¹, Remus Moldovan¹, Flaviu Tabaran³, Lucian Mocan⁴, Cornel Catoi³, Adriana Muresan¹

¹University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca, Romania

²National Institute for Isotopic and Molecular Technologies, Cluj-Napoca, Romania

³University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

⁴Institute of Gastroenterology and Hepatology, Cluj-Napoca, Romania

OBJECTIVE: Single wall carbon nanotubes (SWCNT) represent a material of high medical interest. Recent studies rise concerns regarding their possible oxidative stress –inducing effects. Our aim was to evaluate the in vitro and in vivo induced by various concentrations of SWCNT functionalized with single strand DNA(ssDNA- SWCNT) solutions.

METHODS: ss-DNA-SWCNT water solution was obtained through sonication. In vitro experiments were carried out on Hep G2 cell line. 5, 10 and 20 mg/L concentrated solutions were prepared. MTT assay and 5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate (DCFDA) assay were performed. In vivo experiments were carried out on male Wistar rats (170±10g), i.p. injected with 1.5 ml single walled ss-DNA-SWCNT solutions of different concentrations (70g/l, 250 g/l, 390 g/l). Controls were similarly i.p injected with 1.5ml serum. Malondialdehyde (MDA), carbonylated proteins (PC), hydrogen donor ability (HD), sulfhydryl groups (SH) were assessed in blood at 3, 6, 24 and respectively 48 hours after the SWCNT administration.

RESULTS: We obtained a significant in vitro and in vivo alterations of oxidative balance peaking at 24 hours from administration (p<0.05). Results show significant dependence of observed effects on concentration. At 48 hours from exposure, levels of the analyzed markers remained altered.

CONCLUSIONS: Our results support the ability of ss-DNA-SWCNT to generate oxidative stress, the pattern of alterations depending on the concentration of SWCNT solutions. Present work was supported by National Research Council Grant NANOCITOX 42112/2008.

Keywords: carbon nanotubes, oxidative stress, in vitro, in vivo

2-hydroxy arachidonic acid (2OAA), a new non steroidal anti-inflammatory drug

Daniel Horacio López¹, Maria Antonia Fiol², María Laura Martín², María Antonia Noguera Salvá², Silvia Terés¹, Pablo Escribá², Xavier Busquets²

¹Lipopharma Therapeutics

²Department of Biology-IUNICS, University of the Balearic Islands, Spain

OBJECTIVE: Arachidonic acid (AA) is one of the most abundant lipids in the cellular membrane. AA is then converted into a number of pro-inflammatory mediators including prostaglandins (PGs) and thromboxanes (TXs). Non steroidal anti-inflammatory drugs (NSAIDs) are a family of molecules used in the treatment of inflammation, pain and fever. These compounds block PGs and TXs synthesis inhibiting COX1 and COX2 activities. Our objective is the synthesis of a new NSAID based on the structure of AA.

METHODS: We have synthesized a new non-toxic lipid derivative: the 2-hydroxy arachidonic acid (2OAA) designed by computational docking analysis to interfere with COX1 and COX2. To study the effectiveness of 2OAA in an in vivo model we measured TNF-alpha plasma levels in C57BL6/J mice challenged with LPS (20 µg) and treated with 2OAA at 50, 200 and 500 mg/Kg (5 animals per group).

RESULTS: Our results demonstrated that 2OAA inhibited COX1 activity and COX2 activity and protein/mRNA expression in human U937 monocytes-derived macrophages challenged with lipopolysaccharide (LPS). In addition 2OAA also demonstrated to inhibit iNOS expression and NO production in mouse BV2 microglial cells. To study the effectiveness of 2OAA in an in vivo model we measured TNF-alpha plasma levels in C57BL6/J mice challenged with LPS. Our results demonstrated that oral administration of 2OAA lowered the TNF-alpha plasma levels induced by LPS. The TNF-alpha plasma level inhibition by 2OAA was greater than the inhibition produced by ibuprofen and cortisone in the same experiment.

CONCLUSIONS: These results show the potential of 2OAA as a non-toxic anti-inflammatory/antioxidant drug.

Keywords: inflammation, COX-1, COX-2, NSAID, iNOS, NOS, cancer, Alzheimer disease

Investigating the effect of lactate on myometrial contractility

Jacqui Ann Hanley, Susan Wray

Department of Physiology, University of Liverpool, Liverpool, United Kingdom

OBJECTIVE: Dysfunctional labour is one of the commonest causes for emergency caesarean section (CS) and is characterised by weak, uncoordinated uterine contractions. Lactate has been previously shown to increase in the myometrium during dysfunctional labour (Quenby et al 2004) but currently no work has been done to investigate the effect of lactate on the myometrium. We have therefore examined its effect on contractility.

METHODS: Myometrial strips were taken from late pregnant rats or biopsies obtained with consent from women undergoing elective CS at term. The effects of contractility in response to lactate (1-20mM) and other weak acids (Butyrate, propionate and pyruvate, 5-20mM) were recorded. In some experiments, simultaneous force and intracellular pH analysis were carried out.

RESULTS: Lactate significantly decreased contractility (n=8 rat, n=4 human) A dose dependent decrease in integral force of contraction was seen, which was significant at 5mM (rat=36%±14%, human=44%±17%) relative to control (100%). Other weak acids also significantly affected contractions in a dose dependent manner. Measurements of pHi suggest this response is associated with intracellular acidification.

CONCLUSIONS: Lactate in the physiological range and other weak acids potentially decrease myometrial contractility. Initial pHi measurements suggest its effect is due to increasing acidity of the myometrium, which will decrease Ca²⁺ current. This could therefore cause the decreased contractions seen in dysfunctional labour. Ongoing investigations of Ca²⁺ signalling will shed further mechanistic insight.

Keywords: Uterus, lactate, myometrium, contractility, weak acids, labour

Inhibitory effects of metamizol on prostaglandin F2-alpha-induced contraction of rat myometrium: with reference to in vitro model for primary dysmenorrhea

Damlağül Aydın, Ahmet Ayar

Karadeniz Technical University, Faculty of Medicine, Department of Physiology, Trabzon

OBJECTIVE: Primary dysmenorrhea, also known as menstrual cramping, is believed to result from uterine muscle ischemia through vasoconstriction and myometrial contraction due to excessive production of prostaglandins. The aim of this study was to investigate the effects of metamizole, commonly used for menstrual pain, on the contractility of isolated rat myometrium to determine whether it has also relaxing effect on the myometrium.

METHODS: Myometrial strips were obtained from non-pregnant Wistar rats following decapitation and suspended under 1 gram of resting tension in a double-jacketed isolated tissue bath filled with physiological saline, continuously gassed with 95% O₂-5% CO₂ at pH 7. PGF₂ α (1 μ M) was added to stimulate contractions and cumulatively added concentrations (7, 20, 30 and 50 mM) of metamizole-Na was tested on amplitude, frequency and area-under-contraction-curve (AUC) by 10 minutes intervals. Data were analysed using ANNOVA, p<0.05 being statistically significant.

RESULTS: PGF₂ α significantly stimulated spontaneous contractions which were inhibited by metamizole in a dose-dependant manner. The mean frequency of PGF₂ α -induced contractions was 13.6 \pm 3.8, which was inhibited to 11.9 \pm 3.8 (p>0.05), 10 \pm 1.5 (p<0.05), 8 \pm 1 (p<0.01), 4.6 \pm 1.6 (p<0.001), (n=9 for each). Mean percentage inhibition of the AUC of PGF₂ α -induced contractions was found to be 0% (p<0.05), 16 % (p<0.05), 42 % (p<0.01) and 67 % (p>0.001) after application of 7, 20, 30 and 50 mM metamizole, respectively (n= 9 for each).

CONCLUSIONS: Data from this study indicates that metamizole inhibits the PGF₂ α -induced contractions of rat myometrium in a dose dependent manner, but requiring higher concentrations could be achieved after conventional use.

Keywords: smooth muscle, contraction, dysmenorrhea, metamizole-Na

The effects of transient multiple episodes of hypoxia on spontaneous rat uterine contractility: does hypoxic preconditioning occur?

Mohammed Fahad Alotaibi, Susan Wray

Department of Physiology, Liverpool University, Liverpool, United Kingdom

OBJECTIVE: To investigate in vitro the effect of short multiple episodes of hypoxia on rat uterine contractility.

METHODS: Longitudinal uterine strips (2mm×10mm) from pregnant and non pregnant rats were dissected and mounted in organ bath, bubbled with Hepes buffered, oxygenated physiological saline, for isometric force recording. The effect of repeated 5 min hypoxia was induced by replacing the O₂ for N₂. Paired control experiments were bubbled with O₂ throughout. Contractions were analyzed during and after each hypoxic episode.

RESULTS: In stable, spontaneously active preparations, the effect of a single episode of hypoxia was to significantly decrease or abolish the contractions in both pregnant and non pregnant rats (n=12 and 6 respectively). In pregnant rats, there was a gradual increase in force during and after each hypoxic episode. By the 5th hypoxic episode the amplitude of contractions upon re-oxygenation was significantly higher (141± 8.7% compared to 100% control) than that of the control period and was unchanged in time matched controls. There was also increased contractile activity during the hypoxic period. In contrast, in non pregnant rats hypoxic episodes always abolished contractions and although there was recovery with re-oxygenation, activity never exceeded that seen at the start of the experiments.

CONCLUSIONS: We concluded that transient, repeated hypoxic episodes significantly increase the force amplitude produced by pregnant rat uterus. This suggests that hypoxic preconditioning may be present in the uterus. In non pregnant rats, the phenomenon was not seen, suggesting that this could be part of a mechanism switched on in preparation for labour.

Keywords: Hypoxia, hypoxic preconditioning, spontaneous contractility, uterus

Cyclosporine normalizes oxidative stress and partially protects skeletal muscle from ischemia-reperfusion induced mitochondrial dysfunction: an involvement of cyclophilin D ?

Julien Pottecher¹, Elise Belaidi³, Anne Laure Charles², Max Guillot¹, Jamal Bouitbir², Olivier Collanges¹, Anne Lejay², Joffrey Zoll², Pierre Diemunsch¹, Abdallah Gharib³, Bernard Geny²

¹Pôle Anesthésie Réanimation Chirurgicale, Hôpitaux Universitaires de Strasbourg, Equipe d'Accueil 3072, Université de Strasbourg, France

²Physiologie et d'Explorations Fonctionnelles, Hôpitaux Universitaires de Strasbourg, Equipe d'Accueil 3072, Université de Strasbourg, France

³Laboratoire de Physiologie, Inserm 886, Faculté de Médecine Lyon, France

OBJECTIVE: Cyclosporine (CsA) reduces myocardial infarction through cyclophilin D (CypD) blockade. Preliminary data show that CsA partially decreases gastrocnemius (GC) mitochondrial dysfunction after aortic clamping (1). To further determine the implication of CypD, we determined CypD levels in normal heart and GC and in GC after ischemia-reperfusion (IR).

METHODS: Anaesthetized Wistar rats were randomized to control (n = 8), IR (n = 10, clamping of the infra-renal aorta for 3 h followed by 2 h of reperfusion), or IR+CsA groups (n = 7, 10mg/kg CsA administered intraperitoneally 90 and 30 min prior to reperfusion). Maximal oxidative capacity (V_{max}) and coupling of phosphorylation to oxidation (ACR) were determined in GC permeabilized fibers. Tissue superoxide anion was assessed with dihydroethidium (DHE) staining. CypD expression was assessed by Western-blot.

RESULTS: CypD levels were increased in normal heart as compared to GC (5.52±0.86 vs 2.18±0.38 arbitrary units (A.U.), p<0.05). Compared to Sham, IR significantly decreased GC V_{max} (5.98±0.56 vs 4.08±0.38 μMO₂/min/g; p < 0.05), ACR (1.98±0.20 vs 1.38±0.06; p<0.05) and increased GC superoxide anion (3992±706 vs. 1812±322 A.U.; p< 0.05). IR tended to decrease GC CypD expression (-21%, NS) CsA normalized ACR and superoxide anion, partially restored V_{max} (5.02±0.39 μMO₂/min/g; NS), and unmodified CypD (1.28±0.14 A.U.).

CONCLUSIONS: Aortic cross clamping induced GC mitochondrial dysfunction and ROS overproduction. CsA decreased oxidative stress and partially restored GC mitochondrial function. A lower level of CypD in GC, as compared to the heart might participate in these results.

Reference: 1) Pottecher J et al (2010) Intensive Care Med 32: S143.

Keywords: reperfusion injury, cyclosporine A, skeletal muscle, mitochondria, reactive oxygen species

Effects of estrogen on eccentric exercise induced skeletal muscle damage

Serpil Can¹, Fatma Özabacıgil Gür², Ismail Can³, Kenan Gümüştekin⁴, Serap Yildirim¹, Ali Karadeniz⁵, Bünyami Ünal⁶, Mustafa Gül¹

¹Department of Physiology, Faculty of Medicine, Atatürk University, Erzurum, Turkey

²Biotechnology Application and Research Center, Atatürk University, Erzurum, Turkey

³Department of Histology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey

⁴Department of Physiology, Faculty of Medicine, Abant İzzet Baysal University, Bolu, Turkey

⁵Department of Physiology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey

⁶Department of Histology, Faculty of Medicine, Atatürk University, Erzurum, Turkey

OBJECTIVE: The aim of this study was to investigate whether estrogen can prevent eccentric exercise induced muscle damage in skeletal muscle in rat.

METHODS: Male rats (n=36) were divided equally as estrogen supplemented (n=18) and non supplemented groups (n=18), then these groups were further divided as rest (n=6) and groups exposed to eccentric exercise (n=12). Eccentric exercise groups were further divided as rats killed after 1 h (n=6) and 48 h (n=6) of eccentric exercise. Estrogen (17- β Estradiol, 10 μ g/kg per day) was administered subcutaneously for 30 days. Eccentric exercise was applied as treadmill run (15o downhill, 20 m/min) consisting of periods of 5 min run and 2 min rest repeated 18 times. Leukocyte infiltration in soleus muscle was examined histologically. Activities of glutathione S-transferase, glutathione peroxidase, superoxide dismutase and catalase were also measured spectrophotometrically.

RESULTS: Estrogen alone decreased the glutathione peroxidase activity in soleus muscle compared with the control group. However, when estrogen was combined with eccentric exercise, glutathione peroxidase activity was increased. The increase in oxygen consumption during exercise and consequently increased formation of reactive oxygen species could have caused a compensatory increase in glutathione peroxidase activity, which might have been improved by estrogen. Eccentric exercise increased neutrophil infiltration in skeletal muscle suggesting the presence of the muscle damage. Leukocyte infiltration in skeletal muscle has significantly increased two fold after 48 h compared with the numbers after 1 h, and estrogen supplementation was not able to prevent this infiltration.

CONCLUSIONS: Estrogen seemed to be not very effective to prevent eccentric exercise-induced skeletal muscle damage. Supported by Ataturk University Research Fund (2005/192).

Keywords: Eccentric exercise, estrogen, muscle damage, neutrophil, antioxidant enzymes, rat, soleus muscle.

Hemodynamic Effects of ANP in Global Ischemia-Reperfusion Injury Which has Occurred After the Exercise

Selma Arzu Vardar¹, Orkide Palabıyık¹, Ruhan Topuz², Elif Ezgi Gürel¹, Semra Çalışkan², Serap Topçu¹, Çetin Hakan Karadağ²

¹Department of Physiology, Trakya University Medical Faculty, Edirne, Turkey

²Department of Pharmacology, Trakya University Medical Faculty, Edirne, Turkey

OBJECTIVE: The aim of this study was to compare the hemodynamic effects of atrial natriuretic peptide (ANP) in ischemia followed by reperfusion in exercised rat heart with the non-exercised one.

METHODS: Male Sprague-Dawley rats were divided into exercise (E1, E2, E3, E4, E5) and non-exercise (NE1, NE2, NE3, NE4, NE5, NE6) groups (n=6 each). E groups were exposed to running protocol for 5 days. Global ischemia (35 minutes) and 30-minute-reperfusion were applied in E1 and NE1 groups. ANP (0.1 µM/L) was also administered before ischemia in E2 and NE2 groups. Isatin (0.1-10-100 µM/L), which is known as ANP receptor blocker, was administered before ANP in E3, E4, E5 and NE3, NE4, NE5 and NE6 (without ANP) groups. Left ventricular developed pressure (DP) and the maximum-minimum rates of change in left ventricular pressure (dp/dtmax, dp/dtmin) were recorded during the experiments.

RESULTS: Significantly decreased DP values were shown in E1 (103±13.0 versus 15.4±14.9 mmHg) and NE1 (110.8±12.2 versus 63.4±28.7 mmHg) groups at the 1 sec of reperfusion (p<0.01 and p<0.05). However no significant decrease in DP (80.3±7.9 versus 54.5±19.7 mmHg), +dp/dt (1408.0±330.3 mmHg/s versus 394.5±278.4 mmHg/s) and -dp/dt (-1725.0±181.7 mmHg/s versus -833.6±193.8 mmHg/s) values was observed in E2 group at the 1 sec of reperfusion. Infusion of different doses of isatin did not significantly change hemodynamic values in groups.

CONCLUSIONS: This study indicated that deleterious contractile force occur in reperfusion period following short term treadmill running in the left ventricle. Administration of ANP before ischemia may improve decreased contractile forces in reperfusion period in exercised rat heart.

Keywords: Atrial natriuretic peptide, heart, ischemia, reperfusion

Effects of Endurance Exercise on Nuclear PGC-1 α /MEF2 Association of Skeletal Muscles in AMPK α 2 Transgenic and Knockout Mice

Ying Zhang, Nan Zhang, Haojie Gong
Institute of Sport Science, Beijing Sport University, China 10084

OBJECTIVE: Exercise increases the metabolic capacity of skeletal muscle. At the level of transcription, many genes are regulated by peroxisome proliferator-activated receptor-gamma coactivator 1 (PGC-1) of transcriptional activator, through their interaction with myocyte enhancer factor 2 (MEF2). However, the molecular mechanisms involved remain elusive. The role of AMPK α 2 in regulating the endurance exercise induced nuclear PGC-1 α protein expression and nuclear PGC-1 α association with MEF2 was investigated.

METHODS: This was investigated in muscles from AMPK α 2 over-expression (OE;n=20), AMPK α 2 knockout (KO; n=20) and corresponding wild-type (WT;n=20) mice that had undertaken a program of treadmill training at the speed of 12m/min with a slope of 0 at the speed of 12m/min with a slope of 0, 60min/day for 28 days. The nuclear PGC-1 α protein expression was measured by western blot and nuclear PGC-1 α association with MEF2 by CoIP.

RESULTS: After four weeks of endurance training, the nuclear PGC-1 α content and PGC-1 α associated with MEF2 in OE, KO and WT mice were significantly higher than their control groups respectively, however, OE AMPK α 2 isoform heightened the training-induced increase in nuclear PGC-1 α content and PGC-1 α associated with MEF2 compared with WT mice, while the training-induced increases of nuclear PGC-1 α content and PGC-1 α associated with MEF2 were normal in α 2-KO muscles compared with WT mice despite the reduced AMPK signaling.

CONCLUSIONS: Exercise training can activate nuclear PGC-1 α protein expression and increase PGC-1 α /MEF2 association. And then AMPK activity during exercise is required, though not indispensable, for expression of PGC-1 α protein and PGC-1 α /MEF2 association.

Keywords: AMPK- α 2; nuclear PGC-1 α association with MEF-2; nuclear PGC-1 α protein expression

Time-parameters for comparison of angiotensin II - induced contractions: in vitro experiments with rat gastrointestinal segments

Petya Hadzhibozheva¹, Tsvetelin Georgiev¹, Rosen Iliev², Galina Ilieva¹, Reni Kalfin³, Anna Tolekova¹

¹Department of Physiology, Pathophysiology and Pharmacology, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

²Department of Propaedeutics of Internal Diseases, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

³Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

OBJECTIVE: Angiotensin II (Ang II) has a potent contractile effect on smooth muscles in the gastrointestinal tract (GIT). The present study was designed to compare the registered responses to Ang II of different segments from GIT by application of time-parameters.

METHODS: Longitudinal strips from Wistar rats' stomach and intestine were prepared and influenced by Ang II. The recorded force-vs.-time curves permit determination of amplitudes, area under the curve (AUC) of the smooth muscle contraction, as well as defining of time-parameters: half-contraction time (Thc), contraction time (Tc), half-relaxation time (Thr), contraction plus half-relaxation time (Tchr).

RESULTS: There is a gradual increase in the amplitude of the Ang II - provoked muscle contraction along the rat intestine. The analysis of time-parameters indicated that the stomach response to Ang II required more time to develop - Tc-78.18±5.87 s and Tchr-224.90±18.45 s. All of the recorded intestinal contractions showed similar values for each of the time-parameters with exception of the ileum, which Thr (106.33±9.89 s) and Tchr (141.08±9.48 s) were significantly prolonged.

CONCLUSIONS: The use of time-parameters enables a detailed study of smooth muscle contractions. These parameters significantly contribute to the analysis of the contraction process and permit a good comparison of the Ang II – induced responses. The observed differences in the duration of gastrointestinal contractions may be due to variations in the Ang II receptor subtypes distribution and the corresponding interaction of Ang II with them.

Keywords: angiotensin II, gastrointestinal tract, time-parameters, isolated tissues, smooth muscles

Hypoxic adaptation is associated with the muscle thioredoxin and heat shock protein responses

Şevin Güney¹, Ayhan Korkmaz¹, Ayşe Şebnem İlhan², Mustafa Atalay³, Sibel Dinçer¹

¹Gazi University, Faculty of Medicine Department of Physiology, Ankara, Turkey

²Başkent University, Faculty of Medicine, Department of Physiology, Ankara, Turkey

³University of Eastern Finland, institute of Biomedicine, Department of Physiology, Kuopio, Finland

OBJECTIVE: In the present study we aimed to investigate the levels of HSPs, a major intracellular defense system for protein homeostasis, and antioxidant redox regulator thioredoxin in soleus muscle after the exhaustive exercise in the conditions of hypoxic adaptation.

METHODS: Eight-week-old Wistar Albino male rats were divided in normoxia (n=6) and hypoxia (n=7) groups. Rats in the hypoxia group were exposed to 10% hypoxia in a hypoxic chamber for 2 days. After the hypoxic exposure, all animals were subjected to exhaustive exercise on a treadmill and running time to exhaustion was recorded as exhaustion time. Upon exhaustion, they were sacrificed by cardiac puncture, and soleus muscle samples were collected for further analyses. Levels of HSP 60, HSP70, HSP90 and thioredoxin were measured by western blotting.

RESULTS: There were no statistically significant changes for the measured parameters in hypoxia group, when they compared with their normoxic controls. However there was a positive correlation between Trx and HSP70 in both normoxia and hypoxia groups. There was also a positive correlation between exhaustion time and thioredoxin, HSP70, HSP90 in hypoxia group. Increase in HSP 70 and HSP 90 levels are positively correlated in hypoxia group too.

CONCLUSIONS: Correlations among the measured parameters suggest that hypoxic adaptation might increase the exercise performance and contribute to protection against exercise induced muscle damage by activating HSPs and thioredoxin in the muscle.

Keywords: Hypoxic adaptation, muscle, exercise, heat shock proteins, thioredoxin

Expression of Atrial Natriuretic Peptide in the Hearts of Two Rat Strains: Effects of Two Types of Restraint Stressors

Jana Slavikova¹, Eliska Mistrova¹, Magdalena Chottova Dvorakova¹, Sixtus Hynie², Pavel Sida², Vera Klenerova²

¹Department of Physiology, Faculty of Medicine, Charles University, Plzen, Czech Republic

²Institute of Medical Biochemistry, 1st Faculty of Medicine, Charles University, Prague, Czech Republic

OBJECTIVE: Mammalian cardiomyocytes produce atrial natriuretic peptide (ANP) with regulatory role in body fluid homeostasis. ANP induces suppression of the renin-angiotensin as well as the sympathetic nervous systems to protect cardiovascular homeostasis, which is also deteriorated by the stress. Here, we investigated whether ANP mRNA expression in the heart could be affected by stress.

METHODS: Heart preparations were analyzed by real-time RT-PCR. The two rat strains, Sprague-Dawley (SD) and Lewis (LEW), were used, the latter being known to have a blunted hypothalamic-pituitary-adrenal response. A restraint stressor (immobilization, IMO) and IMO combined with partial immersion of rats into water (IMO+C) were applied to rats for one hour. One or three hours after the stress termination, the rats were decapitated, hearts removed, and each atrium and ventricle were examined separately. Expressions of ANP mRNA were determined in controls and stressed rats (IMO1, IMO3, IMO+C1, IMO+C3).

RESULTS: Expression of ANP mRNA in the right atria of SD rats increased significantly by 47% after IMO1. An increase by 41% after IMO3 was observed in the left atria. IMO1, IMO3, and IMO1+C3 caused a significant increase of the ANP mRNA expression also in the left ventricles. However, a decline by 27% and 29% was shown after IMO1 and IMO+C1, respectively, in the right atria of LEW rats.

CONCLUSIONS: Restraint stressors induced changes in expression of ANP mRNA in cardiomyocytes of atria and left ventricles; these effects may have relevance to ANP-induced cardioprotection. Supported by grants MSM0021620806 and MSM0021620819.

Keywords: rat heart, stress, ANP mRNA expression

Does hypercholesterolemia affect the contractions of the smooth muscles of the urinary bladder?

Z. Dicle Balkanci¹, Bilge Pehlivanoglu¹, Sibel Bayrak¹, Aysen Erdem¹, Ismail Karabulut¹, Serkan Karaismailoglu¹, Gulsen Oner²

¹Department of Physiology, Hacettepe University Faculty of Medicine, Ankara, Turkey

²Department of Physiology, Akdeniz University Faculty of Medicine, Antalya, Turkey

OBJECTIVE: Albeit the effects of hypercholesterolemia on ion channels' and smooth muscle cells' functions have been investigated extensively, limited information on function of urinary bladder is available. Therefore, we aimed to investigate the effects of hypercholesterolemia on contractions of in vitro urinary bladder smooth muscles in rats.

METHODS: Serum lipid profiles and tissue cholesterol levels of adult male Sprague-Dawley rats, fed with standard (C, n=25) or 4% cholesterol diet (HC, n=26) for four weeks were measured. Aorta was examined microscopically for atherosclerosis. Spontaneous contractions of detrusor muscle strips and their responses to 80mM KCl and electrical field stimulation (EFS) or cumulative dose-contraction curves to carbachol (10-8-10-4M) were recorded. Carbachol dose-contraction curves were repeated in the presence of L-type calcium channel blocker nifedipine (10-6M) and/or rho-kinase inhibitor Y27632 (10-5M). Data were analyzed statistically.

RESULTS: Plasma cholesterol was elevated in HC group (C:89.24±3.81, HC:155.38±9.66 mg/dL, P<0.05), tissue cholesterol levels were similar. There was no sign of atherosclerosis. The amplitude of basal spontaneous (P>0.05) and KCl-induced (P<0.05) contractions were greater in HC group (HC:29.1±1.7; C:21.2±1.6 g/100mg tissue). EFS and carbachol-induced contractions were comparable among groups. Nifedipine, Y27632 and nifedipin+Y27632 reduced carbachol-induced contractions to 39%, 23%, 88% of pre-drug controls in the C group and to 68%, 22%, 90% of pre-drug controls in the HC group.

CONCLUSIONS: Increased KCl-induced and spontaneous contractions together with potent attenuation of carbachol-induced contractions with nifedipine strips in hypercholesterolemia group, collectively, suggest that hypercholesterolemia has an impact on both sarcolemmal L-type calcium channels and contractions of detrusor strips.

Keywords: Hypercholesterolemia, smooth muscle, L-type calcium channel, rat

The effect of muscarinic receptors agonists on airway smooth muscle cell proliferation and phenotype

Apostolia Hatziefthimiou¹, Rodopi Stamatou¹, Efrosini Paraskeva¹, Eleni Gianni¹, Konstantinos Gourgoulianis², Paschalis Adam Molyvdas¹

¹Department of Physiology, Medical School, University of Thessaly, Greece

²Department of Respiratory Medicine, Medical School, University of Thessaly, Greece

OBJECTIVE: Chronic airway diseases, such as asthma or COPD, are characterized by excessive acetylcholine release and in some cases airway remodeling. The aim of this study was to investigate the effect of muscarinic agonists (acetylcholine or carbachol) on the proliferation and phenotype of rabbit tracheal airway smooth muscle cells (ASMC).

METHODS: Serum starved cells for 3-15 days were treated with muscarinic agonists and proliferation was estimated using the Cell Titer 96® AQueous One Solution Assay (Promega) or the methyl-[3H]thymidine incorporation method. The involvement of signaling pathways was studied using Western blot analysis in total protein cell extracts and the inhibitors of the PI3K and the MAPK pathways LY294002 and PD89005 respectively. The cell phenotype was studied by indirect immunofluorescence, using antibodies against smooth muscle α -actin and Myosin Heavy Chain (MHC).

RESULTS: In 3 days starved ASMC, both acetylcholine and carbachol increase the ratio of phospho-Akt and phospho-p42/44 to β -actin and DNA synthesis without an effect on cell number. The percentage of cells expressing α -actin or MHC is increased correspondingly to the days of serum starvation. On the contrary in cells treated for 15 days with acetylcholine, carbachol or 10% FBS the percentage of cells expressing α -actin or MHC was significantly reduced compared to control cells. In 7 days starved ASMC the treatment of cells for 24h with acetylcholine or carbachol induced an increase in cell number.

CONCLUSIONS: Muscarinic agonists affect ASMC phenotype and induce proliferation, via activation of the PI3K and the MAPK signaling pathways, in rabbit ASMC that have been exposed to prolonged serum starvation.

Keywords: airway smooth muscle, muscarinic agonist, acetylcholine, carbachol, phenotype, proliferation

Induction of airway smooth muscle proliferation by TGF and bFGF: the effect of sex hormones

Apostolia Hatziefthimiou¹, Rodopi Stamatou¹, Efrosini Paraskeva¹, Anta Salatai¹, Konstantinos Gourgoulialis², Paschalis Adam Molyvdas¹

¹Department of Physiology, Medical School, University of Thessaly, Greece

²Department of Respiratory Medicine, Medical School, University of Thessaly, Greece

OBJECTIVE: Airway wall remodeling, a major characteristic of chronic airway diseases, such as asthma, is believed to contribute to the disease pathogenesis. It is manifested, among others, by the hypertrophy of airway smooth muscle cells (ASMC), caused by the accumulation of inflammatory mediators, including growth factors and cytokines. We have previously shown that 24h exposure of rabbit tracheal ASMC to the genetic hormones, testosterone and 17 β -estradiol, caused a transient induction of cell proliferation. The purpose of this study was to examine the effect of growth factors TGF and bFGF alone or combined with genetic hormones, in ASMC proliferation.

METHODS: Serum starved rabbit tracheal ASMCs were treated with TGF or bFGF and the proliferation was estimated using the Cell Titer 96® Aqueous One Solution Assay (Promega) and the methyl-[3H]thymidine incorporation method. The involvement of the PI3K and the MAPK signaling pathways was studied using the inhibitors LY 294002 and PD 89005 respectively and Western blot analysis in total protein cell extracts.

RESULTS: TGF and bFGF activate the p42/42, p38 MAPK and/or the PI3K signaling pathways, increase DNA synthesis and cell number, after 48h of incubation. The co-incubation of ASMCs for 48h with TGF or bFGF and hormones show that testosterone does not change the effect of either growth factor on ASMC proliferation while 17 β -estradiol abolished the mitogenic effect of TGF but not of bFGF.

CONCLUSIONS: Growth factors, TGF and bFGF induce ASMC proliferation via MAPK and PI3K pathways. Only the mitogenic effect of TGF is affected by 17 β -estradiol.

Keywords: airway smooth muscle, remodeling, TGF, bFGF, 17 β -estradiol, testosterone

Influence of the acetylcholine and the isoproterenol on the membrane potential in the rat pulmonary veins myocardium

Vladislav Stephanovich Kuzmin, Yuriy Vladimirovich Egorov

Laboratory of Heart Electrophysiology, Russian Cardiological Research and Production Center, Moscow, Russian Federation

OBJECTIVE: Much attention is paid to the pulmonary veins (PVs) myocardium as a region responsible for activity triggering atrial fibrillation. However, only several studies dedicated on electrophysiology of rat PVs myocardium. The aim of the present study is to investigate effects of acetylcholine (ACh) and beta-adrenoreceptors agonist isoproterenol (ISO) on membrane potential of rat PVs myocardium.

METHODS: Multicellular preparations including part of left atria and PV of left lung lobe were dissected from male Wistar (200-250 g) rats hearts. Resting (RP) and action potentials (APs) were recorded from left atria appendage (LAA, control) and proximal part of PV with use of standard microelectrode technique. RP (and APs) were recorded in electrically paced and quiescent preparations.

RESULTS: Application of ACh (10 μ M) and ISO (5 μ M) induced slight hyperpolarization in LAA and in PV during pacing. In quiescent preparation (10 min after pacing termination) RP in PV region stabilized at level -60 ± 5 mV. Application of ACh and ISO induced strong hyperpolarization (up to -87 ± 2 and -73 ± 4 mV respectively). Hyperpolarization induced both by ACh and ISO in PV region of paced and quiescent preparations was practically fully abolished in presence of barium ions (50 μ M, 5 min) - blocker of inward rectifier potassium channels. Automatic activity and afterdepolarizations were observed after barium application in PV region (but not in LAA) of ISO-treated preparations.

CONCLUSIONS: Both ACh and ISO induce hyperpolarization in the rat PVs myocardium. Application of barium leads to abolishing of drug-induced hyperpolarization. Thereby, ISO-induced hyperpolarization in rat PVs myocardium, probably, is mediated via activations of IK, ACh as in case of acetylcholine.

Keywords: atrial fibrillation, pulmonary veins, automatic activity, acetylcholine

Impedance plethysmography measurements in peripheral vascular territory

Calin Corciova¹, Daniela Matei¹, Alexandru Salceanu², Flavia Corciova³

¹„Gr. T. Popa” University of Medicine and Pharmacy, Faculty of Medical Bioengineering, Iasi, Romania

²„Gh. Asachi” Technical University, Faculty of Electrical Engineering, Iasi, Romania

³Institute of Cardiovascular Diseases, Iasi, Romania

OBJECTIVE: The aim of this study was to acquire non-invasively and to analyze plethysmographic waves by measuring the electrical impedance of a peripheral segment.

METHODS: We built a device that is designed as an injection module with a microcontroller which generates a sinusoidal pulse with adjustable frequency (10 – 200 kHz) and adjustable intensity. In output, the device delivers an analogical signal representing the variation of the impedance of the explored section. Disposable electrodes were used for the injection of the sinusoidal pulse and the collection of the bioimpedance signal. The acquisition of the bioimpedance signal on PC was made easier by the use of a National Instrument data acquisition device, the NI USB 6009. The bioimpedance signal processing, the user interface and the display were managed by MATLAB.

RESULTS: We synchronized the plethysmographic wave with the electrocardiographic wave and we calculated some important parameters, such as: blood flow, amplitude, height of the dicrote incision, maximum dicrotic height, rise time, index Kunert, sfigmic velocity. We have also calculated the Fourier and the phase coefficients which describe the shape of the pulse curve and are useful for the automatic pulse wave analysis. The relationship between Fourier coefficients allows the evaluation of the elasticity of the arterial vessels in the measured segment.

CONCLUSIONS: The novelty of our method is that it analyses non-invasively the plethysmographic wave in the peripheral segments. Our device assists the physician in the diagnosis and continual monitoring of the peripheral vascular status of the patient. Knowledge of the vascular changes affected by different physiological or pathological conditions (obliterative processes, stenoses, angiopathies) will provide valuable information.

Keywords: impedance, plethysmographic wave, acquisition device, blood flow, physiological parameters

The Effect of Intermittent Fasting and Water Restriction on Ischemia and Reperfusion Induced Arrhythmias in Rats

Salih Tunç Kaya¹, Selçuk Yaşar², Hayriye Soyuturk Orallar², Ömer Bozdoğan²

¹Department of Biology, Duzce University, Duzce, Turkey

²Department of Biology, Abant İzzet Baysal University, Bolu, Turkey

OBJECTIVE: Ischemia and reperfusion induced arrhythmias such as ventricular tachycardia or ventricular fibrillation are one of the most important causes of death in myocardial ischemia in human being. That is why, it is important to find factors increasing resistance against myocardial ischemia. It has been demonstrated that intermittent fasting without water restriction increases life span and reduces myocardial infarct size. There could not be found any research related with the effect of either intermittent fasting or water restriction on the ischemia and reperfusion induced arrhythmias in rats.

METHODS: In this study, thirty male rats in 8-9 months old were used. Six minutes ischemia was produced by ligation of left coronary artery which was followed by six minute reperfusion by releasing of this artery. Control group was fed ad libitum. IFW1 group was subjected to intermittent fasting and water restriction between 8 pm and 8 am for one month. IFW2 group was maintained one month intermittent fasting and water restriction followed by one month normal feeding. Electrocardiogram (ECG) and arterial blood pressure were recorded during ischemia and reperfusion.

RESULTS: There was no significant difference in arrhythmia score calculated from duration and type of arrhythmia among groups. But arrhythmic period during ischemia in IFW1 was longer in respect to other groups ($p < 0.05$). The incidence of arrhythmias in IFW2 group nonsignificantly and risk of infarct zone significantly increased compared to other groups.

CONCLUSIONS: It was found that the intermittent fasting and water restriction that is simulation of an experimental model of Ramadan fasting did not have any effect on the severity of arrhythmia induced by ischemia and reperfusion.

Keywords: Myocardial Ischemia, Reperfusion, Fasting, Water Restriction, Arrhythmia

The acute effect of Shisha smoking on arterial stiffness and pulse wave velocity

Mohamed Al Kubati¹, Abdul Samid Al Kubati², Abdullah Ahmed Gunaid³

¹Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

²NLEP, Taiz, Yemen

³Faculty of Medicine and Health Sciences, Sana'a University, Sana'a, Yemen

OBJECTIVE: The aim of this study was to investigate the acute effects of Shisha Smoking (ShS) on the arterial stiffness, pulse wave velocity (PWV) and other hemodynamic parameters.

METHODS: Ten healthy men (age 31±8 years; mean±SD) were enrolled into this study. Arterial stiffness measurements were investigated by non-invasive Applanation tonometry (Sphygmo-Cor) over the right radial and carotid arteries, while in seated position, after 15 minutes rest, during optimal conditions and at the same durnal time. The measurements for the pulse wave analyses for radial (-ra) and aortic (-ao) blood pressure in mmHg (systolic: SBP, diastolic: DBP, pulse: PP and mean: MBP), heart rate (HR), augmentation pressure (AP), augmentation index (AIx), ejection duration (ED%), subendocardial viability ratio (SEVR%), time of reflection (Tr) and pulse wave velocity (PWV) were taken before (ShS) (after more than 12 hours of smoking cessation with a complete stopping of alcohol, coffee or tea drinking) and repeated after the (ShS) session, which lasted 30 min. for smoking 5 grams of fruit-flavoured tobacco.

RESULTS: Significant increases in the results after (ShS) were found in HR ($p=0.00067$), carotid-radial pulse wave velocity (brachial-PWV) ($p=0.041$), in DBP-ra ($p=0.023$) but non-significant increases in SBP-ra ($p=0.082$), MBP-ra ($p=0.082$), SBP-ao, DBP-ao ($p=0.064$) and MBP-ao. Significant decreases after (ShS) were found in AP ($p=0.0412$), AIx ($p=0.0257$), SEVR% ($p=0.00579$) and non-significant decrease in (Tr), but an increase in ED% ($p=0.00579$) was found also.

CONCLUSIONS: The results confirm that Shisha smoking (ShS) has a significant effect on arterial tone and acutely increases arterial blood pressure, heart rate and arterial stiffness.

Acknowledgement: This study was partially supported by MSM0021622402.

Keywords: Arterial stiffness, Blood pressure, Heart rate, Pulse wave velocity, Shisha smoking

Arterial Blood Pressure in Different Stress Conditions in Medical Students

Smaranda Laura Gotia¹, Smaranda Rodica Gotia¹, Claudia Borza², Corina Serban²

¹Department of Physiology, University of Medicine and Pharmacy, Timisoara, Romania

²Department of Pathophysiology, University of Medicine and Pharmacy, Timisoara, Romania

OBJECTIVE: The increasing obesity among young people, smoking, coffee and alcohol consumption associate with stress represent risk factors of high blood pressure. We proposed to study blood pressure variation in medical students correlated with their lifestyle.

METHODS: Were questioned 27 students, 15 boys and 12 girls, 23 years old, about their lifestyle, coffee, alcohol consumption, and smoking. Blood pressure was determined in the morning, afternoon, periodically from November until February. Also, determinations were performed before and after the exams.

RESULTS: BMI was higher in girls than boys. Coffee consumption was higher in girls (75%) than boys (46.66%), alcohol consumption was found in 41% of cases. Smoking more than 25 cigarettes/day was noted in 33.33% boys and 25% girls. In the boys' group during exams, a moderate increased systolic blood pressure 124.66 ± 11.44 mmHg and diastolic blood pressure 74 ± 8.42 mmHg was noticed, in the afternoon. In girls, before the exam systolic (113.33 ± 11.14 mmHg) and diastolic blood pressure (72.08 ± 5.82 mmHg) increased.

CONCLUSIONS: Blood pressure modification showed a response to the sympathetic vegetative system activation induced by the exam stress, associated with the effect of coffee consumption. Smoking, coffee use associated with stressing situations, as exams, can increase the blood pressure values. So, we recommend periodical check of blood pressure at youths that have high values, informing the youth about the risk factors, and the change of their lifestyle to a healthy life.

Keywords: blood pressure, stress, medical students

Changes in patterns of monophasic action potential morphology during alternating atrial flutter and fibrillation elicited by acetylcholine and rapid pacing in anesthetized rabbits

Isaac Aidonidis, Doulas Konstantinos, Ioannis Makantasis, Paschalis Adam Molyvdas
Dept. of Physiology, Medical School of Larissa, University of Thessaly, Greece

OBJECTIVE: The mechanisms of spontaneous conversions of flutter to fibrillation and inversely still remain an area of investigation. We aimed to assess changes in local atrial repolarization during the transition periods from one arrhythmia form to the other.

METHODS: For that purpose, combination PACING/RECORDING monophasic action potential (MAP) catheters were used in 9 closed-chest ketamine-thiopental scheme anesthetized rabbits. Intravenous acetylcholine was intermittently infused at 0.02 mg/Kg/min to facilitate inducibility of either AF or AFL by rapid burst pacing. MAP recordings were manually analyzed during sustained (>30 s) episodes of the inducible arrhythmia to evaluate changes in action potential frequency and shape preceding the conversion of flutter to fibrillation and inversely. Recordings were made from a stable area during the entire experiment.

RESULTS: We studied 16 episodes of the conversion between AFL and AF. Conversion of flutter into fibrillation was almost always accompanied by 3-5 faster atrial MAPs (115 ± 11 vs. 75 ± 10 ms cycle length, stable AFL vs. transition period). In 12/16 episodes a short interval of zero excitability (isoelectric line) was observed prior to flutter transformation. The transition of fibrillation into flutter was more complex showing a marked diversity of MAP characteristics. In the majority of cases (10/16), however, a period of flutter-like MAP transformation (slower fibrillatory waves more regular in shape) was preceded the onset of stable typical flutter.

CONCLUSIONS: These observations indicate distinct changes in atrial repolarization during alternating AFL/AF, presumably forming one mechanism for functional conduction block leading to either fibrillatory conduction (AFL into AF) or termination of fibrillatory conduction enabling a single reentrant circuit to recover (AF into AFL).

Keywords: unconscious rabbits, acetylcholine, rapid pacing, atrial flutter/fibrillation, monophasic action potentials

Study on the involvement of soluble guanylyl cyclase and its different isoforms in carbon monoxide and carbon monoxide releasing molecule-2 induced vasodilatation

Kelly Decaluwé¹, Bart Pauwels¹, Sara Verpoest¹, Peter Brouckaert², Johan Van De Voorde¹

¹Department of Pharmacology, Ghent University, Ghent, Belgium

²Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium

OBJECTIVE: CO as well as the CO-donor CORM-2 possess vasodilatory properties. The objective was to examine the involvement of sGC and its different subunits in CO and CORM-2 induced vasodilatation within different vascular tissues.

METHODS: Isometric tension recordings were performed on isolated mice aorta, femoral artery as well as corpora cavernosa (CC). To distinguish between the different sGC subunits we evaluated responses to CO and CORM-2 in both sGC α 1^{-/-} and sGC β 1KI/KI mice and their wild-type controls.

RESULTS: CO was unable to relax isolated blood vessels, whereas it induced concentration-dependent relaxations in CC. In CC of wild-type mice, the response to CO was completely inhibited by the sGC inhibitor ODQ. The involvement of sGC was further confirmed by the loss of response to CO in CC isolated from sGC β 1KI/KI mice. Moreover, the vasodilatory responses of CO in the corporal tissue of sGC α 1^{-/-} mice were strongly inhibited although not completely abolished. In contrast to CO, CORM-2 relaxed all vascular tissues examined. ODQ only partially blocked the response to CORM-2 in the aorta. Interestingly ODQ did not affect the CORM-2 induced relaxation in the femoral arteries and the CC, indicating that sGC is not involved, which was confirmed using the transgenic mice.

CONCLUSIONS: Vasorelaxation induced by CORM-2 differs from that of CO. While CO-induced vasorelaxation depends on activation of sGC, primarily the sGC α 1 β 1 heterodimer, the vasorelaxation of CORM-2 is partially or even completely sGC-independent. The observation that CO is more effective in relaxing CC tissues than other cardiovascular tissues suggests that the heme-oxygenase/CO pathway may present a potential new target for treating erectile dysfunction.

Keywords: CO, soluble guanylyl cyclase, blood vessel, aorta, erection, CO-releasing molecules

Role of peroxisome proliferator-activated receptor β agonist on angiogenesis in hindlimb ischemic diabetic rats

Majid Khazaei¹, Ensieh Salehi¹, Bahman Rashidi²

¹Department of Physiology, Isfahan University of Medical Sciences, Isfahan, Iran

²Department of Anatomy, Isfahan University of Medical Sciences, Isfahan, Iran

OBJECTIVE: Peroxisome proliferator activating receptors (PPAR) ligands play a different role in cardiovascular system, however, very little has been known about the function of PPAR β receptor in modulating angiogenesis. The aim of this study was to evaluate the effect of a specific PPAR β agonist, GW0742, on angiogenesis and serum levels of Vascular Endothelial Growth Factor (VEGF), VEGF Receptor-2 (VEGFR-2) and nitrite, the main metabolite of nitric oxide, in hindlimb ischemia in normal and type I diabetic rats.

METHODS: Hindlimb ischemic Wistar rats were divided into four groups: control, diabetic, control and diabetic treated with GW0742 (n=7 each). Diabetes was induced by injection of streptozotocin (55 mg/kg; ip). GW0742 was injected one day after surgery (1mg/kg; sc). After 21 days, blood samples were taken and gastrocnemius muscles were harvested for immunohistochemistry. The study was approved by the local ethics committee.

RESULTS: Results showed that GW0742 significantly increased serum nitrite and VEGFR-2 concentrations and serum VEGF to VEGFR-2 ratio in both control and diabetic rats. The capillary density in hindlimb ischemia was lower in diabetic animals compare to control and GW0742 significantly restored the capillary density in control and diabetic hindlimb ischemic rats.

CONCLUSIONS: PPAR β agonist, GW0742, administration restores skeletal muscle angiogenesis in control and diabetic hind limb ischemia and can be considered for prevention and/or treatment of peripheral vascular complications in diabetic subjects.

Keywords: angiogenesis, PPAR, diabetes, VEGF, nitric oxide

Acid-base balance and artificial controlled ventilation in wistar rats: A chronophysiological view

Pavol Svorc, Jr.¹, Ivana Bacova¹, Erika Svorcova², Benjamin Lee Fulton¹

¹Department of Physiology, Medical Faculty Safarik University, Kosice, Slovakia

²Department of Haematology and Oncohaematology, University Hospital, Kosice, Slovakia

OBJECTIVE: The control of the acid-base balance in the animal experiments with artificial ventilation would be primary effort in all in vivo studies. Problem is that acid-base balance changes after each ventilatory change and results are often compared to averaged reference values irrespective of the circadian dependence. The aim of study was referred to chronobiological aspects the impact of the artificial ventilation on the pH and blood gases in rat experiments in vivo.

METHODS: The experiments were performed in ketamine/xylazine anaesthetized female Wistar rats (ketamine 100 mg/kg + xylazine 15 mg/kg, i.m., 3-4 months, open chest experiments) after 4 weeks of adaptation to the LD cycle (12h:12h). The parameters of the initial ventilation and reoxygenation: respiratory rate 50 breaths/min., tidal volume 1ml/100g. Apnoic episode was simulated by the switching off the ventilator for 2 minutes. The acid-base balance was monitored from blood samples taken from the femoral artery.

RESULTS: Anaesthesia produced the systemic asphyxia in spontaneously breathing rats independently on LD cycle. LD differences were abolished after the apnoae, except pCO₂ with significantly higher values in the light period. 20 min. of reoxygenation evoked acidosis, normocapnia and hypoxia in the light period and alkalosis, hypocapnia and hypoxia in the dark one. LD differences were preserved in all followed parameters.

CONCLUSIONS: It is concluded that the spontaneously breathing rats under the ketamine/xylazine anaesthesia are in asphyxic conditions from the start of in vivo experiments, independently on LD cycle. The recovery of pulmonary ventilation after apnoae does not rectify pH and blood gases to the reference values and changes them in the dependence on LD cycle.

Keywords: Chronophysiology, acid-base balance, artificial ventilation, rats

Anti-apoptotic Effects of Onion (*Allium cepa*) Extract on Doxorubicin-induced Cardiotoxicity in Rats

Seref Alpsoy¹, Cevat Aktas², Ramazan Uygur³, Birol Topcu⁴, Mehmet Kanter⁵, Mustafa Erboga⁵, Osman Karakaya⁶, Asuman Gedikbasi⁷

¹Department of Cardiology, Faculty of Medicine, Namik Kemal University, Tekirdag, Turkey

²Department of Histology and Embryology, Faculty of Medicine, Namik Kemal University, Tekirdag, Turkey

³Department of Anatomy, Faculty of Medicine, Namik Kemal University, Tekirdag, Turkey

⁴Department of Biostatistics, Faculty of Medicine, Namik Kemal University, Tekirdag, Turkey

⁵Department of Histology and Embryology, Faculty of Medicine, Trakya University, Edirne, Turkey

⁶Department of Cardiology, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey

⁷Department of Biochemistry, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey

OBJECTIVE: Doxorubicin (DOX) is one of the effective and useful antitumor drugs. However, the clinical use of DOX has been limited by its cardiotoxic effects which are involved apoptosis in cardiomyocytes. The aim of this study was to investigate the anti-apoptotic effects of onion (*Allium cepa*) extracts (ACE) on DOX-induced apoptosis in cardiomyocytes.

METHODS: The rats were randomly allotted into one of three experimental groups: control, DOX treated and DOX treated with ACE; each group contained 8 animals. Control group received 1 ml/day of saline by orally. The rats in ACE treated group was given a daily dose of 1 ml ACE for 14 days by using intra-gastric intubation. To induce cardiotoxicity, DOX (30 mg/kg body weight) was injected intraperitoneally by single dose and the rats were sacrificed after 48 hours. To date, no such studies have been performed on cardioprotective and anti-apoptotic potential of ACE on DOX-induced apoptosis in cardiomyocytes.

RESULTS: Our data indicate a significant reduction in the activity of in situ identification of apoptosis using terminal dUTP nick end-labeling (TUNEL) in cardiomyocytes of the DOX treated group with ACE therapy. DOX treated with ACE groups induced a significant decrease in MDA levels, increased the activities of SOD, and GSH-Px in comparison with the DOX treated group.

CONCLUSIONS: These biochemical and histological disturbances were effectively attenuated on pretreatment with ACE. The present study showed that ACE may be a suitable cardioprotector against toxic effects of DOX.

Keywords: Doxorubicin, *Allium cepa*, cardiotoxicity, TUNEL, antioxidants

Protective Effects of Onion (*Allium cepa*) Extract on Doxorubicin-induced Aortic Endothelial Dysfunction

Seref Alpsoy¹, Ramazan Uygur², Cevat Aktas³, Birol Topcu⁴, Mehmet Kanter⁵, Mustafa Erboga⁵, Osman Karakaya⁶, Asuman Gedikbasi⁷

¹Department of Cardiology, Faculty of Medicine, Namik Kemal University, Tekirdag, Turkey

²Department of Anatomy, Faculty of Medicine, Namik Kemal University, Tekirdag, Turkey

³Department of Histology and Embryology, Faculty of Medicine, Namik Kemal University, Tekirdag, Turkey

⁴Department of Biostatistics, Faculty of Medicine, Namik Kemal University, Tekirdag, Turkey

⁵Department of Histology and Embryology, Faculty of Medicine, Trakya University, Edirne, Turkey

⁶Department of Cardiology, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey

⁷Department of Biochemistry, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey

OBJECTIVE: The aim of this study was to investigate the protective effects of onion (*Allium cepa*) extracts (ACE) on DOX-induced aortic endothelial dysfunction.

METHODS: The rats were randomly allotted into one of three experimental groups: control, DOX treated and DOX treated with ACE; each group contained 8 animals. Control group received 1 ml/day of saline by orally. The rats in ACE treated group was given a daily dose of 1 ml ACE for 14 days by using intra-gastric intubation. To induce cardiotoxicity, DOX (30 mg/kg body weight) was injected intraperitoneally by single dose and the rats were sacrificed after 48 hours. To date, no such studies have been performed on protective potential of ACE on DOX-induced aortic endothelial dysfunction.

RESULTS: Our data indicate a significant reduction in the activity of in situ identification of apoptosis using terminal dUTP nick end-labeling (TUNEL) in endothelial cells of the DOX treated group with ACE therapy. DOX treated with ACE groups induced a significant decrease in MDA levels, increased the activities of SOD, and GSH-Px in comparison with the DOX treated group.

CONCLUSIONS: ACE pre-treatment could attenuate endothelial dysfunction and it also possibly acts as a free radical scavenger. These results indicate that ACE pre-treatment might be useful in preventing endothelial dysfunction in DOX-induced toxicity in rats.

Keywords: Doxorubicin, *Allium cepa*, aortic endothelial function, TUNEL, antioxidants

Effect of reoxygenation on heart rate variability after apneic episode in Wistar rats: A chronobiological study

Pavol Svorc, Jr.¹, Erika Svorcova², Ivana Bacova¹, Judita Stimmelova¹, Benjamin Lee Fulton¹, Monika Novakova¹

¹Department of Physiology, Medical Faculty, Safarik University, Kosice

²Department of Hematology and Oncohematology Faculty Hospital L.Pasteur, Kosice

OBJECTIVE: The aim was to gain information about the effect of the artificial reoxygenation on heart rate variability (HRV) after apneic episode, as function of the light-dark cycle.

METHODS: The experiments were performed in ketamine/xylazine anaesthetized female Wistar rats (10mg/kg+15mg/kg), after adaptation to the LD cycle of 12:12h. Reoxygenation was simulated by respiratory pump with parameters 1ml/100g and 50 breaths/min. The apnea was induced by the switch off pump for 2min. HRV parameters (RR, VLF, LF, HF and VLF/HF) were evaluated in the intact animal, after 5min of stabilization followed surgical interventions and after 5.,10.,15.,20. minute of reoxygenation after apnea.

RESULTS: In the spontaneous breathing ketamine/xylazine anaesthetized rats, the significant LD differences were found in all parameters, except power VLF and VLF/HF ratio. Only 5 minute of the reoxygenation kept significant LD differences in all followed parameters but this dependence was eliminated by long-term reoxygenation. The dominant parasympathetic tone was preserved during whole experiment in the both lighted parts.

CONCLUSIONS: In the spontaneous breathing ketamine/xylazine anaesthetized rats, the significant LD differences were found in all parameters, except power VLF and VLF/HF ratio. Only 5 minute of the reoxygenation kept significant LD differences in all followed parameters but this dependence was eliminated by long-term reoxygenation. The dominant parasympathetic tone was preserved during whole experiment in the both lighted parts.

Keywords: chronobiology, heart-rate variability, apnoe, reoxygenation, rat

Does the food restriction affect the ischemia induced arrhythmia and infarct size after coronary artery occlusion in rats

Hayriye Soy Turk Orallar¹, Salih Tunç Kaya², Selçuk Yaşar¹, Firdevs Erim¹, Bilgihan Babatürk¹, Ömer Bozdoğan¹

¹Department of Biology, Abant İzzet Baysal University, Bolu Turkey

²Department of Biology, Düzce University, Düzce Turkey

OBJECTIVE: Previous studies have shown that the impairment in myocardial contractility and dysfunction increase in response to food restriction. But the effect of food restriction on the myocardial damage and ischemia induced arrhythmia are still unknown. In this study, it has been aimed to research the effect of food restriction on the arrhythmia and infarct size during myocardial ischemia in rats

METHODS: Eighteen female rats were used in this study. Control and food restricted animals were fed in individual cages. Control animals were fed ad libitum. Food restriction was made by decreasing of dietary food in 50% for 15 days. Left coronary artery was ligated by the silk to produce 30 minutes occlusion in all groups. The blood pressure and ECG were recorded during ischemia. An arrhythmia score was calculated from the duration and the type of arrhythmia. All results were compared after the analyses of variance or Chi-square test.

RESULTS: Non-significant increase in ventricular tachycardia has been observed in food restricted group. There was no effect of individual and group feeding on the arrhythmia or myocardial infarct size in relation to food restricted group.

CONCLUSIONS: Although food restriction did not change the infarct size, the blood pressure and the severity of arrhythmia, significant increased in the arrhythmic period ($p < 0.05$) and non-significant increase in ventricular tachycardia ($p < 0.2$) were observed in food restricted group in respect to control. As a result the findings in the present study support the food restriction promotes myocardial dysfunction.

Keywords: Myocardial, ischemia, arrhythmia, food restriction

Genetic modification of the neural sympathetic regions to control hypertension chronically

Vera Galdes¹, Nataniel J. Rosa¹, Julian F.r. Paton², Isabel Rocha¹

¹Instituto de Fisiologia & Unidade de Sistema Nervoso Autónimo, Faculdade de Medicina and Instituto de Medicina Molecular, Lisbon, Portugal

²School of Physiology & Pharmacology, Bristol Heart Institute, School of Medical Sciences, University Walk, University of Bristol, Bristol, BS8 1TD, UK

OBJECTIVE: Sympathetic activity is controlled by several areas of the central nervous system. The most referred sympatho-excitatory regions are the Paraventricular Nucleus of the Hypothalamus (PVN) and the Rostrolateral Medulla (RLM). Previous data have shown an increase of PVN output on hypertension. The aim of this study was to depress the activity of these sympatho-excitatory regions chronically (PVN / RLM) in order to control Blood Pressure (BP) in Spontaneously Hypertensive Rats (SHR).

METHODS: In telemetry instrumented SHR and normotensive Wistar rats (WKY), a viral vector (mix of LV-TREtigh-Kir-cIRES-GFP 5.4x10E9 and LV-Syn-Eff-G4BS-Syn-Tetoff 6.2x10E9 in a ratio 1:4) was microinjected (0.05µL) using stereotaxic coordinates for both locations. BP and Heart Rate (HR), baro and chemoreceptor reflexes were evaluated.

RESULTS: In SHR the basal values for systolic, diastolic and mean BP were 159±17mmHg, 127±14mmHg, 137±14mmHg, respectively. Microinjected SHR in RLM (n=3) and in PVN (n=4) showed a decrease in systolic (9% p=0.038; 13% p=0.217), diastolic (12% p=0.008; 17% p=0.113) and mean BP (11% p=0.013; 15% p=0.136) between 15 to 30 days post-injection. PVN or RLM saline microinjection in SHR (n=5) and WKY (n=5) did not significantly changed BP, neither did viral microinjection in WKY (n=6). HR and baroreflex gain did not change significantly in any experimental group (p>0.05).

CONCLUSIONS: These data show for the first time that the PVN and RLM play a role in determining the long term levels of BP in conscious rats and hence make them appropriate targets for genetic targeting to control BP.

Keywords: Hypertension, Autonomic Nervous System, Paraventricular Nucleus of the Hypothalamus, Rostrolateral Medulla

Effect of extracorporeal circulation on oxidative stress and viscosity in blood plasma

Cigdem Ozer¹, Fuat Sönmez¹, Deniz Erbaş¹, Aydan Babül¹, Halim Soncul², Emrah Ereren², Tolga Tatar²

¹Gazi University, Faculty of Medicine, Department of Physiology, Beşevler, Ankara, Turkey

²Gazi University, Faculty of Medicine, Department of Cardiovascular Surgery, Beşevler, Ankara Turkey.

OBJECTIVE: Oxidant stress generated through extracorporeal circulation (EC) applied for cardiopulmonary bypass (CBP) is the main cause of morbidity and mortality encountered after surgery. The mechanisms that occur during these processes are not clear yet. In this study we aimed to investigate the effects of extracorporeal circulation on oxidant, antioxidant parameters and plasma viscosity.

METHODS: The study was performed on 22 patients. Blood samples were taken from patients at seven different stages of operation. These stages were in the order of (A) preoperative intensive care, (B) anesthesia, (C) entering pump, (D) after aortic cross clamping, (E) after release of aortic cross clamping, (F) after pump out, (G) post operative intensive care. In all stage plasma Malondialdehyde (MDA), NOx (Nitrate and Nitrite), total sulfhydryl group (RSH) levels and plasma viscosity were determined.

RESULTS: MDA and NOx levels began to increase and RSH levels began to decrease at the A and B stages together with C stage. The lowest level of RSH and highest levels of MDA and NOx were determined at the F stage. Viscosity values also decreased with starting at the C stage and increased at the F stage.

CONCLUSIONS: The data obtained from the MDA and RSH levels were compatible with the oxidant stress and with lowered antioxidant capacity caused by surgical trauma and nonphysiologic EC circulation surface. Increased NOx levels may be the results of the mechanical effect of extracorporeal circulation on endothelium because of the surgery and the expression of eNOS by red blood cells exposed to mechanical stress.

Keywords: Extracorporeal circulation, MDA, NOx, RSH, Viscosity

Evaluation of hemorheological parameters in patients with slow coronary flow

Erdem Demir¹, Ibrahim Susam¹, Burcu Uludağ¹, Melek Bor Küçükataş², Dursun Dursunoğlu¹, Yalın Tolga Yaylalı¹, Emine Kılıç Toprak², Gülten Erken³

¹Pamukkale Üniversitesi Tıp Fakültesi, Kardiyoloji Anabilim Dalı, DENİZLİ

²Pamukkale Üniversitesi Tıp Fakültesi, Fizyoloji Anabilim Dalı, DENİZLİ

³Balıkesir Üniversitesi Tıp Fakültesi, Fizyoloji Anabilim Dalı, BALIKESİR

OBJECTIVE: Slow coronary flow (SCF) is delayed progression of the contrast seen at the coronary angiography in the absence of epicardial stenosis and/or of other conditions associated with decreased coronary flow velocity. While microvascular abnormalities are suspected to underlie the mechanism of SCF, less attention has been paid to intrinsic properties of blood that can also impair microcirculatory flow. In this study we aimed to evaluate erythrocyte deformability, erythrocyte aggregation, whole blood and plasma viscosity in SCF.

METHODS: Thirty patients with SCF (53.70 ±1.73 years, 11 male) and 20 subjects with normal coronary arteries (11 male, 54.05±2.69 years) were included in the study. Coronary flow was quantified by means of thrombolysis in myocardial infarction (TIMI) frame count. Aggregation and deformability of erythrocytes were measured by an ektacytometer. Viscosities were measured by a cone-plate viscometer. This study was approved by the local ethics committee. Independent samples t-test and Mann–Whitney U test were used for statistics.

RESULTS: There was no statistically significant difference between the groups with respect to age, sex, family history, body mass index, fibrinogen levels, hematological parameters, diabetes, hypertension and smoking. Erythrocyte deformability measured at 0.30 Pascal (Pa) (0.040±0.003 vs. 0.048±0.002, respectively, p< 0.05); 0.53 Pa (0.056±0.003 vs. 0.070±0.004, respectively, p< 0.05); 0.95 Pa (0.114±0.007 vs. 0.138±0.006, respectively, p< 0.05); 1.69 Pa (0.205±0.009 vs. 0.330±0.007, respectively, p< 0.05); 3.00 Pa (0.305±0.009 vs. 0.330±0.007, respectively, p< 0.05) were higher in SCF patients. Erythrocyte aggregation parameters and viscosities were similar between the two groups.

CONCLUSIONS: Increments in erythrocyte deformability may serve as an advantageous factor to regulate blood flow in SCF.

Keywords: Slow coronary flow, erythrocyte deformability, erythrocyte aggregation, plasma and whole blood viscosity

The effects of organic dried apricot on some hematological parameters in female rats

Ismet Yılmaz

Department of Pharmacology, Pharmacy Faculty of Inonu University, Malatya, Turkey

OBJECTIVE: The main purpose of this study was to investigate the medicinal importance of organic dried apricot (*Prunus armeniaca* L.) consumption on hematological parameters, and the secondary purpose was to determine the best ratio and/or period(s) of organic dried apricot supplementation to standard diet in female rats.

METHODS: One-hundred and twenty female rats were randomly divided into five groups as follows: Group I (control) were fed with standard rat chow, Group II were fed 1%, Group III were fed 2.5%, Group IV were fed 5% and Group V were fed 10% organic dried apricot supplemented to standard diet were given for 30, 60, and 120 days study periods. At 30th, 60th and 120th days, blood samples were taken and hematologic parameters were measured.

RESULTS: Hematological parameters such as RBC, HCT, HGB, MCV, MCH, MCHC, RDW, PLT, MPV, PDW and PCT were improved when organic dried apricot supplementation was given at 5 or 10 of percent for periods of 60 or 120 days ($p < 0.05$).

CONCLUSIONS: Organic dried apricot has led to beneficial changes in hematologic parameters in female rats. I conclude that organic dried apricot could support reticulo-endothelial system and bone marrow in female rats.

Keywords: Organic dried apricot, hematologic parameters, female rat.

myeloperoxidase, cellular adhesion molecules and neopterin in metabolic syndrome patients with coronary artery disease

Gita Krievina¹, Peteris Tretjakovs², Antra Jurka¹, Inga Bormane¹, Karlina Elksne², Jurijs Verbovenko³, Guntis Bahs³

¹Department of biology, University of Latvia, Riga, Latvia

²Riga Stradins University, Riga, Latvia

³Riga Eastern Clinical University Hospital, Riga, Latvia

OBJECTIVE: The aim of the present study was to evaluate differences in serum levels of neopterin, adhesion molecules and myeloperoxidase (MPO) between coronary artery disease and metabolic syndrome (CAD-MetS) patients with stable and unstable angina pectoris (SAP, UAP), and to clarify relationships between neopterin and other biomarkers.

METHODS: The study included 60 patients with CAD-MetS who were classified into two groups, 30 patients with SAP and 30 patients USP. 20 healthy subjects were selected as controls (C). Serum soluble vascular cell adhesion molecule-1 (sVCAM-1), intercellular cell adhesion molecule-1 (sICAM-1), sE-selectin and MPO levels were measured by Luminex xMAP technology, but serum neopterin concentrations were measured by radioimmunoassay.

RESULTS: Serum levels of neopterin, MPO, sVCAM-1, sICAM-1, and sE-selectin were significantly higher in patients with UAP in comparison with the group of healthy controls ($p < 0.05$). Patients with SAP also had higher levels of these biomarkers than healthy controls ($p < 0.05$), except for sE-selectin. The biomarkers did not differ between the two patient groups, except for MPO, which was significantly higher in the USP group ($p < 0.05$). Neopterin was significantly correlated only with sVCAM-1 ($p < 0.05$).

CONCLUSIONS: CAD-Met patients with SAP have more apparent elevations of serum sICAM-1 and sVCAM-1 levels, simultaneously with higher MPO and neopterin concentrations than healthy subjects, but UAP is also associated with more substantial changes in MPO and significantly increased sE-selectin levels. Neopterin has a close correlation only with sVCAM-1.

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Keywords: Myeloperoxidase, neopterin, vascular cell adhesion molecule-1, metabolic syndrome

The effect of adenosine and atropine combination on the arrhythmia that has occurred following coronary occlusion in rats

Selçuk Yaşar¹, Salih Tunç Kaya², Hayriye Soyuturk Orallar¹, Ömer Bozdoğan¹

¹Department of Biology, Abant İzzet Baysal University, Bolu, Turkey

²Department of Biology, Duzce University, Duzce, Turkey

OBJECTIVE: Adenosine has been commonly used in the treatment of supraventricular tachycardia in humans. Otherwise adenosine is a hypotensive agent that usually induces bradycardia, even cardiac arrest after coronary artery ligation. Hypotensive and bradycardic effects of adenosine make its usage unsafe in the treatment of arrhythmia. In this study, it has been aimed to research the effect of adenosine and atropine combination on the heart rate, blood pressure and on the arrhythmia induced by acute coronary artery occlusion in rats.

METHODS: Twenty male Sprague Dawley rats were used in this study. Myocardial ischemia was produced by ligation of left coronary artery for 6 minutes and reperfusion by the releasing of this artery for 15 minutes. A1 Adenosine receptor agonist (2 Chloro - N6 – Cyclopentyl-adenosine, CCPA) 5 µg/kg/100ul and atropine in 1mg/kg /1ml intravenously at 2 min. of ligation were given alone or in combination. The arrhythmia score using the incidence and the duration of all type of arrhythmia were determined from recorded ECG.

RESULTS: Adenosine increased the incidence of bradycardia $P<0.05$. Bradycardic effect of adenosine disappeared when adenosine combined with atropine. Although the arrhythmia score was not different among groups, the incidence of ventricular tachycardia decreased in atropine and combination group in respect to control. The arrhythmic period in atropine and combination group was also longer than the control group ($p<0.01$).

CONCLUSIONS: Bradycardic effect of adenosine was abolished by atropine. Adenosine and atropine combination was found to be effective to decrease the incidence of ventricular tachycardia. The usage of adenosine with atropine in the treatment of supraventricular tachycardia may be safer than the usage of adenosine alone.

Keywords: myocardial ischemia, reperfusion, arrhythmia, adenosine, atropine

Duration of Arrhythmias during reperfusion

Table 1. The duration of arrhythmias during reperfusion. * P<0.05, Different from Control and adenosine, ^cP<0.05, different from control

Groups	N	Onset of arrhythmia (min)	Arrhythmic Period (Min)	Length of Arrhythmia (min)			
				VT	Other	Total	Bradycardia
Control	7	0,40±0,15	4,04±1,66	0,73±0,20 (n=7)	0,83±0,34	1,56±0,45	0
Adenosine	6	0,46±0,32	2,58±0,83	0,34±0,14 (n=5)	0,52±0,21	0,75±0,28	0
Atropine	4	0,13±0,06	9,50±2,40*	0,07±0,01 (n=2)	0,64±0,25	0,45±0,18 ^c	0
Adenosine + atropine	5	0,35±0,11	10,43±1,75*	0,11 (n=1)	0,78±0,07	0,80±0,09	0

Data are mean ±SE

Total= VF+ VT + Other arrhythmias including VPC, ventricular gemini, AV nodal arrhythmia and salvo.

Survival Rate and Incidence of Arrhythmias during 15 min reperfusion

Table 2. The survival rate and the incidence of arrhythmia during 15 min of reperfusion.

*P<0,05, **P<0,01

Groups	N	Survival rate (N / %)	Incidence of arrhythmia (n / %)				The score of arrhythmia
			VF	VT	Others	Bradycardia	
Control	7	7 / 100	0 / 0	7 / 100	7 / 100	0 / 0	3,4 ±0,37
Adenosine	8	6 / 68	1 / 13	7 / 88	8 / 100	4 / 50*	3,0 ±0,65
Atropine	4	4 / 100	1 / 25	2 / 50**	4 / 100	0 / 0	2,7 ±0,48
Combination (Adenosine+Atropine)	6	5 / 84	1 / 17	2 / 33*	6 / 100	1 / 17	3,7 ±0,50

Heart Rate Variability Changes After Coronary Artery Bypass Graft Surgery

Sirma Osman¹, Ümmühan Isoglu Alkac¹, Sacit Karamürsel¹, Enver Dayıođlu²

¹Istanbul Faculty of Medicine Department of Physiology, Istanbul University, Istanbul, Turkey

²Istanbul Faculty of Medicine Department of Cardiovascular Surgery, Istanbul University, Istanbul, Turkey

OBJECTIVE: The aim of this study is to evaluate Heart Rate Variability (HRV) as an important marker of autonomic nervous system (ANS) modulation before and after coronary artery bypass graft (CABG) surgery in patients (n=10) with the diagnose of coronary artery disease (CAD).

METHODS: Five minute electrocardiography (ECG) epochs were recorded before and 4-5 weeks after the surgery by using MITSAR recording system in order to calculate Heart rate (HR) data. Spectra of HR were divided into low (LF: 0.02-0.05Hz)- and high (HF: 0.2-0.35 Hz)-frequency bands. All statistical analyses were performed with t-test by using SPSS-PC 16.0.

RESULTS: The average values of LF and HF band powers of HRV data were decreased after the surgery without any statistically significance (LF: p=0.47, HF: p=0.81). The decrease in LF/HF ratio after surgery was statistically significant (p= 0.04).

CONCLUSIONS: Reduced LF/HF (marker of sympathovagal balance as a measurement of HRV) indicates higher parasympathetic activation after surgery. Although the preliminary results point the effects of CABG surgery on ANS modulation of heart rate, to increase reliability of the results, more patients and recordings in later periods (6-12 months after surgery) would be beneficial. The present work was supported by the Research Fund of Istanbul University. Project No. 6285

Keywords: Coronary Artery Bypass Graft (CABG), Heart Rate Variability (HRV), Electrocardiogram (ECG), Coronary Artery Disease (CAD).

Central Administration of trout pituitary adenylate cyclase - activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) decrease baroreflex sensitivity in trout: a transfer function analysis

Frédéric Lancien¹, Nagi Mimassi¹, John Michael Conlon², Jean Claude Le Mével¹

¹Université Européenne de Bretagne. Université de Brest; INSERM U650, Laboratoire de Neurophysiologie, IFR 148 ScInBioS, CS 93837, 29238 Brest Cedex 3; CHU de Brest, France

²Department of Biochemistry, Faculty of Medicine and Health Sciences, United Arab Emirates University, 17666 Al Ain, United Arab Emirates

OBJECTIVE: Although pituitary adenylate cyclase - activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) exert diverse actions on heart and blood vessels along the vertebrate phylum, no information is currently available concerning the potential role of these peptides on the regulation of the baroreflex response, a major mechanism for blood pressure homeostasis. Consequently, the goal of this study was to examine in our experimental model, the unanesthetized rainbow trout, whether PACAP and VIP are involved in the regulation of the cardiac baroreflex sensitivity (BRS).

METHODS: For this purpose, we investigated the effects of intracerebroventricular (ICV) and intra-arterial (IA) injections of trout PACAP and VIP (25-100 pmol) on BRS in unanesthetized trout. Cross spectral analysis techniques using a fast Fourier transform algorithm were employed to calculate the coherence, phase and gain of the transfer function between spontaneous fluctuations of systolic arterial blood pressure and R-R intervals of the electrocardiogram. The BRS was estimated as the mean of the gain of the transfer function when the coherence between the two signals was high and the phase negative.

RESULTS: Compared with vehicle, ICV injections of trout PACAP and trout VIP dose-dependently reduced the cardiac BRS to the same extent with a threshold dose of 50 pmol for a significant effect. When injected IA at the same doses as for ICV injections, only the highest dose of VIP (100 pmol) significantly attenuated the BRS.

CONCLUSIONS: Our findings indicate new roles for PACAP and VIP, functioning as neurotransmitters or neuromodulatory peptides, for the control of neural pathways involved in the cardiac baroreflex sensitivity in trout.

Keywords: PACAP; VIP; R-R interval and systolic blood pressure variabilities; Baroreflex; Intracerebroventricular injection; Intra-arterial injection; Transfer function analysis; Teleost

Autonomic Dysfunction in Teenagers with High Prevalence of Atherosclerotic Risk Factors

Daniela Matei¹, Bogdan Ignat², Calin Corciova³, Cristian Dinu Popescu², Dan Zaharia³

¹University of Medicine and Pharmacy “Gr.T. Popa” Iasi, Romania, Faculty of Biomedical Engineering, Biomedical Sciences

²University of Medicine and Pharmacy “Gr.T. Popa” Iasi, Romania, Faculty of General Medicine, Department of Neurology

³University of Medicine and Pharmacy “Gr.T. Popa” Iasi, Romania, Faculty of Biomedical Engineering, Biomedical Instrumentation

OBJECTIVE: The aim of this study was to investigate autonomic cardiac control in teenagers with high prevalence of atherosclerotic risk factors.

METHODS: Study was made on 25 subjects with the average age of 25 ± 2.12 who have ≥ 2 cardiovascular risk factors, one of them had to be major (smoking, high blood pressure, dyslipidemia) and antecedence in the family of diabetes mellitus and 25 normal subjects (control group). Using Biopac Acquisition System, we monitored the electrocardiogram, heart rate variability (HR) in basal condition and during a cold pressor test. From these measurements, HR variability parameters were calculated: low- and high-frequency bands – LF and HF, LF/HF index), time-domain parameters (mean RR interval, SDNN, SDANN, SDNN index, rMSSD and pNN50%).

RESULTS: The teenagers with high prevalence of atherosclerotic risk factors had an increase in the mean heart rate at rest ($P < 0.001$), a decrease in standard deviation of R-R interval as well as in PNN50 ($P < 0.001$), and an increase in the LF/HF component ratio ($P < 0.01$) indicating a vagal–sympathetic dysfunction in teenagers with atherosclerotic risk factors compare with control group.

CONCLUSIONS: Our results suggest a subclinical impaired of the autonomic nervous system in teenagers with high prevalence of atherosclerotic risk factors with marked parasympathetic dysfunction and sympathetic predominance.

Keywords: atherosclerotic risk factors, diabetes mellitus, electrocardiogram, low-and high-frequency bands

Effects of L-Carnosine on Blood Rheological Properties in Density-Separated Erythrocytes of Aged and Young Rats

Gülten Erken¹, Melek Bor Küçükataş², Emine Kılıç Toprak², Beyza Akdağ³, Vural Küçükataş²

¹Department of Physiology, Balıkesir University, Balıkesir, Turkey

²Department of Physiology, Pamukkale University, Denizli, Turkey

³Department of Biostatistics, Pamukkale University, Denizli, Turkey

OBJECTIVE: This study aimed to investigate alterations in hemorheology induced by carnosine, an anti-oxidant dipeptide, and to determine their relationship to oxidative stress in density-separated erythrocytes of aged and young rats.

METHODS: Twenty-eight male Sprague Dawley rats were divided into 4 groups as aged (Aca), young (Yca) carnosine groups (250 mg/kg L-carnosine, i.p.) and aged (As), young (Ys) control groups (saline, ip). Density separation was further performed to these groups in order to separate erythrocytes according to their age. Blood samples were used for the determination of erythrocyte deformability, aggregation; and oxidative stress parameters. Statistical significance was accepted as $P < 0.05$. Kruskal-Wallis and Mann-Whitney U with the Bonferonni Correction Tests were used for statistically analyses.

RESULTS: Erythrocyte deformability of Yca group measured at 0.53 Pa was lower than As and Aca. Similarly, deformability of least-dense (young) erythrocytes of Yca group was decreased compared to least-dense erythrocytes of As and Aca groups. Erythrocyte aggregation in the Aca group was found to be increased compared to Ys group. Total antioxidant status (TAS) of Aca and Yca groups was higher and oxidative stress index (OSI) lower than As group.

CONCLUSIONS: Although carnosine resulted in an enhancement in TAS of both young and aged rats, this favorable effect was not observed in erythrocyte deformability and aggregation in the dose applied in this study.

Keywords: aged rat, density separation, erythrocyte deformability, total antioxidant status, total oxidant status

The involvement of carbon monoxide the contraction responses in mesenteric vascular bed in young and old rats

Filiz Gündüz, Günnur Koçer, Seher Ülker, Ümit Kemal Şentürk
Akdeniz University, Medical Faculty, Department of Physiology, Antalya, TÜRKIYE

OBJECTIVE: It is known that aging is associated with increasing in cardiovascular disorders and, endothelial dysfunction has a pivotal role in this process. Endothelial dysfunction observed in aged subject seems to be related with decreased NO production and bioavailability. On the other hand carbon monoxide (CO) is another mediator derived from endothelial cells and it also contributes the regulation of vascular tonus. However, the possible role of CO to endothelial dysfunction in aged rats is not investigated. The aim of this study was to investigate how CO affects the vascular tonus in aged animals.

METHODS: Young (4 months, n=10) and aged (24 months, n=10) Wistar rats were used in this study. Mesenteric arteries and its first (1A), second (2A) and third (3A) branches were dissected and mounted wire myograph. The contraction responses of vessel segments to phenylephrine (Phe) were determined in the presence and absence of heme oxygenase inhibitor (CRMP) and all protocols were achieved in the presence of L-NAME.

RESULTS: The contraction response to Phe in the presence of CRMP was significantly increased in main mesenteric arteries obtained from both of young and aged animals ($p<0.001$). Maximal response values to Phe in the presence of CRMP were significant decreased in old compared to young animals ($p<0.001$). CRMP did not alter vasoconstriction responses to Phe of other mesenterial arterial segments.

CONCLUSIONS: These results demonstrated that endogen CO is involved in the regulation of vascular tonus in conduit type in mesenteric bed, but not in small arteries, and, this involvement is attenuated in aged rats.

Keywords: carbon monoxide, arteries, aging, rat

The effects of clonidine on blood pressure and cardiac autonomic activity in conscious two-kidney, one-clip hypertensive rats

Zehra Gül Koçaklı, Kubra Akillioglu, Besim Özaykan

Department of Physiology, Faculty of Medicine, University of Çukurova, Balcalı, 01330 Adana/Turkey

OBJECTIVE: Two kidney one clip (2K1C) hypertension in rats is associated with sympathetic hyperactivity. The alpha-2 agonist, clonidine, reduces sympathetic activity. The aim of the present study was to investigate the effects of clonidine on mean arterial blood pressure (MAP), heart rate (HR), cardiac sympathetic tonus (ST), and parasympathetic tonus (PT) in conscious 2K1C hypertensive rats.

METHODS: For this purpose, a one week after renal artery clipping or sham operation, we treated the rats with either clonidine (200 µg/kg/d, orally) or distilled water for two weeks. Then, the rats were instrumented with catheters in the femoral artery and vein to measure MAP and to infuse the drugs, respectively. After a one day of recovery, cardiac ST and PT were determined by HR response to atenolol (1 mg/kg) and atropine methyl nitrate (1 mg/kg), respectively.

RESULTS: Clonidine increased MAP in both sham-operated (70 mmHg vs 79) and hypertensive rats (112 mmHg vs 134; $p<0.001$). Resting HR tended to decrease in clonidine-treated sham-operated rats ($p=0.058$). Blockade of β_1 receptors decreased HR in only distilled water treated sham-operated rats ($p<0.01$). Clonidine did not affect cardiac ST, and PT in both sham-operated and hypertensive rats. Intrinsic HR was less in clonidine-treated sham-operated rats than distilled water-treated sham-operated rats (361 beats/min vs 402; $p<0.01$).

CONCLUSIONS: We conclude that clonidine increases MAP and does not change cardiac sympathetic and parasympathetic activity in 2K1C hypertensive rats. In addition, clonidine decreases intrinsic HR in normotensive rats. Consequently, cardiac sympathetic and parasympathetic pathways may not contribute to the increase in MAP after clonidine treatment in normotensive and hypertensive rats.

Keywords: Clonidine, renovascular hypertension, autonomic nervous system

Bilateral difference in superficial and deep femoral artery blood flow

Zbignevs Marcinkevics¹, Zane Lukstina², Anzela Turkina², Juris Imants Aivars¹

¹Department of Human and Animal Physiology, Faculty of Biology, University of Latvia, Riga, Latvia

²Institute of Atomic Physics and Spectroscopy, University of Latvia, Riga, Latvia

OBJECTIVE: The endothelium plays a vital role on the control of vascular functions. Clinically revealed, non-uniform distribution of peripheral vascular diseases throughout the arterial tree suggests that localized factors such as hemodynamic forces can modulate the endothelial dysfunction. The Doppler blood flow measurement in the lower extremities has become a routine procedure, however, there is limited knowledge concerning bilateral differences of blood flow pattern. Therefore we examined blood flow patterns of femoral arteries (superficial femoral-SFA; deep femoral- DFA) in paired legs.

METHODS: Young, physically inactive, healthy volunteers (10 females, 5 males; age 21.2 ± 2.6 years) participated in this study. During rest conditions beat-per-beat arterial pressure (MAP), heart rate (HR), blood velocity (TAM), and artery diameter (D) were registered from SFA and DFA in the left and right leg by an ultrasound Doppler. The mean blood flow (Q), anterograde and retrograde mean blood velocities (TAMa), anterograde (TAMr) and retrograde shear rates as well as oscillatory shear index rates were computed and compared for both legs.

RESULTS: During entire recording systemic hemodynamic parameters were similar for all subjects (MAP: 92.1 ± 5.2 mmHg, HR: 65.3 ± 5 bpm). There was a significant bilateral asymmetry for flow parameters in DFA: D=10.1%, Q=30.4%, TAM=24.2%, TAMa=30.3%, TAMr=14.5%; SFA:D=3.3%, Q=20.1%, TAM=23.3%, TAMa=21.1%, TAMr=10.2%. We observed correlation between flow differences in SFA and DFA for paired leg.

CONCLUSIONS: The asymmetrical values of hemodynamic parameters between the femoral arteries in paired legs are observed in even healthy, young individuals who perform low level daily physical activity. Possibly, hydrodynamic factors which influence the endothelium are asymmetrical.

Keywords: femoral arteries, pulsatile blood flow, bilateral difference.

The effects of aged garlic extract and S-allylcysteine on blood pressure and baroreflex sensitivity in two kidneys, one clip hypertensive rats

Besim Özaykan, Zehra Gül Koçaklı

Department of Physiology, Faculty of Medicine, University of Çukurova, Balcalı, 01330Adana, Turkey

OBJECTIVE: The effect of garlic preparations on blood pressure (BP) regulation is highly controversial. The aim of this study was to investigate the effects of aged garlic extract (AGE) and its major component, S-allylcysteine (SAC), on BP and baroreflex sensitivity in two kidneys, one clip hypertensive rats.

METHODS: Left unilateral renal artery constriction or sham operation was performed on rats. Two weeks after clipping or sham operation, AGE (4 mL/kg/d) or SAC (150 mg/kg/d) was given orally to two different groups of clipped rats for 7 days. Sham-operated and clipped control groups were treated with distilled water for the same period. Then, the rats were instrumented with catheters to record BP via femoral artery. The next day, control BP and heart rate were measured and then, to determine the acute effect, an additional AGE, SAC or distilled water was administered to the animals. Three hours later, BP was recorded to calculate MAP and, to determine baroreflex sensitivity by sequence method.

RESULTS: Renal clipping increased MAP and heart rate and, decreased baroreflex sensitivity (1.45 beats/mmHg vs 2.25; $p < 0.05$). Neither a one day after treatment with AGE or SAC for 7 days nor 3 hours after an additional AGE or SAC treatment, the MAP and baroreflex sensitivity in clipped rats changed, significantly.

CONCLUSIONS: We conclude that AGE and SAC did not decrease BP in two kidney one clip hypertensive rats unlike reduced renal mass-saline and spontaneously hypertensive rats. In addition, these garlic preparations did not improve the baroreflex sensitivity in this hypertension model.

Keywords: Aged garlic extract, S-allylcysteine, renovascular hypertension, baroreflex sensitivity

Elucidation of the roles of α 1- and α 2-adrenoceptor subtypes in blood pressure responses

James R Docherty

Department of Physiology, RCSI. Dublin 2, Ireland

OBJECTIVE: Peripheral control of blood pressure involves α 1- and α 2-adrenoceptors, and the receptor subtypes involved can be studied in the pithed rat in the absence of central or reflex control. Confusion in this area occurs because yohimbine, which has been employed in a number of studies to identify α 2-adrenoceptor components to pressor responses, has affinity for α 1D-adrenoceptors. We have re-examined the actions of yohimbine in the pithed rat.

METHODS: We have re-examined the actions of yohimbine in rats pithed under anaesthesia and ventilated with 100% O₂ at 60 per min. The carotid artery and jugular vein were cannulated for pressure recording and drug injection.

RESULTS: Pressor nerve responses to 1 Hz stimulation were markedly inhibited by the α 1A-adrenoceptor antagonist RS 100329 (0.1 mg/kg) and by the α 1D-adrenoceptor antagonist BMY 7378 (0.1 mg/kg). Yohimbine (1 mg/kg) significantly reduced pressor nerve responses, but subsequent to BMY 7378 (0.1mg/kg), yohimbine failed to produce any further inhibition. The α 2A-adrenoceptor antagonist BRL 44408 (1mg/kg) did not inhibit pressor nerve responses. Pressor responses to exogenous agonists involve α 1A-and α 1D-adrenoceptors, but again the role of α 2-adrenoceptors was re-examined. Yohimbine or BRL 44408 (both 1mg/kg) significantly shifted, but BMY 7378 (5mg/kg) failed to affect, the pressor potency of the α 2-adrenoceptor agonist xylazine. In addition, the potent α 2A-adrenoceptor antagonist methoxyidazoxan produced great shifts in xylazine potency than the same dose of yohimbine or BMY 7378.

CONCLUSIONS: Pressor nerve responses in the pithed rat involve both α 1A-and α 1D-adrenoceptors, but responses to exogenous agonists involve additionally α 2A-adrenoceptors but there is no clear evidence for the involvement of α 2-adrenoceptors in pressor nerve responses.

Keywords: α 1-Adrenoceptors, α 2-adrenoceptors, blood pressure control

Effect of high fat diet on ovalbumin sensitized rats

Irina Luciana Dumitriu¹, Liliana Georgeta Foia⁴, Petru Plamadeala², Lucian Dragos Gorgan³, Didona Ungureanu⁴, Elena Cojocaru¹, Bogdan Gurzu¹

¹Department of Functional Sciences, "Gr T Popa" Iasi University, Iasi, Romania

²Laboratory Patology, "St Mary" Emergency Childrens Hospital, Iasi, Romania

³Genetics Laboratory, Faculty of Biology, "Al I Cuza" University Iasi, Romania

⁴Department of Biochemistry, "Gr T Popa" Iasi University, Romania

OBJECTIVE: Recent studies have demonstrated that not only obesity but, also the diet could modulate the immune response and contributes to the pathogenesis of immune-mediated diseases. The aim of this study was to determine if and to what extent the high fat diet (HFD) could modulate the development of allergic lung diseases.

METHODS: OR-CD (obese resistant) rats were sensitized against ovalbumin and divided into 2 groups: group I was fed a standard rat chow diet (12% calories as fat) and group II was fed a HFD (32.5% of calories as fat). The specific airway resistance (sRaw), cellularity of bronchoalveolar lavage fluid (BAL), antioxidant status and lung histological aspects were comparatively assessed on both rats groups.

RESULTS: There was no significant difference between the body weights of rats from both groups. On HFD rats both the sRaw increased in response to aerosolized acetylcholine and the total number of cells recovered in BAL fluid were significantly higher. Histological examination revealed that HFD increased infiltration of inflammatory cells on the walls of lobar and segmental bronchi and in peribronchiolar space from this level. Plasma total antioxidant capacity decreased with more than 70% on HFD group.

CONCLUSIONS: The HFD had unfavourable effects on both the hyperactivity and inflammation related with OVA sensitization. These effects appeared to be mediated, by increasing of oxidant-antioxidant imbalance associated to pulmonary allergic disease.

Keywords: high fat diet, ovalbumin, asthma, obese resistant rat

Responses of Cardiovascular and Autonomic Nervous Systems to Sudden Standing with Lower Abdominal Tension

Mohamed Al Kubati¹, B. Fiser²

¹a)- Department of Physiology, Faculty of Medicine, Masaryk University. b)- First Clinic of Cardiovascular Medicine, St. Anne's University Hospital, Brno, Czech Republic.

²Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic.

OBJECTIVE: To examine the effect of Lower Abdominal Tension (LAT) on blood pressure (BP in mmHg), heart rate (HR) and cardiac baroreflex sensitivity (BRS in ms/mmHg, and BRSf in Hz/mmHg).

METHODS: We studied seven healthy volunteers of age 30 ± 3 years (mean \pm SD). We recorded beat-to-beat arterial blood pressure: systolic (SBP), diastolic (DBP), mean (MBP); and inter-beat-interval (IBI in ms) with breathing controlled by metronome. Measurements were repeated 3 times (Finapres Ohmeda): First: in supine position. Second: after 20 seconds of a sudden standing from the supine position without abdominal tension. Third: after 20 seconds of a sudden standing with abdominal tension (each measurement lasted 5 minutes). We used spectral analysis to determine BRS and BRSf. The values of the first measurements were taken as base line values.

RESULTS: When compared with the base line values, we found that the increase in heart rate during standing with LAT (non-significant=NS) is less than the increase during standing without tension (NS). Increases in systolic, diastolic, and mean blood pressure during standing with LAT were found to be ($P=0.031$, $P=0.018$, and $P=0.018$ consequently), and during standing without tension were ($P=0.025$, $P=0.009$ and $P=0.011$). The baroreflex sensitivity BRS and BRSf non-significantly increased during standing with LAT more than during standing without tension.

CONCLUSIONS: We found that in orthostatism, increases in HR, baroreflex sensitivity and in the blood pressure during LAT are closely the same as during standing without tension. These results indicate that the use of the LAT alone to avoid orthostatic hypotension is not enough. This study was supported by: MSM0021622402

Keywords: Heart rate, blood pressure, baroreflex sensitivity, low abdominal tension, orthostatic hypotension

Alterations of central hypercapnic respiratory response induced by acute central administration of serotonin re-uptake inhibitor, fluoxetine

Gülderen Sahin¹, Ibrahim Guner¹, Nermin Yelmen¹, Onur Yaman¹, Murat Mengi¹, Gonul Simsek¹, Sevtap Sipahi²

¹Department of Physiology, Cerrahpasa Medical Faculty, Istanbul University, Turkey

²Department of Internal Medicine, Cerrahpasa Medical Faculty, Istanbul University, Turkey

OBJECTIVE: The role of increased central concentration of serotonin(5-HT) by inhibiting its re-uptake via fluoxetine on the central hypercapnic ventilatory response (CHVR) is complex and little is known. We aimed to research the effect of acute intracerebroventricular (ICV) injection of fluoxetine on CHVR in the absence of peripheral chemoreceptor impulses. In addition we determined the effect of ketanserin (5-HT₂ receptor antagonist) on these responses.

METHODS: Anesthetized rabbits were divided as Fluoxetine and Ketanserin groups. For ICV administration of fluoxetine and ketanserin, a cannula was placed in the left lateral ventricle by the stereotaxic method. Respiratory frequency (fR), tidal volume (VT) and ventilation minute volume (VE) were recorded in both groups.

RESULTS: ICV fluoxetine (10.12 mMol/kg) injection during normoxia caused increases in VT and VE ($P < 0.01$) in the fluoxetine group. When the animals were switched to hypercapnia f/min, VT and VE increased significantly. The increases in percentage values in VT and VE in Fluoxetine + Hypercapnia phase were higher than those during hypercapnia alone ($P < 0.01$, $P < 0.05$). There was an increase in CO₂ sensitivity Index in the Fluoxetine +Hypercapnia phase ($P < 0.01$). On blocking of 5-HT₂ receptors by ketanserin (0.25 mMol/kg), the degree of increases in VT and VE in the Ketanserin + Hypercapnia phase were lower than those during hypercapnia alone ($P < 0.01$, $P < 0.001$). CO₂ sensitivity Index also decreased ($P < 0.01$).

CONCLUSIONS: We concluded that acute central fluoxetine increases normoxic ventilation and also augments the stimulatory effect of hypercapnia on respiratory neuronal network by 5-HT₂ receptors in the absence of peripheral chemoreceptor impulses.

Keywords: Acute central fluoxetine, serotonin, hypercapnic ventilatory response, CO₂ sensitivity Index, ketanserin

Effects of Anaerobic Threshold on Respiratory Patterns during Incremental Exercise Test in Sedentary Male Subjects

Ihsan Serhatlioglu¹, Gulsen Cakmak³, Seda Ugras², Oguz Ozcelik¹

¹Firat University, Faculty of Medicine Department of Biophysics

²Firat University, Faculty of Medicine Department of Physiology

³Firat University, Faculty of Engineering, Department of Bioengineering

OBJECTIVE: The objective of this study was to analyse the effects of anaerobic threshold (AT) on respiratory patterns and regulation of ventilation (VE) during incremental exercise test.

METHODS: Ten sedentary male subjects (19.7 ± 0.2 yr, 68.3 ± 2.3 kg) were performed an incremental exercise test (15 W/min) until the limit of tolerance using an electromagnetically-braked cycle ergometer. Before the study, each subject gave signed written informed contents which were approved by the local ethical committee. Respiratory patterns (total time, Ttot, inspiratory time, Ti and expiratory time, Te) and VE were measured using spirometry. The relationships between VE and work rate were used to estimate AT. A paired t- test was used to evaluate values ($P < 0.05$).

RESULTS: Ti/Ttot and Te/Ttot did not change below the AT. However, above the AT, Ti/Ttot increased 10% ($p=0.01$) but Te/Ttot decreased 7% ($p=0.01$). VE increased 19.9 ± 1.0 L/min (warm-up) to 43.0 ± 1.7 L/min (at the AT) and 125.8 ± 6.3 L/min (at the maximal exercise). Below AT, VE increased due to the increase (74%) in tidal volume (VT). However, above AT, VE increased with closely increasing breathing frequency (115%). VT/Ti was 0.767 ± 0.05 L/s at warm-up and 1.762 ± 0.1 L/s at the AT (139% increase), and 4.440 ± 0.1 L/s at maximal exercise (506% increase).

CONCLUSIONS: The aerobic and anaerobic regions of incremental exercise test have a marked influence on respiratory patterns. The end productions of anaerobic metabolism above the AT, mainly affects Te and breathing frequency. The widely evaluation of aerobic and anaerobic regions on respiratory patterns during exercise is essential to determine its role in impaired exercise tolerance, especially in patient with respiratory system problems.

Keywords: Ventilation, anaerobic threshold, inspiratory Time, expiratory time, VT/Ti

Interactions between systemic hemodynamics and cerebral blood flow during attentional processing

Stefan Duschek, Natalie Werner

University of Munich

OBJECTIVE: Extensively used neuroimaging methods such as PET and fMRI are based on the functional interaction between nerve-cell activity and cerebral blood flow. While changes in brain perfusion are mostly attributed to flow metabolism coupling, it is commonly assumed that they are not affected by modulations in systemic hemodynamics. This assumption was challenged by the present study, which explored the interaction between systemic and cerebral hemodynamics during attentional processing with a specific focus on the temporal dynamics of this linkage.

METHODS: Using transcranial Doppler sonography, blood flow velocities in the middle cerebral arteries (MCA) of both hemispheres were recorded while 50 subjects performed a cued reaction time task. The task involved motor reactions on a visual stimulus, which was preceded by an acoustic warning signal (interstimulus interval 5 s). Blood pressure and heart rate were also continuously monitored.

RESULTS: Doppler sonography revealed a right dominant blood flow response. The extent of the increase measured in second two of the interstimulus interval showed a clear positive association with reaction speed. Task related changes in blood pressure and heart rate proved predictive of changes in MCA flow velocities in limited time windows of the response.

CONCLUSIONS: Besides an association between cerebral blood flow and attentional performance, the results suggest a marked impact of systemic hemodynamics on the blood flow response. The observed time-dependence of all interactions underlines the importance of the temporal aspect in the investigation of relationships between hemodynamic, neural and psychological processes, and therefore emphasizes the suitability of methods enabling high time resolution analyses.

Keywords: Cerebral blood flow, attention, transcranial Doppler sonography, blood pressure, heart rate

Trasplantation of encapsulated astroglial grafts ameliorate lesion-induced motor deficits in adult rats

Margarita Heredia, Fuente Antonio, Criado José María, Jiménez Díaz Lydia, Yajeya Javier, Nava Mauricio, Riobos Adelaida

Department of Physiology and Pharmacology. INCyL. Salamanca University, Salamanca, Spain

OBJECTIVE: We have previously shown the functional recovery of skilled forelimb use in rats after grafting of the frontal cortex lesion with cortical or amygdalar fetal tissue when rats were obliged to use the impaired limb. Here, we aimed to explore the effects of astrocyte grafts on the motor impairments caused by frontal cortex lesion in adult rats.

METHODS: Animals were conditioned in a paw-reaching-for-food task and the preferred paw was determined. Animals were anaesthetised in all stages of subsequent procedures (Equithesin, 20 mg/kg i.p.). Experimental animals were lesioned by aspiration in the motor cortex contralateral to the preferred paw. Astrocytes encapsulated in alginate spheres were implanted in the lesion cavity in one group of rats; fetal cortical tissue in a second group; and empty alginate spheres in a third group. The three groups were compared with a sham-operated control group.

RESULTS: Three months later, both rats with encapsulated astrocyte or fetal cortical transplants ameliorated the motor deficit caused by the lesion. Rats with empty alginate sphere implants showed no improvement. Animals with alginate sphere grafts showed an increase in the lesion size.

CONCLUSIONS: In summary, our findings show that transplants of astrocytes encapsulated in alginate spheres induce a long-term improvement of motor deficits caused by the lesion, however they produce side effects on the host. This work was supported by the MAPFRE Foundation. The authors thank to Noelia González and Javier Blanco for their excellent technical assistance.

Keywords: motor cortex, astrocyte transplants, alginate, encapsulation, adult rat.

Galanin-like immunoreactivity in the brain of the desert lizard *Uromastyx acanthinura* during activity season

Hammouche Sadjia¹, Bennis Mohamed²

¹Aride Area Research Laboratory, Biological Sciences Faculty, University of Sciences and Technology of Houari Boumediene, P.B. 39, El-Alia, Bab Ezzouar, Algiers. Algeria

²Pharmacology, Neurobiology and Comportement Laboratory, Department of Biology, Sciences Faculty, University of Cadi Ayyad, Marrakech, Morocco.

OBJECTIVE: The localization and distribution of the galanin in the brain of desert lizard *Uromastyx acanthinura* and the research of the relationship between this peptide and reproduction.

METHODS: The distribution of galanin-like immunoreactive perikarya and nerve fibers in the brain of the desert lizard *Uromastyx acanthinura* was studied by means of immunofluorescence using an antiserum against rabbit galanin.

RESULTS: In the wild, these animals experience successive arid and wet seasons that alternately stimulate their antidiuretic and diuretic systems. In this study, animals were captured during the activity season before vitellogenesis (March) and during eggs retention in the oviduct (June). Immunoreactive neurons were mostly detected in the infundibular recess nucleus. Immunoreactive fibers were found in the telencephalic, diencephalic and mesencephalic areas. Differences in galanin expression between females collected under both sets of conditions indicate changes during the reproductive cycle. Few galanin immunoreactive neurons and fibers were observed in the females at eggs retention stage, while many neurons and fibers were expressed before vitellogenesis.

CONCLUSIONS: Furthermore, the distribution of labelled neurons in the brain of this lizard was less restricted than that described in a snake. The wide hypothalamic and extrahypothalamic distribution of labelled fibers suggests that galanin peptides may have hypophysiotropic, neuromodulator and neurotransmitter roles in the lizard *U. acanthinura*.

Keywords: Galanin; Neuropeptides; Brain; Reproduction; Immunohistochemistry; Reptiles

Protective effects of snake venom on the vestibular compensation and regeneration of LVN neurons following unilateral labyrinthectomy

Naser Khalaji, Vaghinak H Sarkisian

Uremia Medical University of Science, Department of Physiology, Iran

OBJECTIVE: Damage of the vestibular apparatus following the traumatic brain injury is one of the common brain injuries, which in consequence leads to partial or even total disability. Neurodegeneration of the central and peripheral nervous system is one of the major sociomedical problems. The aim of this work to study of electrophysiological and histochemical features of lateral vestibular nucleus (LVN) and vestibular compensation by mean of NOX venom.

METHODS: Experiments were carried out on adult; mail Albino rats (230 ± 30 g). In the first stage animals were randomly categorized into three groups (normal, unilateral labyrenthectomy(UL) and UL with administration of NOX (10% LD50, im three next days after UL). The histochemical properties of LVN neurons were tested by means of recording the activity of Ca^{2+} - dependent acidic phosphates. For electrophysiological patterns of LVN neurons were evoked by bilateral stimulation of Paraventricular and supra optic nuclei with high frequency stimulus.

RESULTS: The findings of this study were compared with those of normal group. In histochemical results of UL groups, it was shown that neuron leads to neurodegenerative pattern, whereas in the case of NOX administration the recovery of neurons was found. The electrophysiological pattern of LVN neurons demonstrated that after UL there is asymmetric between ipsi and contra lateral sides. But in NOX group was the same as norm group.

CONCLUSIONS: The findings of this study indicate that following vestibular damage the snake venom probably leads to the recovery of Deiters' neurons and also enhanced the vestibular compensation during recovery.

Keywords: vestibular compensation; unilateral labyrinthectomy; neurodegeneration; electrophysiological; histochemical; Paraventricular; supra optic; snake venom.

Gadolinium inhibits membrane depolarisation-induced calcium signalling in isolated rat trigeminal ganglion neurons

Haluk Kelestimur¹, Murat Baykara¹, Emine Kacar¹, Mete Ozcan², Ahmet Ayar³

¹Department of Physiology, Faculty of Medicine, Firat University; Elazig, Turkey

²Department of Biophysics, Faculty of Medicine, Firat University; Elazig, Turkey

³Department of Physiology, Faculty of Medicine, Karadeniz Technical University; Trabzon, Turkey

OBJECTIVE: Chelates of gadolinium are used to provide enhanced contrast between healthy and diseased tissue in nuclear magnetic resonance imaging of different organs. Gadolinium is known to block many types of calcium channels such as stretch-activated and voltage-gated calcium channels. Free gadolinium is highly toxic and can cause a variety of abnormalities. Therefore, it is crucially important that gadolinium should be strongly attached to a ligand to avoid its toxic effects. Nevertheless, some chelates of gadolinium can release gadolinium ion, and they can bring about a wide variety of changes in physiology. In this study, the effects of gadolinium on intracellular calcium concentration were investigated in isolated rat trigeminal neurons.

METHODS: Trigeminal ganglion neurons were loaded with 1 μ mol Fura-2 AM and calcium responses were assessed by using the fluorescent ratiometry (excitation at 340 and 380 nm, and emission at 510 nm). All data were analyzed by using an unpaired t test, with a 2-tailed P level of <0.05 defining statistical significance.

RESULTS: Gadolinium caused significant ($P<0.001$) reductions in KCL-induced increase in intracellular calcium concentration. In the presence of gadolinium, intracellular calcium increase evoked by KCL was reduced to $86.6\pm 4.1\%$ ($n=11$).

CONCLUSIONS: These results indicate that gadolinium can inhibit increase in intracellular calcium concentration in these sensory neurons, which might result in side effects in trigeminal functions.

Keywords: Gadolinium, trigeminal ganglion, fluorescence calcium imaging

The NMDA receptor subunits NR2C and NR2D in frog and turtle retinal glial cells

Liliya Alexandrova Vitanova, Petia Nikolova Kuppenova
Dept. physiology, Medical university-Sofia, Sofia 1431, Bulgaria

OBJECTIVE: The glutamate NMDA (N-methyl-D-aspartate) receptors are widely distributed in the central nervous system (CNS) where they are involved in many cognitive processes as memory and learning, in motor control etc. In retina, which may be regarded as a biological model of CNS, glutamatergic neurotransmission mediated by NMDA receptors is extensively studied. However, data mainly concerns mammalian retinas, while the lower vertebrate ones are poorly studied. To the largest extent it is true for NMDA receptor subunits NR2C and NR2D which possess special features different from the features of other subunits. That's why the aim of the present study was to investigate their possible distribution in the mixed retina of frog *Rana ridibunda* and the cone retina of turtle *Emis orbicularis*.

METHODS: The indirect immunofluorescent method was used.

RESULTS: The NR2C and NR2D immunoreactivities had similar patterns of staining, both to each other and in the two types of retinas. Bright immunofluorescence was present in all retinal layers. In the inner nuclear layer the cell bodies of Müller cells, the main glial cells in retina, were evident. Their processes directed to both plexiform layers could also be followed. The inner and the outer limiting membranes were stained as well. No synaptic staining for these subunits was revealed.

CONCLUSIONS: The pattern of staining, which is indicative for the Müller cells, shows that retinal glia possesses NMDA receptors containing NR2C and NR2D subunits. A conclusion could be drawn that the retinal glial NMDA receptors differ in their subunits composition from the neuronal NMDA receptors which may be important for the neuron-glia interactions.

Keywords: retina, glutamate NMDA receptors, Muller glial cells, frog, turtle

Exercise affects skeletal muscle reinnervation: role of trk receptors

Stefano Sartini, Fanny Bartolini, Lattanzi Davide, Ciuffoli Stefano, Ambrogini Patrizia, Cuppini Riccardo
Department of Earth, Life and Environment Sciences, University of Urbino "Carlo Bo", Urbino, Italy

OBJECTIVE: Following nerve injury and regeneration, a transient phase of multiple innervation of muscle cells occurs. Successively, 1:1 ratio of innervation is progressively reached. Two mechanisms have been proposed to explain these processes: nerve-terminal competition for muscle released growth factors and the different pattern of axon activity. We previously showed running to enhance muscle expression of BDNF, a trophic factor inducing sprouting. Thus we investigated the role of running early in post-traumatic reinnervation.

METHODS: We used intracellular recordings (n= 67) and miography (n= 14) to evaluate soleus muscle reinnervation following nerve crush, in runner (n= 22) and sedentary rats (n= 29).

RESULTS: In sedentary rats, about 10% of recorded muscle cells were found to be multiply innervated from 7 to 45 days from nerve crush. In runners, multiple innervations reached 34% 10 days after nerve crush and this percentage gradually decreased during the following days, although it remained significantly higher with respect to sedentary group (Fig. 1). This effect of running was reversible (n= 5) and disappears blocking trk receptors (n= 11) (Fig. 2). Both in runner and sedentary rats all axons were showed to be regenerated 10 days after nerve crush, but in runners recovery of muscle strength was higher and muscle reinnervation was almost complete.

CONCLUSIONS: We hypothesize that intense motoneuron-muscle activity might induce up-regulation of one or more neurotrophic factors muscle-released that, via trk receptors, causes an increase of nerve terminal sprouting and massive muscle cell multiple innervation. This model allows to unify nerve terminal competition and axon activity hypotheses of muscle reinnervation mechanism and sheds light on exercise protocol planning during rehabilitation.

Keywords: Muscle reinnervation, muscle activity, nerve sprouting, multiple innervation, trk receptors, growth factors

Effects of dopamine D1 receptor blockade on the frog ERG under different conditions of light adaptation

Elka Popova, Petia Nikolova Kупenova

Department of Physiology, Medical University, Sofia, Bulgaria

OBJECTIVE: Dopamine is the predominant catecholamine in the vertebrate retina, acting through five subtypes of dopamine receptors. It is supposed that dopamine may be related to the process of retinal adaptation. The aim of this study was to investigate the possible contribution of D1 dopamine receptors to retinal sensitivity control under different conditions of adaptation.

METHODS: The effect of dopamine D1 receptor blockade by SCH 23390 on the intensity-response function of the ERG ON and OFF response was investigated in dark and light adapted frog eyes.

RESULTS: We obtained that the blocker enhanced the amplitude of the b- and d-waves in both conditions of adaptation. The enhancing effect of the blocker was relatively more pronounced on the rod- than cone dominated responses for the both ERG waves. The absolute sensitivity of the b-wave was not altered, but that of the d-wave was significantly increased. The intensity-response function of the b-wave, but not that of the d-wave, was shifted to the left along the intensity axis. The b-wave $V - \log I$ function had steeper slope and narrower dynamic range in both dark and light adapted eyes after the D1 receptor blockade. The latency of the responses was not altered, but the implicit time of the rod-dominated responses was significantly increased.

CONCLUSIONS: The results obtained indicate that the endogenous dopamine, acting through D1 receptors, does not play a crucial role in the process of retinal adaptation, although it changes in a specific manner the intensity-response function of both the ERG b- and d-waves.

Keywords: light adaptation, dopamine, electroretinogram, frog, retina

Participation of the *Drosophila* glial histamine receptor HCLB in visual sensitivity modulation under different ambient light conditions

Petia Nikolova Kuppenova¹, Shazie Yusein Myashkova²

¹Department of Physiology, Medical University, Sofia, Bulgaria

²Department Structure and Function of Chromatin, Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria

OBJECTIVE: Histaminergic transmission between photoreceptors and second-order visual neurons in arthropods is mediated by ionotropic receptors, encoded by two genes, *hclA* and *hclB*. The lack of *HCLA* receptors, which are expressed in the second-order neurons, completely blocks histaminergic transmission. However, null mutations in *hclB*, which is expressed in the glial cells, result in increased amplitude of the second-order neuron components of the electroretinographic (ERG) responses. In this work, the *HCLB*-mediated influences on the ERG responses were studied in different conditions of ambient illumination.

METHODS: The intensity-response functions ($V(\log I)$) of the ERG responses of the *hclB* null mutant *hclBT2* were studied under dark and light adaptation. The dynamics of the dark sensitivity recovery following short light adaptation was also characterized.

RESULTS: The amplitudes of the ERG ON and OFF transients were increased in the *hclBT2* mutant, the relative increase being independent on both stimulus intensity and state of adaptation. As a result, the amplitude scales and absolute sensitivities of the ERG transients were increased in a similar manner under both dark- and light-adaptation. The relative sensitivity of the transients and the adaptational shift of the $V(\log I)$ curves along the intensity axis were not altered. The *hclBT2* mutant flies showed delayed dark sensitivity recovery with lacking postadaptational potentiation of the ERG transients.

CONCLUSIONS: Our results show that *HCLB* receptors are involved in visual sensitivity modulation under both dark and light adaptation conditions but they do not alter significantly the process of light adaptation. They modulate the characteristics and the dynamics of the dark sensitivity recovery following short light adaptation.

Keywords: *hclB*, histamine, glia, *drosophila*, ERG, light adaptation

Functional and morphological study in patients with retinitis pigmentosa

José Antonio Sáez Moreno, Inmaculada Concepción Domínguez Hidalgo, Rafael Mancebo Azor, José Manuel Rodríguez Ferrer

Laboratorio de Neurociencia Visual y Cognitiva. Institute of Neuroscience, Granada University, Granada, Spain

OBJECTIVE: Retinitis pigmentosa (RP) is the most common inherited progressive retinal neurodegenerative disease. Eleven patients with RP, after complete ophthalmic exploration, were studied by electrophysiological and morphological techniques.

METHODS: Flash (ERG) and pattern (pERG) electroretinogram recordings and optical coherence tomography (OCT) images were obtained from the retina of the patients. Evaluation of the visual cortex was done by recording pattern reversal visual evoked potentials (VEPs).

RESULTS: All of the patients, except one with reduced amplitude, had non recordable flash ERG. In 3 patients, pERG recordings were non detectable and 6 patients showed reduced amplitude and 2 had normal pERG. All patients had recordable VEPs. In 9 of them, the amplitude of the P100 was reduced in different extent. OCT images show the absence of the layer of photoreceptors in 3 patients and its presence only in macular region in 3 cases. In the rest, the photoreceptors layer was found throughout the retina. In 7 of the patients, other alterations of the retina, as macular oedema or epiretinal membrane, were observed. These results show first that VEPs can be obtained in patients with undetectable flash and pattern ERG and, what it is more interesting, in patients that showed absence of the photoreceptors layer by OCT exam. Secondly, retinal pathologies other than the alteration of the photoreceptor layer are commonly found in RP patients.

CONCLUSIONS: Our results point out the importance of performing a complete electrophysiological and morphological study to correct interpretation of the clinical aspect of RP and evaluation of the results from the different diagnostic tests.

Keywords: Retinitis pigmentosa, ERG, VEPs, OCT

Figure 1

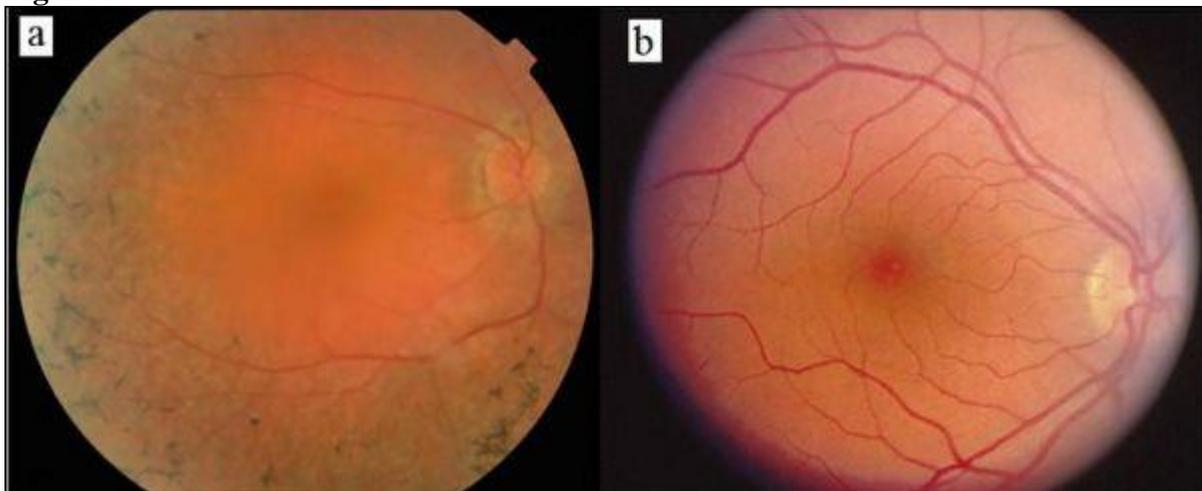
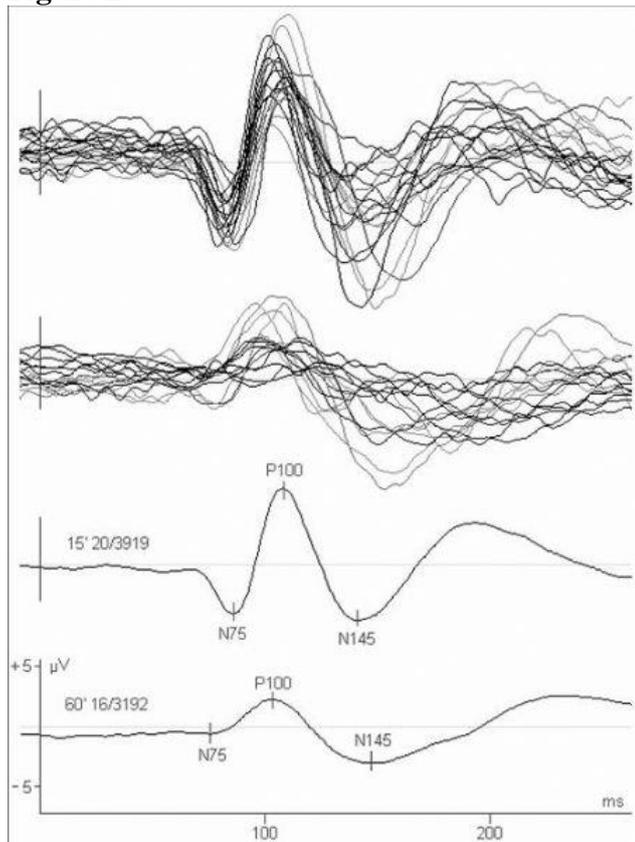


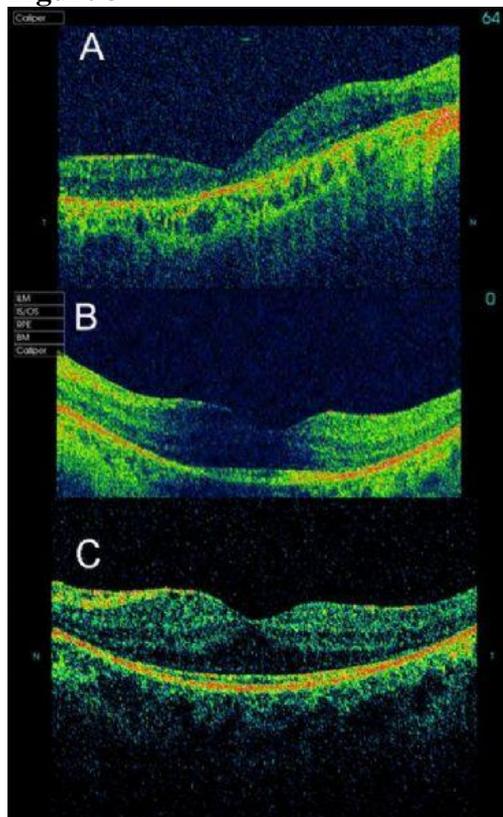
Figure 1. (a) Right fundus of a patient with RP. Note the attenuated retinal vessels and the bone spicule-like pigmentary deposits throughout the periphery, that characterize RP. (b) Right fundus of a healthy subject.

Figure 2



VEPs obtained in: (a) healthy control subjects and (b) RP patients with the check width that produced the largest amplitude of the P100; 15 min and 60 min, respectively. In (c) and (d) averaged VEPs of controls subjects and patients, respectively. The peak VEPs components N75, P100 and N145 are indicated.

Figure 3



OCT images of three RP patients showing different degrees of alteration of the layer of photoreceptors's external segment.

The Evaluation of the Visual Cognitive Function for the Professional Dancers and Sportsmen

Sernaz Demirel Temel¹, Gökçer Eskikurt², Tan Temel¹, Ümmühan Isoglu Alkac³, Ilker Yücesir⁴, Bülent Bayraktar⁵

¹The Art and Design Faculty Department of Music and Performing Arts, YTU, Istanbul, Turkey

²Institute of Experimental Medicine, Department of Neuroscience, Istanbul University, Istanbul, Turkey

³Istanbul Faculty of Medicine, Department of Physiology, Istanbul University, Istanbul, Turkey

⁴School of Physical Education and Sports, Istanbul University, Istanbul, Turkey

⁵Istanbul Faculty of Medicine, Department of Sports Medicine, Istanbul University, Istanbul, Turkey

OBJECTIVE: Since visual attention is important for the activities of the dance and sport, the main aim of this study was to investigate visual cognitive responses of professional dancers and sportsmen.

METHODS: Ten healthy right-handed professional modern dancers and 10 basketball player participated in this study (5 female, 5 male; mean age: 24.7±1.2). Electroencephalographic (EEG) activity was recorded from 19 sites with MITSAR. Since, the P300 component of the Event Related Potentials (ERPs) can be used to identify cognitive functions as attention, Visual Oddball Paradigm (VERPs; P300 Paradigm) applied to dancers and sportsmen. All statistical analyses were performed using SPSS-PC 16.0 (Repeated Measured ANOVA).

RESULTS: In VERPs paradigm, P300 responses of professional dancers had larger in amplitude than of sportsmen especially for the Fz, F4 and C4 locations. The P300 responses of the sportsmen had larger amplitude than of dancers for the F3, C3, Cz, P3, Pz, P4, O1 and O2 locations. The channel effect was significantly different between dancers and sportsmen [$F(18,198)=31,09$; $p<0.0001$]. The group x channel effect was different statistically [$F(18,198)=2,06$; $p<0.008$]. There is no any significant difference for the latency of P300 responses between the dancers and sportsmen. The cognitive responses of the dancers were higher in sportsmen for the anterior-right locations. On the other hand, the cognitive responses of sportsmen were higher than in dancers for the anterior-left and posterior locations of brain.

CONCLUSIONS: The present study is important to demonstrate the electrophysiological differences of the visual cognitive functions between dancers and sportsmen.

Keywords: Visual Evoked Potentials, EEG, Dancer, Sportsmen

The Properties of the Components of Primer Visual Processing and P300 Responses by Visual Oddball Paradigm

Gökçer Eskikurt¹, Ümmühan Isoglu Alkac²

¹Institute of Experimental Medicine, Department of Neuroscience, Istanbul University, Istanbul, Turkey

²Istanbul Faculty of medicine, Department of Physiology, Istanbul University, Istanbul, Turkey

OBJECTIVE: The aim of this study was to determine the hemispheric differences of primer visual processing and P300 responses by using Visual Oddball Paradigm.

METHODS: Ten healthy volunteers (5 male, 5 female) participated the study. Brain electrical activity (EEG) recorded by using WinEEG program (MITSAR) from 19 different locations. Volunteers are instructed to press a button every time when they see the blue square at the bottom of edge (target stimulus) and not to press button when they see the blue square at the top of the edge (standart stimulus). In average 330 stimuli were presented with percent 20 target stimulus (I.U. Ethics Commision File No: 2009/2648-43). Repeated measured ANOVA (SSPSS-PC 16.0) test is used for the statistical analysis.

RESULTS: There was no significant difference on the amplitudes [$F(1,9) = 2.918, p = 0.122$] and latency [$F(1,9)=0.289, p=0.604$] of P300 responses between left and right hemisphere. On the other hand, the amplitudes of N100 component for left hemisphere were higher than in right hemisphere [$F(1,9)=5.249, p<0.05$] but there was no difference in latency of N100 component [$F(1,9)=2.716, p=0.134$]. Amplitudes of N100 component is especially higher at the centro-posterior region [$F(7,63)=2.554, p<0.05$].

CONCLUSIONS: The present study is important to show that the hemispheric properties of primer visual processing and also cognitive responses. This work was supported by scientific research projects coordination unit of Istanbul University. Project number 4965.

Keywords: Visual Oddball, Primer Processing components, Dominant hemisphere.

Do different muscle activities have different effects on spontaneous electrical activity of the brain?

Tan Temel¹, Gökçer Eskikurt², Sernaz Demirel Temel¹, Numan Ermutlu³, Ilker Yücesir⁴, Bülent Bayraktar⁵, Ümmühan Isoglu Alkac⁶

¹The Art and Design Faculty Department of Music and Performing Arts, YTÜ, Istanbul, Turkey

²Institute of Experimental Medicine, Department of Neuroscience, Istanbul University, Istanbul, Turkey

³Medical Faculty Department of Physiology, Istanbul Bilim University, Istanbul, Turkey

⁴School of Physical Education and Sports, Istanbul University, Istanbul, Turkey

⁵Istanbul Faculty of Medicine, Department of Sports Medicine, Istanbul University, Istanbul, Turkey

⁶Istanbul Faculty of Medicine, Department of Physiology, Istanbul University, Istanbul, Turkey

OBJECTIVE: The frequency properties of the spontaneous brain activity have revealed valuable insights into brain correlates underlying brain functions. Because of previous studies showed that the muscle activity affects directly the brain activity, in the present study we investigated the differences of spontaneous electrical activity (EEG) of the brain between professional dancers, sportsmen and control groups.

METHODS: Ten healthy and right-handed professional dancers, 10 sportsmen and 10 healthy volunteers participated in this study (5 female, 5 male for each groups). Spontaneous EEG was recorded during the two different conditions: 5 min EEG sequences under resting conditions were recorded with eyes open and closed. The properties of frequencies of the spontaneous brain electrical activity were analyzed. All statistical analyses were performed using SPSS-PC 16.0 (Repeated Measured ANOVA).

RESULTS: In condition with eyes closed: There were significant differences for the brain frequencies between groups (control, dancers and sportsmen). In condition with eyes open: There were significant differences for the brain frequencies between groups. Sportsmen had higher frequency power especially for the theta and alpha than in dancers and controls for the conditions with eyes open and also closed.

CONCLUSIONS: The present study is important to demonstrate the effect of the muscle activity on the electrical activity of the brain in dancers and sportsmen.

Keywords: EEG, Spontaneous brain electrical activity, Frequency components, Dancer, Sportsmen

The effect of cannabinoid WIN55-212,2 in rapid eye movement sleep induced hyperalgesia

Osman Haluk Sönmezocak¹, Levent Öztürk¹, Elif Ezgi Gürel¹, Ahmet Ulugöl²

¹Department of Physiology Trakya University Faculty of Medicine, Edirne, TURKEY

²Department of Pharmacology Trakya University Faculty of Medicine, Edirne, TURKEY

OBJECTIVE: The aim of this study was obtain evidence for explaining the neurochemical mechanism between sleep and pain by investigating the effects of WIN55-212,2, a cannabinoid substance, on 96-hours rapid eye movement (REM) sleep deprivation induced hyperalgesia.

METHODS: Forty Wistar albino rats (weight range, 200-220 g) were used. Four experimental groups, each having 10 animals, were as follows: CONT, REM deprivation+Placebo; WIN1, REM deprivation+Win55 (1 mg/kg); WIN3, REM deprivation+Win55 (3 mg/kg); WIN10, REM deprivation+Win55 (10 mg/kg). All animals underwent pain threshold measurements by hot-plate test and REM sleep deprivation by modified multiple platform technique. Then, Group 1 had DMSO 50%, Group 2, 3 and 4 had 1 mg/kg, 3 mg/kg, and 10 mg/kg WIN55-212,2 intraperitoneal injection (5 mL/kg), respectively. Sixty minutes after drug administration, pain threshold measurements were repeated. Pain threshold measurements were tested by Kolmogorov-Smirnov, the difference between the mean results of study groups were tested by ANOVA. In addition, post-hoc Tukey test was used. $P < 0.05$ accepted as statistically significant.

RESULTS: Pain threshold values significantly decreased after REM-deprivation in CONT, WIN1 and WIN3 groups (9.4 ± 1.5 vs 7.6 ± 1.6 ; 12.0 ± 3.3 vs 6.5 ± 1.6 ; and 12.0 ± 2.6 vs 6.7 ± 1.1 ; $p < 0.05$ for all), whereas in WIN10, hyperalgesia disappeared by 10 mg/kg i.p. Win-55 (11.3 ± 3.0 vs 10.5 ± 4.8 ; $p > 0.05$).

CONCLUSIONS: Intraperitoneal 10 mg/kg WIN55-212,2 prevented REM sleep deprivation induced hyperalgesia which suggests a role for cannabinoids in pain modulation during REM sleep loss.

Keywords: REM sleep, cannabinoid, hyperalgesia, deprivation

The effect of citalopram, a selective serotonin reuptake inhibitor, on memory alterations induced by selective rapid eye movement sleep deprivation in rats

Nurcan Arıcanlı¹, Çetin Hakan Karadağ¹, Levent Öztürk²

¹Department of Pharmacology Trakya University Faculty of Medicine, Edirne, TURKEY

²Department of Physiology Trakya University Faculty of Medicine, Edirne, TURKEY

OBJECTIVE: We aimed to investigate the effects of citalopram, a selective serotonin reuptake inhibitor, on memory alterations induced by selective rapid eye movement (REM) sleep deprivation.

METHODS: Sixty male Wistar albino rats (average weight, 200- 300g) were equally divided into five groups: Group 1 (n=12) normal sleep + placebo (%0.9 NaCl); Group 2 (n=12) REM sleep deprivation by modified platform technique + placebo; Group 3, 4, and 5 (for each n= 12) REM sleep deprivation and 5, 10, and 20 mg/kg single dose citalopram, respectively. All the study groups, except Group 1, underwent REM sleep deprivation between the 1st -13th days of the study. Drug interventions were applied between the 8th – 13th days of the study. The rats were trained 4 times daily between Day 8 and Day 12 of the study (acquisition phase). At the 13th day, the platform was removed, and the probe test was performed for 30 seconds (retrieval).

RESULTS: REM sleep deprivation led to statistically significant impairments in various learning parameters ($p<0.05$), and increased thigmotaxis ($p<0.001$); these impairments were not seen in citalopram groups. However, a dose-response relationship was not found in the effects of citalopram. In probe trials, REM sleep deprivation did not cause any significant impairment.

CONCLUSIONS: REM sleep deprivation retards learning by increasing thigmotaxis and the negative effects of REM sleep deprivation on learning may be prevented by citalopram.

Keywords: REM sleep, citalopram, learning, memory, deprivation

The validity of linguistic specificity for N400 component in cognitive visual evoked potentials technique using a Romanian patented psycho-verbal stimulation interface

Bogdan Mircea Dionisie

University of Medicine "Gr.T.Popa", Iasi, Romania

OBJECTIVE: This study was planned to demonstrate the validity of linguistic specificity for N400 component in cognitive visual evoked potentials technique whatever is the level and the strategy of decoding, using a Romanian patented psycho-verbal stimulation interface.

METHODS: The acquisition of visual evoked potentials was performed in Cz, Pz, Fz, Oz leads, using a group of 50 adult persons between 19 – 21 year old, students who made two recognition tasks using decoded of 500 strings of symbols and decoding 500 strings of letters (words and non-word) with an average number of 7 characters. EPs was analyzed in a 1000 ms interval, after it used Grand AVG function for 50 person's recordings at each task, on 17 of 100 ms interspersed intervals, looking through SPSS software using ANOVA method which assess the highest difference in amplitude for each interval.

RESULTS: Averaged EPs was poor in component after 300 ms for non-linguistic strings of symbols, but for linguistic stimuli it appeared a pronounced negative wave between 350 – 450 ms ($t=6,25$, $p<0,001$) and others negative and positive reverberation was present after this latency ($t=1,10$, $p>0,05$), especially in Fz and Pz. P300 was present on both tasks.

CONCLUSIONS: The N400 component is related with linguistic stimuli, regardless of semantic or non-semantic types (pseudo - words) which confirm the findings of other studies.

Keywords: Cognitive visual evoked potentials, N400, lexical recognition, neurosciences & biomedical engineering.

Oxytocin inhibition of pentylenetetrazole-induced convulsion and its identification by behavioral measurement and thalamic EEG in the rat

Oytun Erbaş, Vedat Evren, Gönül Ö. Peker

Ege University, Faculty of Medicine, Department of Physiology. Izmir, TURKEY

OBJECTIVE: Oxytocin's (OX) recently attributed features other than its well known endocrine effects have been on the agenda lately. In this study, we hypothesized that OX might possibly exert anticonvulsant effect, and we investigated this in pentylenetetrazol (PTZ) induced generalized seizure and absence seizure rat model, identifying the action by behavioral measurement and thalamic EEG recordings.

METHODS: Using 60 (8-12 week old) Sprague Dawley male rats, we set 10 groups (n=6 / group), administered i.p. 10, 20, 40, 80 and 120 U/kg OX to Groups 1-5, and saline only to Group 6 (control); 5 minutes later, we administered 70 mg/kg PTZ, and determined the dose-response ratio. Evaluating seizures by using Racine's Convulsion Scale, we determined the absence seizure inducing PTZ dose as 35 mg/kg, and the suppressive OX doses as 80 and 120 U/kg. We recorded EEG on the Biopac MP30 System by bipolar EEG electrodes implanted in the left nucleus of posterior thalamus using stereotaxy (AP: -3.6 mm, L: +2.8 mm, V: -5.0 mm; Paxinos). We administered saline only, 35 mg/kg PTZ only, and 80 and 120 U/kg OX plus PTZ to the Groups 7, 8, 9 and 10, respectively. We affirmed electrode location histologically following euthanasia. Processing recordings by "Power Spectral Analysis" using the "Fast Fourier Transform", we determined the percentage dominance of EEG waves.

RESULTS: The potent anticonvulsant effect emerging at 40 and 80 U/kg doses ($p < 0.05$) and peaking at 120 U/kg dose of OX, and its significant ($p < 0.005$) overall delta enhancing and theta attenuating effect ($p < 0.005$) were definitely demonstrative.

CONCLUSIONS: Our results suggest OX as a putative anticonvulsant in treating epilepsy. We aim to identify the specific mechanism mediating this action.

Keywords: Thalamic EEG, Oxytocin (OX), Racine scale, Absence and generalized convulsion, Pentylenetetrazol (PTZ)

Insulin induces GABA-A channels that generate tonic currents

Bryndis Birnir¹, Zhe Jin¹, Yang Jin¹, Suresh Kumar Mendu¹, Eva Degerman², Leif Groop²

¹Department of Neuroscience, Uppsala University, Uppsala, Sweden

²Lund University Diabetic Center, Lund University, Lund, Sweden

OBJECTIVE: The objective was to examine effects of metabolic hormones on neuronal excitability with the initial focus on insulin. Insulin signaling to the brain is important not only for metabolic homeostasis but also for higher brain functions such as cognition.

METHODS: We used the patch-clamp whole-cell and single-channel recordings to examine the neuronal excitability.

RESULTS: GABA (γ -aminobutyric acid) decreases neuronal excitability by activating GABA-A channels that generate phasic and tonic currents. The level of tonic inhibition in neurons varies. Here we show in acute rat hippocampal slices that 1 nM insulin “turns on” new extrasynaptic GABA-A channels in CA1 pyramidal neurons resulting in decreased frequency of action potential firing (1). The channels are activated by more than million times lower GABA concentrations than synaptic channels, generate tonic currents and show outward rectification. The single-channel current amplitude is related to the GABA concentration resulting in a single-channel GABA affinity (EC₅₀) in intact CA1 neurons of 17 pM with the maximal current amplitude reached with 1 nM GABA. They are inhibited by GABA-A antagonists but have novel pharmacology as the benzodiazepine flumazenil and zolpidem are inverse agonists.

CONCLUSIONS: The results show that tonic rather than synaptic conductances regulate basal neuronal excitability when significant tonic conductance is expressed and demonstrate an unexpected hormonal control of the inhibitory channel subtypes and excitability of hippocampal neurons. The insulin-induced new channels provide a specific target for rescuing cognition in health and in diseases like diabetes, dementia and Alzheimer disease.

Reference: 1) Jin et al. (2011) PLOS One 6: e16188.

Keywords: inhibition, GABA, GABA-A, insulin, current, benzodiazepine, hippocampus

The effect of co-administration of the NMDA blocker with agonist and antagonist of CB1-receptor on penicillin-induced epileptiform activity in rats

Duygu Çakıl¹, Mehmet Yıldırım², Mustafa Ayyıldız³, Erdal Agar³

¹Department of Physiology, Gaziosmanpaşa University, Tokat, Turkey

²Department of Physiology, Karadeniz Technical University, Trabzon, Turkey

³Department of Physiology, Ondokuz Mayıs University, Samsun, Turkey

OBJECTIVE: Memantine is an uncompetitive NMDA receptor antagonist, that have potentially wide applications including epilepsy. On the other hand, cannabinoid system plays a key role in regulating seizure activity in brain. In this study we examined the interaction between memantine and cannabinoid system on penicillin induced epileptiform activity.

METHODS: Rats were anesthetized with urethane (1.25 g/kg, i.p) and placed in stereotaxic frame. Left cerebral cortex was exposed by craniotomy. The epileptic focus was produced by 500 units penicillin G injection. Combinations of Memantine, AM251 and ACEA were used to examine their effects on epileptiform activity. The electrocorticography (ECoG) activity was monitored on a four-channel recorder.

RESULTS: Memantine, at doses of 2.5 and 5mg/kg, significantly decreased the epileptiform activity. The most effective dose was 5 mg/kg. AM-251 caused a significant increase in epileptiform activity. It also caused development of status epilepticus-like activity. ACEA significantly decreased the epileptiform activity.

AM-251 (0.25 µg, i.c.v) did not influence anti-epileptiform effect of memantine. The best and earlier anti epileptiform effect appeared in the presence of memantine+ACEA.

CONCLUSIONS: Memantine presented bell-shaped dose-effect relationship in this study. An additional anti-epileptiform effect was seen in the presence of Memantine + ACEA, which was blocked by AM-251. The proconvulsant effect of AM-251 on penicillin-induced epileptiform activity disappeared in the presence of memantine + AM-251, suggesting an interaction between cannabinoid system and NMDA receptors.

Keywords: epilepsy, penicillin, AM251, ACEA, Memantine, Cannabinoids, NMDA

Inducible nitric oxide synthase activity involves in the anticonvulsant effect of grape seed extract on penicillin-induced epileptiform activity in rats

Sedat Per¹, Abdulkadir Taşdemir², Mehmet Yıldırım³, Mustafa Ayyıldız⁴, Nusret Ayyıldız², Erdal Agar⁴

¹Department of Biology, Bozok University, Yozgat, Turkey

²Department of Biology, Erciyes University, Kayseri, Turkey

³Department of Physiology, Karadeniz Technical University, Trabzon, Turkey

⁴Department of Physiology, Ondokuz Mayıs University, Samsun, Turkey

OBJECTIVE: Grape seed extract (GSE) has been known to be neuroprotective due to its antioxidant properties. The aim of the present study was to examine both the effect of GSE on penicillin-induced epileptiform activity in rat and the role of the NO pathway in the effects of GSE.

METHODS: In this study, sixty-six adult male Wistar rats weighing 225–260 g were used. The ECoG activity was continuously monitored on a four-channel recorder. All recordings were made under anesthesia and stored on a computer. The frequency and amplitude of epileptiform ECoG activity was analyzed offline.

RESULTS: Intracortical injection of penicillin induced epileptiform activity. The best and earlier effects of selective inducible nitric oxide synthase inhibitor, aminoguanidin appeared at dose of 60 mg/kg, i.p. The effective dose of aminoguanidine (60 mg/kg, i.p.) partially reversed the anticonvulsant activity of GSE. The administration of the NO substrate, L-arginine (500 mg/kg, i.p.) 10 minutes before the effective dose of GSE (200 mg/kg, i.p.) blocked the influence of aminoguanidine on the anticonvulsant effect of GSE on penicillin-induced epileptiform activity. 7-NI significantly decreased the frequency of epileptiform ECoG activity without changing amplitude after 7-NI administration. However, the mean frequency of epileptiform activity was significantly decreased in the 50 min in the presence of L-arginine +GSE.

CONCLUSIONS: The anticonvulsant effect of GSE was diminished by the selective inducible NOS inhibitor, aminoguanidine, which was inhibited by L-arginine. Moreover, the nonspecific NOS inhibitor, L-NAME partially inhibited the anticonvulsant effect of GSE. Therefore, these findings imply that inducible-NOS participates in the anticonvulsant activity of GSE.

Keywords: Epilepsy, Grape seed extract, Nitric oxide, Nitric oxide synthase, Penicillin

The Interactions of Memantine and Vitamins on Penicillin-Induced Epileptiform Activity in Rats

Duygu Çakıl¹, Mehmet Yıldırım², Mustafa Ayyıldız³, Erdal Agar³

¹Gaziosmanpaşa University, Department of Physiology, Tokat, Turkey

²Karadeniz Technical University, Department of Physiology, Trabzon, Turkey

³Ondokuzmayıs University, Department of Physiology, Samsun, Turkey

OBJECTIVE: Memantine is an uncompetitive NMDA receptor antagonist that have the potential therapeutic benefits in numerous neurological disorders including epilepsy. The neuroprotective properties of ascorbic acid, pyridoxine and α -tocopherol have been documented in in some experimental epilepsy models. The objective of the present study was to investigate the effects of co-administration of memantine with ascorbic acid, pyridoxine and α -tocopherol on penicillin-induced epileptiform activity in rats.

METHODS: Rats were anesthetized with urethane (1.25 g/kg, i.p) and placed in stereotaxic frame. The left cerebral cortex was exposed by craniotomy. The epileptic focus was produced by 500 units penicillin G injection into the somatomotor cortex. Pyridoxine (40 mg/kg), alpha-tocopherol (500 mg/kg) and ascorbic acid (100 mg/kg) were intraperitoneally administered 15 min after memantine (5 mg/kg, i.p.) application.

RESULTS: Memantine (5mg kg, i.p) significantly decreased the epileptiform activity in the 50th minute after penicillin injection. Statistically significant reductions in epileptiform activity were started at 40th, 40th and 30th minutes after penicillin injection in the memantine+ascorbic acid, memantine+alpha- tocopherol and memantine+pyridoxine administrated groups, respectively.

CONCLUSIONS: The results of the present study indicate that co-administration of vitamins with memantine did not significantly change the anticonvulsant activity of memantine against penicillin-induced epileptiform activity.

Keywords: memantine, epilepsy, penicillin, ascorbic acid, alpha-tocopherol, pyridoxine

The Comparative Effects of Acetaminophen and Diazepam on Penicillin-Induced Epilepsy in Rats

Ibrahim Mumcuoğlu¹, Zeynep Kasap², Duygu Çakıl², Fatih Ekici², Gülsüm Semiha Kurt¹

¹Department of Neurology, Gaziosmanpaşa University, Tokat, Turkey

²Department of Physiology, Gaziosmanpaşa University, Tokat, Turkey

OBJECTIVE: Acetaminophen is a widely used analgesic and antipyretic agent. AM404 (N-arachidonoyl-phenolamine) the active metabolite of acetaminophen was reported to reduce epileptic activity by activating the endocannabinoid system in some models of experimental epilepsy. Diazepam is a benzodiazepine with well known anticonvulsant effects. The objective of the present study was to investigate the effects of acetaminophen and diazepam comparatively on penicillin induced epilepsy in rats.

METHODS: Rats were anesthetized with urethane (1.25 g/kg, intraperitoneal) and placed in stereotaxic frame. Body temperature was maintained at 37°C by a heating blanket. The left cerebral cortex was exposed by craniotomy. The epileptic focus was produced by 500 units penicillin G injection into the somatomotor cortex. Acetaminophen (100 mg/kg, intravenous), diazepam (5 mg/kg, intravenous) and diazepam + acetaminophen were administrated 30 minutes after penicillin injection and their effects were examined comperatively on epileptiform activity. The electrocortigography (ECoG) activity was monitored for 2 hours.

RESULTS: Intracortical injection of penicillin (500 units) induced epileptiform activity in all groups. Statistical analysis was estimated by one-way ANOVA and Post Hoc LSD tests. Diazepam (5mg kg, intravenous) significantly decreased the epileptiform activity in the 10th minute after penicillin injection. The administration of acetaminophen (100 mg/kg, intravenous) did not influence the penicillin induced epileptiform activity. Also no significant diffirence was found between antiepileptic effects of diazepam and diazepam + acetaminophen groups.

CONCLUSIONS: The results of present study indicate that acetaminophen did not influence the penicillin-induced epileptiform activity and there are no interactions between acetaminophen and diazepam on penicillin-induced epilepsy. Further investigations are needed concerning the fundimental mechanisms of acetaminophen on epilepsy.

Keywords: penicillin, epilepsy, acetaminophen, diazepam

Brain wave synchronization and entrainment to binaural beat stimulation of rabbit

Mesud Soydan, Ahmet Koyu, Nurhan Gumral

Department of Physiology, Süleyman Demirel University, Isparta, Turkey

OBJECTIVE: It is well known that different brainwave frequencies show the synchronies related to different perceptual, motor and cognitive states. Brainwaves have also been shown to synchronize with external stimuli by repetition rates of 10-40 Hz. Based on the human studies concerning brain physiology, it has been demonstrated that brain activities could also synchronize to external stimuli. But this physiology has not been investigated in any animal models yet.

METHODS: We performed the analysis of brain waves entrainment to different binaural beat frequencies in rabbit, which are alpha, beta and theta. Rabbit was listened to binaural beat tapes, followed by shaving scalp and placing EEG electrode with paste at frontal and occipital scalp area. During this procedure, we observed that brainwaves had the potential to entrain the desired frequency in rabbit.

RESULTS: According to our findings, the theta binaural stimulation led to increase the theta brainwaves, contrast to beta. In addition, alpha and beta binaural stimulation caused the increases in alpha and beta brain waves, respectively.

CONCLUSIONS:

Keywords: brain wave entrainment binaural beat rabbit

Influence of carbenoxolone on the anticonvulsant efficacy of phenytoin in pentylenetetrazole kindled rats

Fatih Sefil¹, Faruk Bağırıcı², Meryem Dilek Acar², Cafer Marangoz²

¹Department of Physiology, Faculty of Medicine, Mustafa Kemal University, Hatay, Turkey

²Department of Physiology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

OBJECTIVE: Irregular synchronized neuronal discharges mediated by gap junctions have an important role in epileptic seizures. Analysis of anticonvulsant drugs acting on gap junctions is still a priority in epilepsy research. Therefore, the present study was planned to investigate the effect of carbenoxolone (CBX), a gap junction blocker, on the anticonvulsant efficacy of phenytoin on pentylenetetrazole (PTZ) kindling model of epilepsy in rats.

METHODS: Male Wistar albino rats, 14 weeks of age, were used. In the first step of study, animals were given PTZ 35 mg/kg intraperitoneally (i.p.) three times a week until kindling was produced. Then, permanent screw electrodes allowing EEG monitoring from awake rats, were implanted into the cranium of kindled rats. In this way, we were able to record EEG and evaluate seizure stage at the same time. In the second step, combination of CBX (40 mg/kg i.p.) and phenytoin (60 mg/kg, i.p.) was administered. The data analysis was performed using a one-way ANOVA with LSD post-hoc test.

RESULTS: Phenytoin prevented generalized seizures by 66.6 % and reduced seizure severity and score ($P<0.05$). The combination of CBX and phenytoin prevented generalized seizures by 66.6 % and decreased seizure severity and score ($P<0.05$).

CONCLUSIONS: The results of this study show that there is no significant difference between CBX-phenytoin combination and phenytoin on all parameters. Thus, CBX does not increase the anticonvulsant efficacy of phenytoin in PTZ model of epilepsy in rats.

Keywords: Carbenoxolone; Phenytoin; Epilepsy; Pentylenetetrazole; Kindled rats

Effects of enrichment environment on sympathetic system in pentylenetetrazol-kindled rats

Nazan Dolu¹, Seval Keloglan², Soner Bitiktas², Setenay Cuğ²

¹Erciyes University Faculty of Medicine, Physiology EU, Kayseri

²Erciyes University, Institute of Health Sciences, Department of Physiology

OBJECTIVE: Skin conductance (electrodermal activity, EDA) measurements reflect sympathetic tone. EDA is frequently used as an indirect measure of stress. Selective depletion of forebrain noradrenaline has been shown to potentiate various types of experimentally induced seizures. The aim of this study was to investigate sympathetic activity and stress condition with EDA in pentylenetetrazole-kindled rats and was to test whether environmental enrichment alters responsiveness of pituitary-adrenocortical and sympathetic response in these rats.

METHODS: We separated animals into four different groups. The control group in normal cage (CN), the control group in enrichment cage (CE), epileptic group in normal cage (EN), the epileptic group in enrichment cage (EE). Serum physiologic and PTZ (35 mg/kg, ip) were injected to control or epileptic group, respectively once every alternate day for a period of 38 days. After a week from the last injection, EDA was recorded from rat's planter surface of posterior extremities using Ag/AgCl electrodes. Tonic and phasic (response to 15 auditory stimuli) skin conductance levels (SCL) were taken.

RESULTS: There were significant differences among the groups for tonic and phasic SCL. CN group had significantly higher SCL than CE, EN, EE groups. SCL of the control group when housed in enrichment cage were diminished. But, in epileptic group, SCL were not diminished. There were no significant differences between rats housed in normal and enrichment cages.

CONCLUSIONS: The data suggest that epileptic rats had diminished electrodermal activity due to by their relatively low noradrenaline content. Enrichment in environment did not seem to have an improving effect on sympathetic system in the epileptic rats.

Keywords: pentylenetetrazol-kindled, rat, enrichment environment, electrodermal activity

An Unintended Dietary Constituent -Acrylamide- Exerts Anticonvulsive Effect In Mice

Okan Arıhan, Neslihan H. Dikmenoğlu

Hacettepe University, Faculty of Medicine, Department of Physiology

OBJECTIVE: Epilepsy is a common neurological disorder, in which diet can be important in prevention or induction of convulsions. Acrylamide is an unintended dietary constituent formed by reaction of carbohydrates and proteins during food processing at high temperatures. Since acrylamide is neurotoxic, we aimed to evaluate the pro/anticonvulsive effect of acrylamide in mice which can be further extrapolated to food intake of epileptic patients.

METHODS: Male Swiss-albino mice, weighing 30-35 g were divided into 5 groups. Control group received saline. Acrylamide groups were divided according to dose (10 and 50 mg/kg) and time of PTZ injection (½ and 4 hours after acrylamide injection). The drugs were dissolved in saline and were administered intraperitoneally in a volume of 10 ml/kg of body weight. Changes in time interval before the initiation of the first epileptic activity, time required for the generalized convulsions and the duration of the convulsions were accepted as measures of pro/anticonvulsive effect. Kruskal-Wallis test was used for statistical analysis

RESULTS: In acrylamide groups, period before the initiation of epileptic activity was prolonged; 49.0 (½ hour-10 mg/kg), 49.9 (½ hour-50 mg/kg), 56.51 (4 hour-10 mg/kg) and 61.17 percent (4 hour-50 mg/kg). Similarly time required for the generalized tonic-clonic convulsions were found to be increased in all of the acrylamide groups. Percent prolongation for acrylamide groups for this parameter were; 7.5 (½ hour-10 mg/kg), 29.3 (½ hour-50 mg/kg), 29.6 (4 hour-10 mg/kg) and 33.4 percent (4 hour-50 mg/kg).

CONCLUSIONS: The outcome of this preliminary study suggests that acrylamide exerts an anticonvulsive activity in mice, but it was not shown statistically.

Keywords: Epilepsy, convulsion, PTZ, acrylamide

Electroencephalographic Modifications in Motor/Virtual Activities

Marius Daniel Georgescu¹, Daniel Georgescu¹, Denisa Enescu Bieru², Catalin Bogdan¹, Veronica Sfredel¹, Maria Iancu¹

¹University of Medicine and Pharmacy of Craiova, Romania

²University of Craiova, Romania

OBJECTIVE: Our research aimed at finding efficient methods of evaluating the role of cerebral plasticity in learning motor tasks

METHODS: The subject group was made up of 31 sportsmen (handball, volleyball, fencing), average age 20 years (standard deviation 2.9). The electroencephalographic (EEG) modifications were tracked during activity: closing right fist (A), closing left fist (B), the command of closing right fist without actual movement (C), command of closing left fist without actual movement (D). Each activity was followed by a relaxation period (R). We concentrated our analysis on finding synthetic quantifiable indicators of the frequency specter. Spectral analysis of EEG tracks was made using EEG Mapping QP-220AK program, concentrating on: Peak frequency, Median frequency, Average frequency, Edge frequency (the frequency that, at a previously given value, establishes the left area/full spectrum area ratio).

RESULTS: Statistical study of the analyzed synthetic indicators did not yield significant differences between activities of A, B, C, D for the entire group or for subgroups H, V, F but it did highlight the capacity of the EDGE index to differentiate between subgroups H, V, F (Student test offers significant p values in the cortical areas implicated in the activity of A, B, C, D).

CONCLUSIONS: In the case of the analyzed group the EDGE index proved to be the most effective indicator, offering the possibility of constructing models which will characterize specific training in certain types of activities.

Keywords: EEG

The Protective Effects of Medical Ozone Treatment on Learning-Memory Functions in Diabetic Rats

Osman Genç¹, Gülten Erken², Ceylan Ayada³, Haydar Ali Erken⁴, Gulsah Demirkaya Gundogdu³, Hasan Doğan⁵

¹Department of Physiology, Dumlupinar University, Kutahya, Turkey

²Department of Physiology, Balikesir University, Balikesir, Turkey

³Department of Physiology, Pamukkale University, Denizli, Turkey

⁴Balikesir State Hospital, Balikesir, Turkey

⁵Ozone Treatment Center, Denizli, Turkey

OBJECTIVE: Oxidative stress is suggested to have an important role in the development of neurological complications in diabetes. Some studies have demonstrated that medical ozone treatment reduced reactive oxygen species by stimulation and/or preservation of the endogenous antioxidant systems in experimental models. The aim of this study was to investigate the protective effects of medical ozone treatment on learning- memory functions in diabetic rats.

METHODS: Thirty adult female Sprague Dawley rats were divided into five groups (6 rats in each); Control group (C), Diabetes group (D), Ozone group (O), Diabetes+Ozone group (DO), Diabetes+Insulin group (DI). For induction of experimental DM, Streptozocine was injected intra peritoneally. Ozone and insulin injections in diabetic rats were given to intraperitoneally. After fifteen days, seven day Morris Water Maze protocol was used to evaluate for learning process. Kruskal-Wallis and Mann-Whitney U tests were used for the differences between groups, $P < 0.05$ accepted as statistically significant.

RESULTS: Memory retrieval was increased DO and DI groups compare to D groups but this increase was not statistically significant.

CONCLUSIONS: A longer period of medical ozone treatment may prevent memory retrieval impairment induced by diabetes mellitus.

Keywords: diabetic rat, learning, medical ozone, memory, morris water maze

Bright light therapy in aged subjects with mild cognitive impairment changes the emotional response

Jose A Rubiño, Daniel Ottmann, Roser Aguiló, Margalida Ramis, Cristina Nicolau
Iunics, Universitat de les Illes Balears, Spain

OBJECTIVE: Disturbances in emotional processing are a typical feature of dementia and mild cognitive impairments in elderly patients. These disturbances have been attributed to age-related changes in the circadian clock. This report aims at studying changes in emotional responses using the Self Assessment Manikin (SAM) ratings of the International Affective Picture System (IAPS) before and after exposure to bright light used to entrain the biological clock in institutionalized subjects with mild cognitive impairments

METHODS: Six elderly (mean age 75) institutionalized subjects of both sexes with mild cognitive impairment were studied during two weeks. The first week served to set the baseline level. On the second week, each subject was submitted to 90 min of bright light. Before and after the treatment, the subjects' emotional state was evaluated using 30 pictures selected from the IAPS. Of them, 13 were pleasant, 8 neutral, and 10 unpleasant. Each picture was shown together with the 9-point SAM scale. The subjects rated their response to each picture according to a three dimensional scale: valence (pleasantness), arousal (activation level) and dominance (capacity to modify the state).

RESULTS: The exposure to bright light caused modifications in the valence and the arousal. Regarding valence, the pleasantness and unpleasantness rating decreased and increased respectively. The arousal changed in the same way, increasing the activation caused by the unpleasant pictures and decreasing for pleasant ones. The dominance remained without changes.

CONCLUSIONS: The bright light therapy changed the emotional valence and arousal of the subjects at the levels reported in the bibliography for young subjects.

Keywords: bright light, emotional response, ageing

Short-term rehabilitation post-injury is able to improve the deficit in motor ability produced by frontal cortex lesion in adult rats

Adelaida Riolobos, Víctor Santamaría, Fuente Antonio, Criado José María, Nava Mauricio, Yajeya Javier, Margarita Heredia

Department of Physiology and Pharmacology. INCyL. Salamanca University, Salamanca, Spain

OBJECTIVE: Lesions of the frontal cortex produce motor deficits which are evident at three months post-injury. The aim of this study was to investigate whether short-term rehabilitation in a critical period post-injury improves the deficit in motor ability produced by motor cortex lesions in adult rats. Additionally, we studied the expression of the brain-derived neurotrophic factor (BDNF) in the brains of animals injured and rehabilitated.

METHODS: Rats were conditioned in a paw-reaching-for-food task and the preferred paw was determined. Animals were lesioned in the forelimb motor cortex contralateral to the preferred paw and retested to evaluate the effectiveness of the lesion. Fourteen days post-injury rats were re-tested with the forced use of the paw affected by the lesion by placing a bracelet on the non-preferred paw.

RESULTS: Our findings showed that: a) rehabilitation therapy produced an improvement of motor deficits induced by the frontal cortex lesion when animals were forced to use the paw affected by the injury; b) the effectiveness of the rehabilitation therapy was determined by its application within a critical period post-injury; c) the immunohistochemical results suggested an overexpression of BDNF in the peri-lesional area.

CONCLUSIONS: Our findings provide evidence that rehabilitation therapy within a critical period post-injury induce an amelioration of motor deficits, suggesting that there is a timeframe when the brain provides optimal conditions for the beneficial effect of rehabilitation. Supported by MAPFRE Foundation. The authors thank to N.González and J.Blanco for their excellent technical assistance.

Keywords: Adult rats, Cortex motor lesion, Short-term rehabilitation, Paw-reaching-for-food task.

The role of cholinergic system in glucocorticoid-induced impairment of memory reconsolidation in mice

Abbas Ali Vafaei, Ali Rashidy Pour, Zahra Jafarian, Faraji Afsaneh
Physiology Research Center, Semnan University of Medical Sciences, Semnan, Iran

OBJECTIVE: Recent evidence suggests that glucocorticoids can impair memory reconsolidation, but the underlying mechanism(s) are not clear. The aim of this study was to determine the role of cholinergic system in glucocorticoid-induced impairment of memory reconsolidation in mice.

METHODS: Experiments were performed on 120 male albino mice (about 30 gr). The animals were trained in an inhibitory avoidance task (1 mA shock for 3 seconds). In experiment 1, effects of corticosterone were determined. Immediately after reactivation test, the animals received of vehicle or corticosterone (0.5, 1 and 3 mg/kg). In experiments 2 and 3, effects of corticosterone were examined in the presence or absence of atropine (a blocker of muscarinic cholinergic receptors 0.5 and 2 mg/kg) or mecamylamine (a blocker of nicotinic cholinergic receptors 0.5 and 2 mg/kg), respectively. In all experiments, memory retention tested 2, 5, 7 and 9 days after reactivation.

RESULTS: Results from experiment 1 indicated that corticosterone impaired memory reconsolidation in a dose-dependent manner ($P < 0.01$). Blockade of muscarinic cholinergic, but not nicotinic receptors suppressed corticosterone-induced impairment of memory reconsolidation. Both blockers alone did not change memory reconsolidation as compared with control group.

CONCLUSIONS: Findings above showed that the memory impairing effects of corticosterone on reconsolidation process, at least in part, may mediate via muscarinic cholinergic receptors.

Keywords: Atropine, Mecamylamine, Corticosterone, Nicotinic receptor, Muscarinic receptors, Memory reconsolidation, Mice

Neonatal NMDA Receptor Blockade Effect on Short-Term Memory and Anxiety in Adult C57BL/6 Mice

Kubra Akillioglu, Secil Binokay

Division of Neurophysiology, Department of Physiology, Medical Faculty, University of Çukurova, Adana, Turkey

OBJECTIVE: It is known that N-methyl-D-aspartate (NMDA) type of glutamate receptors in the brain play important roles in the development of neuronal migration and establishment of synaptic connections. NMDA receptor system in the neonatal or postnatal periods is supposed to play a crucial role in the neuropathology of schizophrenia. In our study, NMDA receptors that are known important for development of brain, in neonatal period blockade effects on short-term memory and anxiety have been investigated in adult C57BL/6 mice.

METHODS: MK-801 (0.25 mg/kg, 0.1ml/20gr body weight, i.p.) was applied to C57BL/6 male mice between postnatal 7-10 days. The same volume of saline was injected into the control group. Anxiety behaviors were evaluated by using an open field (saline treated n=10, MK-801 treated n=13) and elevated plus maze test (saline treated n=13, MK-801 treated n=14) during the adult period. Short-term memory performances were evaluated by passive avoidance test (saline treated n=11, MK-801 treated n=10).

RESULTS: The intraperitoneal treatment with MK-801 increased center latency ($p<0.05$) and the speed, decreased the time spent in center ($p<0.01$) of C57BL/6 mice compared to saline group in the open field test. NMDA receptor blockade with MK-801 decreased the time spent in open arm and number of entries to open arm ($p<0.05$) in the elevated plus maze. In the passive avoidance test, decreased the escape latency in C57BL/6 mice ($p<0.05$).

CONCLUSIONS: NMDA receptor blockade increased level of anxiety and locomotor activity in neonatal period, caused a decrease in the short-term memory performance. Neonatal NMDA receptor hypofunction may cause schizophrenia-like behaviors in the adults.

Keywords: Elevated Plus maze, MK-801, Neonatal, NMDA receptors, Open-field, Passive avoidance

The Effects of NMDA Receptors Blockade and Environmental Enrichment During Different Critical Developmental Windows of the Nervous System on the Emotional and Cognitive Function at Adult Age in the Balb/c mice

Kubra Akillioglu¹, Emine Babar Melik², Enver Melik², Sayad Kocahan³

¹Division of Neurophysiology, Department of Physiology, Medical Faculty, Çukurova University, Adana, Turkey

²Retired faculty member of Çukurova University

³Health School, Adiyaman University, Adiyaman, Turkey

OBJECTIVE: In our study, we evaluated in the situation of NMDA receptor hypoactivity during critical developmental period, the effects of environmental enrichment on locomotor activity, emotional and cognitive functions of the brain at adult age.

METHODS: For this aim, three "developmental windows", between 5-10 postnatal days as first window, 15-20 postnatal days as second window and 25-30 postnatal days as third window were selected as a model. NMDA receptor hipofunction was performed during these devepomental windows by using MK-801 (for 5 days 0.25 mg / kg twice a day, intraperitoneal). In Balb/c mice treated with MK-801 and reared in physical standart and enriched environmental conditions, emotional behaviors were assessed by using open field, elevated plus maze tests, and cognitive functions were assessed in the Morris water maze.

RESULTS: In the first development window (between 5-10 postnatal days), enriched environmental condition did not restore deteriorating effects of MK-801 in locomotor activity ($p<0.001$), emotional ($p<0.01$) and cognitive functions ($p<0.01$). In the second developmental window (between 15-20 postnatal day), MK-801 did not affect fear responses to open field and height, enriched environmental condition ($p<0.05$) restored MK-801-induced decreases in risk assessment behavior ($p<0.01$), spatial exploration and impairment in cognitive functions ($p<0.05$). In the third developmental window (between 25-30 postnatal days), enriched environmental condition ($p<0.05$) restored MK-801-induced decrease in risk assessment behavior ($p<0.05$), but not cognitive functions.

CONCLUSIONS: Physical environmental enrichment can restore disturbances in emotional and cognitive functions dependent on the NMDA receptor hypofunction only during second developmental window. Practically, the physical environmental enrichment could restore NMDA receptor system impairment at early childhood period.

Keywords: Balb/c mice, Critical developmental period, Emotional and cognitive function, MK-801, Physical environmental enrichment.

An interaction between Ginkgo Biloba and acute stress and corticosterone on fear memory retrieval and extinction in mice

Ali Rashidy Pour, Abbas Ali Vafaei, Fatemeh Dehbashi, Neda Alizadeh
Physiology Research Center, Semnan University of Medical Sciences, Semnan, Iran

OBJECTIVE: stress or glucocorticoids modulate various phases of learning and memory.

METHODS: In this study, we tested preventive effects of a natural medicine the extract of Ginkgo Biloba (EGB 761) on acute stress and corticosterone-induced impairment of fear memory retrieval and extinction.

RESULTS: Our findings indicate that acute stress or corticosterone impaired and facilitated fear memory retrieval and extinction in an inhibitory avoidance task, respectively. EGB 761 suppressed the effect of corticosterone, but not acute stress on retrieval and extinction of fear memory.

CONCLUSIONS: We conclude that, in mice, administration of EGB 761 prevents corticosterone-induced impairment and enhancement of fear memory retrieval and extinction.

Keywords: Extinction, Glucocorticoids, Ginkgo Biloba, Fear Memory, Retrieval, Acute stress

Effects of Pinealectomy and Exogenous Melatonin on Passive Avoidance Learning in Rats

Metehan Akça¹, Mehmet Yıldırım²

¹Institute of Health Sciences, Karadeniz Technical University, Trabzon, Turkey

²Department of Physiology, Karadeniz Technical University, Trabzon, Turkey

OBJECTIVE: Melatonin (N-acetyl-5-methoxytryptamine) is an endogenous indoleamine secreted by the pineal gland especially in response to darkness. It has many functions including acting as an antioxidant and assisting to regulate circadian rhythms. Furthermore, it has been recently reported that melatonin may affect on learning and memory in mice, and it can modify electrophysiological procedures associated with memory. The aim of the present study was to analyze the effects of pinealectomy and exogenous melatonin administration on passive avoidance response in adult male Sprague Dawley rats.

METHODS: Animals were separated into six groups (n=8) as control, 5% ethanol, sham-operated, melatonin, pinealectomy, pinealectomy+melatonin groups. The two groups of rats were exposed to pinealectomy, and seven days after surgery, animals in the melatonin and pinealectomy+melatonin groups were administrated daily with melatonin (10 mg/kg i.p.) for 14 days. Cognitive functions were assessed using step-through latency on the passive avoidance apparatus.

RESULTS: As a result of the statistical analysis of the obtained data, pinealectomy and exogenous melatonin administration was not any effect on the passive avoidance learning in rats. In addition, there was no significant difference in the avoidance latency in sham, ethanol and pinealectomy + melatonin groups compared to the control group.

CONCLUSIONS: In conclusion, we suggest that melatonin does not act directly on the learning of passive avoidance behavior in rats.

Keywords: Learning, Melatonin, Pinealectomy, Passive avoidance, Rat

Effects of adult hypothyroidism on short-term memory on rats

Demet Timur, Nazan Dolu, Aise Seda Artis, Seval Keloglan, Sehrazat Kavraal
Physiology Department, Erciyes University Medical Faculty, Kayseri, Turkey

OBJECTIVE: While a large body of literature is available on the effect of thyroid hormone deficiency on learning and memory functions during the developmental stage, electrophysiological and behavioral findings, particularly on propylthiouracil administration to adult normothyroid animals, are not satisfactory. Therefore we aimed to evaluate short-term spatial working memory and short-term synaptic plasticity in adult rats with hypothyroidism.

METHODS: The experiments were carried out on adult rats after receiving approval from Ethics Committee of Erciyes University. Hypothyroidism was induced by administering 6-n-propyl-2-thiouracil in their drinking water for 21 days at a concentration of 0.05%. Spatial learning performance of hypothyroid and control rats were studied on Y-maze. The in vivo electrophysiological recordings were taken from the dentate gyrus of hippocampus. A pair of pulses of equal intensity was administered at different inter-pulse intervals (IPI) to analyze the paired-pulse index.

RESULTS: The level of spontaneous alternation was different between the groups ($p < 0.01$). The percentage of alternation was significantly lower in the experimental group than the control group ($p < 0.05$). In the control animals paired pulse ratio of population spike amplitudes increased with IPI, became maximal at IPI of 80 ms, and then declined at longer intervals. Similar pattern was also observed in the hypothyroid group with a significantly less facilitation than the control ($p < 0.05$).

CONCLUSIONS: The present study provides in vivo evidence for the action of propylthiouracil leading to impaired paired pulse facilitation which might explain deficit in spatial memory tasks in adult hypothyroid rats.

Keywords: hypothyroidism, short-term spatial memory, synaptic plasticity, Y-maze, paired pulse response

How hyperthyroidism affects short-term memory?

Seval Keloglan¹, Aise Seda Artis², Soner Bitiktas¹, Nazan Dolu², Demet Timur²

¹Erciyes University, Institute of Health Sciences, Department of Physiology

²Erciyes University Faculty of Medicine, Physiology Department, Kayseri

OBJECTIVE: Adult-onset hyperthyroidism is known to induce cognitive deficits in learning and memory. However, studies evaluating memory with hyperthyroidism are controversial and scarce in number. We aimed to evaluate short-term spatial working memory (by Y maze) and synaptic plasticity in hyperthyroid adult rats.

METHODS: The experiments were carried out on adult rats after receiving approval from Erciyes University, Ethics Committee. Rats were randomly assigned into 2 groups: control and hyperthyroid groups. To induce hyperthyroidism, L-thyroxine (0.2 mg/kg/day ip) was given. Y-maze was used to test the alternation behavior. The in vivo electrophysiological recordings were taken from the dentate gyrus of hippocampus. A pair of pulses of equal intensity was administered at different inter-pulse intervals to analyze the paired-pulse index.

RESULTS: The level of spontaneous alternation was different within the groups ($p < 0.01$). In the hyperthyroid rats the percentage of alternation was significantly lower than the control ($p < 0.05$). During the analysis of paired pulse response similar pattern was observed in both groups with a significantly less facilitation in hyperthyroid group than the control ($p < 0.05$).

CONCLUSIONS: We have shown that elevation of thyroid hormones could impair short-term spatial working memory of adult rats. Our results also suggest that the changes in thyroid hormone metabolism can disturb synaptic plasticity in the dentate gyrus which denotes spatial short-term memory. Since almost all studies focused on the effect of low levels of thyroxine on learning and memory, the worse performance of the hyperthyroid rats on the short-term memory measures seems to be an interesting finding.

Keywords: hyperthyroidism, short-term memory, rat

How cold stress affects hippocampal function in female rats?

Aise Seda Artis, Cem Suer, Nazan Dolu, Leyla Sahin, Erdem Başaran
Physiology Department, Erciyes University Medical Faculty, Kayseri, Turkey

OBJECTIVE: Females cope differently with stressful situations than males. We aimed to investigate the effects of stress induced by exposure to cold on females on the synaptic plasticity in the dentate gyrus and behavioral spatial memory performance.

METHODS: Approval from Erciyes University Ethical Committee was obtained. Rats were divided into 3 groups as the control (kept at 22oC), acute cold stress (kept 2hr/day at +4oC for one day) and chronic cold stress (kept 2hr/day at +4oC for 21 days). Spatial memory was tested by Morris water maze. Also long-term potentiation (LTP) recordings were taken and blood was obtained for corticosterone measurements.

RESULTS: Both of the cold exposed groups showed significant increase in the corticosterone levels when compared to the control. The corticosterone levels of the acute stress group were also significantly higher than the chronic stress group. Both experimental groups reached to the platform later than the control rats in Morris water maze test. Input-output relationship and LTP responses obtained by perforant path stimulation were found to be more depressed in the chronic stress group than the acute stress and control groups.

CONCLUSIONS: In the present study, chronic stress decreased LTP together with an impairment of spatial memory task. However, poor memory performance in the acute stress group was not in parallel with LTP findings. We suggest that impaired LTP caused by chronic exposition to cold is the result of exposure of long-lasting genomic effects of moderate level increase in corticosterone level.

Keywords: Hippocampus, Long-term potentiation, Spatial memory, Morris water-maze, Synaptic plasticity

Improving effects of Resveratrol chronic treatment on cognitive ability in aged rats

Ruben Victor Rial, Fiorella Sarubbo, Carmen Colom, Margalida Ramis, Joan Bestard, Andrea Ruiz, Antoni Miralles, Susana Esteban
Iunics, Universitat de les Illes Balears

OBJECTIVE: The chronic effects of the antioxidant polyphenol resveratrol on cognitive performance in old rats have been analyzed.

METHODS: To evaluate the possible improvements in memory processes, two different tests have been used, the spatial working memory in the 8-arm radial maze, and the novel object recognition as a form of non-spatial working memory test. Old (24 months) Wistar male rats (500-550g) were treated with resveratrol (20 mg/kg) or vehicle (corn oil, 1 ml/kg) for 28 days, intraperitoneally. Trials on radial maze were judged complete when rats had chosen all 8 baited arms or spent 20 min in the trial. Re-entered or non entered arms were computed as errors.

RESULTS: A significant improvement in memory processes was observed after chronic treatment with resveratrol in old animals. Chronic vehicle treatment did not change task performance respect to the test made just before the treatment. However, chronic resveratrol treatment improved task performance in the radial test with respect to controls, and with respect to the test made just before the treatment with a 33% reduction in trial time, 41% errors reduction (90% errors reduction due to none entered into some arms and 29% errors reduction due to re-entered arms). Moreover, the time spent exploring the novel object compared to familiar object increased 1.6 times in the group of rats treated with resveratrol, while control rats spent the same time at the beginning and end of treatment.

CONCLUSIONS: Resveratrol enhanced working memory. The results suggest the usefulness of resveratrol treatment to recover from the age associated behavioural deficits observed in cognitive capacities.

Keywords: working memory, aging, resveratrol, rats

Neuro-epithelial Stem Cell Transplantation Together with Adjacent Mesenchymal Tissue into Hippocampal Region of Offspring of Hypothyroidic Rat Dams Results in Successful Engraftment and Improvement of Impaired Cognitive Performance

Sema Tulay Koz¹, Giyasettin Baydas², Suleyman Koz³, Ahmet Kavaklı⁴, Nusret Akpolat⁵

¹Malatya State Hospital, Laboratory Department, 44000 Malatya, Turkey

²Rectorate, Bingol University, 12000 Bingol, Turkey

³Department of Nephrology, Turgut Ozal Medical Centre, Inonu University, 44300, Turkey

⁴Department of Anatomy, Faculty of Medicine, Firat University, 23119 Elazig, Turkey

⁵Department of Pathology, Turgut Ozal Medical Centre, Inonu University, 44300, Turkey

OBJECTIVE: Maternal thyroid hormone deficiency can impair proper brain development in fetus. We aimed to explore whether neuroepithelial and mesenchymal tissue transplantation into hippocampal regions of offspring of hypothyroidic rat dams could result in successful engraftment or not, and if successful, could allograft transplantation improve cognitive performance or not.

METHODS: Five pregnant Wistar albino rats received 10 mg/kg/day propylthiouracil via drinking water throughout pregnancy. Offspring were grown until 75 days and at this time they were divided into transplantation and sham groups. Neuroepithelial stem cell with adjacent mesenchymal tissue obtained from mesencephalic neural plate and adjacent ventral mesenchyme of 10.5-days-old embryo of pregnant rats was stereotaxically injected into hippocampus of transplantation group animals. Sham group was stereotaxically injured with a Hamilton syringe but not implanted with embryo tissue. Three weeks after transplantation (at postnatal 95th day) all rats were tested on Morris water maze and probe test for cognitive functions. After the test for cognitive functions all rats were sacrificed and whole brain was examined immunohistochemically, for glial fibrillary acidic protein (GFAP) and vimentin, markers of astrocyte maturation. For all the immunohistochemical procedures, areas adjacent to the engraftment implanted regions served as negative controls.

RESULTS: Brain sections of transplanted animals showed marked increase of vimentin and GFAP expression in areas of stem cell implantation, implying successful engraftment. Cognitive performance of transplanted animals on Morris water maze and the probe trial was significantly better than sham controls.

CONCLUSIONS: Mesencephalic neural plate cells with adjacent mesenchymal tissue can successfully be implanted to cognitively impaired animals and this may help cognitive recovery of animals.

Keywords: Maternal Hypothyroidism, Neuroepithelial stem cells, Transplantation, Cognitive functions.

Effects of acute footshock stress on hippocampal cells and spatial learning and memory in the adolescent rats

Nazan Uysal Harzadin¹, Ali Riza Sisman², Ayfer Dayi¹, Seda Ozbal³, Ferihan Cetin¹, Basak Baykara⁴, Ilkay Aksu¹, Aysegul Tas¹, Secil Ayca Cavus³, Sevil Gonenc Arda¹

¹Department of Physiology, Dokuz Eylul University, Izmir, Turkey

²Department of Biochemistry, Dokuz Eylul University, Izmir, Turkey

³Department of Histology and Embriyology, Dokuz Eylul University, Izmir, Turkey

⁴High school of Physical Therapy and Rehabilitation, Dokuz Eylul University, Izmir, Turkey

OBJECTIVE: Depending on the type and duration, stress affects cognitive functions. It is known that acute stress improves cognitive function in adults, but it is unknown how it affects in adolescents. The purpose of this study is to study the effects of low and high intensity of acute stress in hippocampus in the adolescent period.

METHODS: Groups: both sexes, high intensity (1.6 mA) and low intensity (0.2 mA) stress groups and controls. Learning and memory performances were evaluated using Morris water tank, after 1 hour, 20 minute foot shock stress. VEGF and BDNF levels were measured by ELISA. Also, VEGF immune staining was performed.

RESULTS: Stress affected learning processes significantly in all groups. In probe trial, the stressed groups had more time in target quadrant, had less time in opposite quadrant ($p < 0.003$, $p < 0.004$). Increased the cell number in CA1 and gyrus dentatus, increased VEGF and BDNF levels of hippocampus ($p < 0.05$). Also, VEGF + cells were determined by VEGF immune staining. Gyrus dentatus and CA1 cell counts, levels of VEGF and BDNF were positive correlated with time spent in target quadrant ($r=0.548$, $p=0.001$; $r=0.341$, $p=0.042$; $r=0.423$, $p=0.020$; $r=0.438$, $p=0.016$), were negative correlated with time spent in opposite quadrant ($r=-0.643$, $p=0.000$; $r=-0.455$, $p=0.005$; $r=-0.376$, $p=0.040$; $r=-0.388$, $p=0.034$). There was no difference between basal corticosterone levels in all groups.

CONCLUSIONS: These results suggest that of low and high intensity of acute stress showed similar positive effect on hippocampus in the adolescents, whose hypothalamus-pituitary-adrenal axis were not in the adult form yet.

Keywords: footshock, acute stress, adolescent, hippocampus, VEGF, BDNF

Effects of Oxytocin on Cognitive Impairment Due to Chronic Restraint Stress in Adolescent Rats

Ayfer Dayi¹, Ferihan Cetin¹, Ali Riza Sisman², Ilkay Aksu¹, Aysegul Tas¹, Sevil Gonenc Arda¹, Nazan Uysal Harzadin¹

¹Dokuz Eylul University, Faculty of Medicine, Physiology, Izmir, Turkey

²Dokuz Eylul University, Faculty of Medicine, Biochemistry, Izmir, Turkey

OBJECTIVE: It is known that stress can cause many diseases and affects hippocampus. While the effects of chronic stress on hippocampus are temporary in adults, in the adolescents where the brain development continues the effects are permanent. Oxytocin reduces anxiety and affects behavior. The aim of this study is to investigate the effects of chronic restraint stress on hippocampal cells in adolescence, and the role of oxytocin on these effects.

METHODS: Thirty-eight days-old Wistar Albino rats of both sexes were used. Groups: chronic restraint stress+intranasal saline, chronic restraint stress+intranasal oxytocin (2µg/kg), control. Stress procedure; 1 hour/day for 7 days. On the 8th day, learning and memory test was evaluated with Morris' Water Maze. When rats were 50 days old, they were sacrificed and hippocampal VEGF and BDNF levels were evaluated with ELISA.

RESULTS: It is shown that stress has adverse affects on learning process (females, $p<0.004$, $p<0.001$; males, $p<0.05$, $p<0.004$). Oxytocin treatment shortened the escape latency. ($p<0.05$, $p<0.001$ in females; $p<0.05$ in males). In probe trials, restraint stress group spent significantly less time in the target quadrant, more time in the opposite quadrant than other groups ($p<0.002$ for both sexes). Oxytocin treatment improved spatial memory in probe ($p<0.001$ for both sexes). VEGF and BDNF levels were increased in the oxytocin-applied groups compared to the others (females; VEGF, $p<0.001$, BDNF, $p<0.05$; males; VEGF, $p<0.002$, BDNF, $p<0.05$). Furthermore, positive correlation was found between VEGF and BDNF levels and the time spent in the target quadrant ($r=0.533$, $p=0.002$ with VEGF; $r=0.434$, $p=0.017$ with BDNF).

CONCLUSIONS: Results suggest that impaired hippocampal functions due to stress, can be affected positively by oxytocin.

Keywords: chronic restraint stress, oxytocin

Effect of Propolis on Learning and Memory of Streptozotocin-Induced Diabetic Rats

Burcu Kırımhan Köksal, Memet Hanifi Emre

Department of Physiology, Inonu University, Malatya, Turkey

OBJECTIVE: Purpose of this study was to investigate effects of propolis application on learning and memory of diabetic rats exposed to streptozotocin (STZ).

METHODS: The study was conducted for four weeks by using radial maze with eight arms and included 51 male Sprague Dawley rats divided into four experimental groups; control (C) (n=14), STZ application (STZ) (n=12), STZ exposure after propolis application (P+STZ) (n=12) and propolis application after STZ exposure (STZ+P) (n=13). The study focused on latent learning rate, short-term (STMF) and long-term memory failure (LTMF) rates, and duration for memory tasks. In addition, fasting blood glucose level, change in weights, plasma trace element levels and oxidative stress indicators were investigated.

RESULTS: According to the findings of the study, fasting blood glucose levels of STZ group were higher than the other groups and propolis application decreased the glucose levels. Weight lost was also determined in the STZ group. Plasma Zn²⁺ levels of the rats exposed to propolis were higher than the other groups while there were no differences between groups in terms of other trace element and oxidative stress indicator levels. The most frequent STMF was in the STZ group while the most frequent LTMF was in the P+STZ group. The least frequent STMF and LTMF were in the STZ+ P group.

CONCLUSIONS: The STZ group entered less number of total and correct arms in spite of their staying for longer time in the maze. The rats exposed to propolis entered similar number of total and correct arms with control group rats in similar time interval. Propolis may have an effect on memory and learning in diabetic disorders.

Keywords: Diabetes, Learning, Memory, Propolis, Radial Maze, Streptozotocin

The role of the inhibitory mechanism of histamine in hippocampal long term potentials

Nazan Dolu¹, Cem Suer¹, Aise Seda Artis¹, Soner Bitiktas², Erdem Başaran²

¹Erciyes University Faculty of Medicine, Department of Physiology, Kayseri

²Erciyes University, Institute of Health Sciences, Department of Physiology, Kayseri

OBJECTIVE: The dentate gyrus of hippocampal formation receives the strongest histaminergic innervation and also contains the highest density of H3 receptor binding sites. The histamine H3 receptor is a presynaptic autoreceptor which controls histamine release. Thioperamide, H3 receptor antagonist, blocks the effect of histamine. We aimed to investigate whether histamine acts on long term potentials through H3 receptor or other histaminergic receptor and neurotransmitters. For this purpose, we infused histamine and thioperamide in the dentate gyrus of rats to evoke excitatory postsynaptic potentials, in vivo.

METHODS: The approval from Erciyes University Local Ethical Committee was obtained. A bipolar stimulating electrode was placed to the medial perforant path and a double-barrel glass micropipette was placed in the dentate gyrus as recording electrode. Artificial cerebrospinal fluid (to control group), histamine (10 μ M), thioperamide (10 μ M) or thioperamide+histamine (10 μ M) were infused to dentate gyrus. Three minutes after the infusion, high frequency stimulation protocol was applied to evoke long-term potentiation.

RESULTS: The population spike amplitudes of the histamine group showed a depressed response when compared to control group; whereas thioperamide or thioperamide+histamine groups were not different than the control. When we compared the slope of excitatory postsynaptic potentials we observed no difference among the groups.

CONCLUSIONS: Our results showed the presence of a histaminergic tract ending at the dentate gyrus, that is activated that by stimulation of the perforant pathway. Although the predominantly presence of the H3 receptors on this tract, we need new studies to understand whether this occurs via direct or indirect mechanisms.

Keywords: long term potentials, histamine, thioperamide, rat

Topiramate improves water maze performances in young adult rats exposed to maternal systemic hypotension

Sibel Bayrak¹, Bilge Pehlivanoglu¹, Hamit Ozyurek², A. Meltem Sevgili¹, Z. Dicle Balkanci¹, Banu Anlar²

¹Department of Physiology, Hacettepe University Faculty of Medicine, Ankara, Turkey

²Department of Pediatrics, Pediatric Neurology Unit, Hacettepe University Faculty of Medicine, Ankara, Turkey

OBJECTIVE: Maternal systemic hypotension associated with fetal hypoxia/ischemia may cause impaired spatial learning and memory. Topiramate, an antiepileptic drug, exhibited neuroprotective effects in animal models of hypoxia/ischemia, excitotoxic insults and stroke. Therefore, we investigated water maze performances of 70-day-old rats to explore the possible favorable effects of topiramate against the outcomes of maternal systemic hypotension-induced neuronal damage.

METHODS: Pregnant Wistar rats were assigned to control (n=7), control+topiramate (n=5), hypotension (n=3), hypotension+topiramate (n=6) groups. Hypotension groups were subjected to transient systemic hypotension by blood withdrawal for 30 minutes on the 15th day of pregnancy. Randomly selected animals were injected intraperitoneally with topiramate (40 mg/kg/day) or saline 15 minutes after the termination of the hypotensive period. After spontaneous vaginal delivery, cognitive functions of pups were evaluated via Morris water maze test on postnatal day 70. The latency to reach the platform (seconds) and percentage of the time spent in the target quadrant were measured. Data were analyzed statistically.

RESULTS: Morris water maze performances improved in all groups over time ($P<0.05$, 1st vs. 5th day). Animals in the hypotension group took longer time to reach the hidden platform ($P>0.05$) and spent significantly less time in the target quadrant in comparison with other groups. Both parameters were improved with topiramate treatment ($P<0.05$).

CONCLUSIONS: Since topiramate treatment immediately after hypoxic-ischemic insult significantly improved long-term cognitive functions, it may offer a therapeutic potential for neuroprotection over a prolonged period in the developing rat brain.

Keywords: Topiramate, water maze, maternal systemic hypotension, rat brain

The Effect of Magnetic Field to Blood-Brain Barrier in Right Hemisphere, Left Hemisphere, And Cerebellum in Febrile Convulsive Rats

Tuncer Demir¹, Sefa Gültürk²

¹Department of Physiology, Gaziantep University, Gaziantep, Turkey

²Department of Physiology, Cumhuriyet University, Sivas, Turkey

OBJECTIVE: The objective of this study was to determine the effect of magnetic field (MF) in right hemisphere, left hemisphere, and cerebellum blood-brain barrier (BBB) in febrile convulsive (FC) rats.

METHODS: Totally, 36 male and 21 days-old Wistar-Albino rats were used. Rats were divided into six groups: sham; exposed to no MF, FC; febrile convulsion, MF; exposed to MF, FCMF; exposed to MF after FC, MFFC; exposed to MF before FC, MFFCMF; exposed to MF before and after FC. In all groups, the BBB was investigated in right hemisphere, left hemisphere, and cerebellum. FC was created by application of hyperthermic water. Magnitude of 5.0 mT with 50 Hz frequency modulation MF was applied.

RESULTS: When the evans-blue (EB) values, obtained from different regions in FC, MF, FCMF, MFFC, MFFCMF were compared; cerebellum values were significantly higher than both hemispheres ($p<0.05$). The cerebellum, right and left hemispheres' EB values of MFFCMF group were the highest ($p<0.05$).

CONCLUSIONS: In FC, the application of MF had more influence on cerebellum comparing to both hemispheres in terms of BBB. This result suggested that cerebellum was more sensitive to MF in terms of BBB. Only MF and FC application had deteriorative effect on BBB of rats. Additionally, in FC rats BBB was more deteriorated when MF was applied. It was shown that the application of MF after and before FC caused no differences in terms of BBB. However, BBB had more deterioration by the application of MF on FC rats both after and before seizures. It was seen that in FC exposure to MF in longer periods affected BBB more.

Keywords: blood-brain barrier, febrile convulsion, magnetic field,

The effects of zinc treatment on the blood-brain barrier permeability and brain element levels during convulsions

Fatma Burcu Seker¹, Hatice Yorulmaz², Ertuğrul Yalçın³, Baria Öztaş⁴

¹Department of Physiology, Yeditepe University Faculty of Medicine, Istanbul, Turkey

²Department of Physiotherapy and Rehabilitation, Haliç University, Istanbul, Turkey

³Department of Biology, Marmara University Faculty of Sciences and Arts, Istanbul, Turkey

⁴Department of Physiology, Istanbul University Istanbul Faculty of Medicine, Istanbul, Turkey

OBJECTIVE: We evaluated the effect of zinc treatment on the blood-brain barrier (BBB) permeability and we also determined the levels of Zinc (Zn), Natrium (Na) and Copper (Cu) in the brain tissue during epileptic seizures.

METHODS: Wistar-albino rats were divided into four groups each as follows: Control Group, Zn Group: Rats treated with ZnCl₂ 227ml/kg added in drinking water for two months, PTZ (Pentylentetrazole) Group: Rats treated with PTZ (80 mg/kg, i.v.) to induce epileptic seizures, Zn+PTZ Group. The brains were divided into left, right and cerebellum+brain stem regions. Evans blue was used as BBB tracer. Element concentrations were analyzed by Inductively Coupled Plasma Optical Emission Spectroscopy.

RESULTS: The BBB permeability showed an increase in PTZ and Zn+PTZ groups ($p < 0.01$). Additionally the BBB permeability of cerebellum+brain stem in the Zn group ($p < 0.05$) and the Zn concentrations of the left and right hemispheres in PTZ and Zn+PTZ groups ($p < 0.05$) showed an increase. In all experimental groups the Cu concentrations of all brain regions decreased, whereas Na concentrations showed an increase ($P < 0.05$).

CONCLUSIONS: During convulsions Zn treatment did not show protective effect on the BBB permeability. Nevertheless we have shown that Zn itself might have adverse effects on the BBB. Our results indicated an increase in Na levels, which plays a role in neuronal membrane excitability. The results showed a decrease in Cu levels. Whereas Cu has anti-oxidative effects, we can conclude that this decrease might play a role in epileptic seizures-induced brain damage.

Keywords: Blood-brain barrier, epilepsy, zinc, copper

The effect of estrogen on ethanol induced blood brain barrier disruption in an in vitro model

Sabriye Karadenizli¹, Gülay Üzüm¹, Serap Erdem Kuruca¹, Kadriye Ak Gün Dar², Muzaffer Beyza Cetin¹, Ayşegül Kapucu²

¹Department of Physiology, Medical faculty of istanbul,Istanbul University, Istanbul, Turkey

²Department of Biology, Facultyof Science, Istanbul University, Istanbul, Turkey

OBJECTIVE: The blood-brain barrier (BBB) is formed by the presence of tight junction proteins (TJPs)between brain endothelial cells that restrict paracellular permeability and it is essential for central nervous system normal function. The effect of estrogen on BBB function is unclear. It is reported that age and estrogen reseptors are important for its effects. We performed this study to determine 17beta-estradiol could affect blood-brain barrier disruption caused by ethanol using an in vitro BBB model.

METHODS: Measurements of transendothelial electrical resistance (TEER) and expression of TJPs (occludin and claudin 1-2) were performed to analyze BBB integrity and inducibility in an in vitro co-culture model of human umbilical vascular endothelial cells (HUVEC) and rat glioma cells (C6). HUVEC express estrogen receptor beta but not alfa (It is intresting in terms of imitating menopause period).

RESULTS: Coculture of HUVEC/C6 caused increase in TEER and TJPs levels indicating BBB formation. Adding ethanol into the culture media after BBB formation, BBB integrity was destroyed (decrease in TEER and TJPs). Effect of 17 beta estrodiol on BBB: long-term application of estrodiol, its single application, its application before and after ethanol caused decrease both in TEER and in the amount of TJPs. This decrease was higher when ethanol and estrogen were applied together.

CONCLUSIONS: Preventing the formation of the BBB in HUVEC line and increasing of ethanol induced disruption of barrier with addition of estradiol, let us think that estrogen treatment (especially in menopause) can create a risk for causing cerebrovascular diseases.

Keywords: invitro blood brain barrier, tight junction proteins, transendothelial electrical resistance, ethanol,17 beta estrodiol

Changes of pain thresholds in rats under different pain states

Simon Vaculin, Miloslav Franek

Dpt. of Normal, Pathological and Clinical Physiology, Third Faculty of Medicine, Charles University in Prague, Czech Republic

OBJECTIVE: Pain threshold measurement is one of the most frequent methods to evaluate pain in experimental animals. In plantar test, pain threshold is measured as a latency to withdraw a limb in response to heat stimulation. Decrease of the threshold is traditionally interpreted as allodynia/hyperalgesia, while increase as analgesia. The aim of the present study was to demonstrate other possible interpretations.

METHODS: Pain threshold measurement is one of the most frequent methods to evaluate pain in experimental animals. In plantar test, pain threshold is measured as a latency to withdraw a limb in response to heat stimulation. Decrease of the threshold is traditionally interpreted as allodynia/hyperalgesia, while increase as analgesia. The aim of the present study was to demonstrate other possible interpretations.

RESULTS: It was shown (1) that both neuropathic and visceral pain evoked increase of pain thresholds in unaffected limbs; (2) that the increase of pain threshold was diminished after the treatment, and (3) that the pain score negatively correlated to changes of pain thresholds of the limbs.

CONCLUSIONS: It is concluded that increase in pain threshold of unaffected limb could be interpreted as the presence of heterotopic pain of neuropathic and/or visceral origin. Subsequent decrease of the thresholds after the treatment supports this interpretation. Further, the increase of pain thresholds of unaffected limb could be attributed to activation of diffuse noxious inhibitory control, since pain thresholds change negatively correlated to visceral pain score in colorectal distension model. Supported by SVV 262708/2011 and RG 0021620816.

Keywords: visceral pain, neuropathic pain, pain thresholds

The Effect of NMDA Receptor Blockade During the Early Development Period on the Emotional Behaviours in Adolescence

Sayad Kocahan¹, Kubra Akillioglu², Secil Binokay²

¹Health School, University of Adiyaman

²Department of Physiology, Medical Faculty, University of Çukurova

OBJECTIVE: Adolescence is the period of physical, cognitive, and social maturation between childhood and adulthood. The N-methyl-D-aspartate (NMDA) receptor plays an important role in the regulation of neuronal development, learning and memory, neurodegenerative diseases, and neurogenesis. The role of NMDA receptor blockade during the early development period on emotional behaviours and the role of NMDA-mediated glutamate transmission remain to be elucidated. The aim of the present study was to investigate the role of NMDA receptor blockade during the early development period on anxiety and response to novelty in adolescence.

METHODS: Male Wistar rats were used. NMDA receptor blockade was performed between postnatal 7-10 days using MK-801 treatment. Rats were intraperitoneally injected with MK-801 twice a day for a period of 4 days (dizocilpine either at a dose of 0.25 mg/kg or in a volume of 0.1 ml/20 g body weight). The OF (open field) and EPM (elevated plus maze) tests were used to evaluate emotional behaviours in adolescence (30-50 days of age).

RESULTS: In the OF test, the frequency of rearing in the center area ($p < 0.05$) velocity and distance movement ($p < 0.01$) increased in the the MK-801 group than in the saline group. In the EPM test, the time spent in the open arm of the maze ($p < 0.01$) and headdipping ($p < 0.01$) was increased while the latencies to enter open arm of the maze decreased ($p < 0.05$) in group treated with MK-801.

CONCLUSIONS: We propose that the NMDA receptor blockade during the early development period increased locomotor activity as detected by open field test and reduced to anxiety as measured by elevated plus maze in adolescence.

Keywords: Adolescence, NMDA Receptor Blockade, MK-801, Anxiety, Rat.

Maternal regular aerobic exercise decreased anxiety of pups correlated increased prefrontal cortex BDNF and VEGF

Ilkay Aksu¹, Ferihan Cetin¹, Ali Riza Sisman², Ayfer Dayi¹, Celal Gencoglu³, Aysegul Tas¹, Sevil Gonenc Arda¹, Nazan Uysal Harzadin¹

¹Department of Physiology, Dokuz Eylul University, Izmir, Turkey

²Department of Biochemistry, Dokuz Eylul University, Izmir, Turkey

³High school of Sports Science Technology, Dokuz Eylul University, Izmir, Turkey

OBJECTIVE: Anxiety which is known as the status of increasing concern without any threatening around generates in amygdala. The evidences obtained in recent years point to that the medial prefrontal cortex edits and checks the amygdala outputs. Regular exercise during pregnancy is known to affect positively offspring learning and memory. The purpose of this study is to examine the effects of aerobic exercise during pregnancy on the offspring's anxiety and the levels of VEGF and BDNF which have anxiolytic effects in the prefrontal cortex where anxiety-related region of the brain.

METHODS: Groups; maternally exercised and sedentary. Early evaluation at 26 days old, late evaluation 4 months old. Before pregnancy, familiarization was performed at 5m/ min-10min for 5 days; during pregnancy, 30 min/day, 8m/min, 5 days/week; last 10 days of pregnancy, exercise intensity was reduced to 6m/min.

RESULTS: Unrelated to sex, all offsprings of mothers who have exercised during pregnancy, at prepubertal and adult period, had increased locomotor activity, had lower anxiety levels and took more risk (all groups, $p < 0.001$). VEGF and BDNF levels in the prefrontal cortex also were increased in both age groups and sexes (prepubertal, $p < 0.001$; adult, $p < 0.003$). In addition, it was found that there was highly positive correlation between the open-field test results with the levels of BDNF and elevated + maze with VEGF levels (BDNF, $r = 0.718$, $p < 0.000$; VEGF, $r = 0.606$, $p < 0.000$). There was no difference in blood cortisol levels of the groups.

CONCLUSIONS: As a result, this study indicates that exercise during pregnancy positively affects the prefrontal cortex of offspring and protects them from anxiety in early and late periods of their lives.

Keywords: pregnancy, exercise, VEGF, BDNF, prefrontal cortex

Cortical connectivity changes following nociception stimulation during anesthesia in rat

Bogdan Pavel, Radu Ionut Braga, Alexandru Calin, Andrei Daneasa, Ana Maria Radulescu, Leon Zagrean
Department of Physiology, University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania

OBJECTIVE: Cortical connectivity is considered to be one of the most sensitive parameters for estimating brain function. The aim of this study was to evaluate changes in cortical connectivity induced by nociceptive stimulation during anesthesia, in rats.

METHODS: In the present study we used Wistar rats (n=5) of about 250-300 gr. Anesthesia was induced and maintained with chloral hydrate, at an anesthetic depth estimated by a median frequency (MEF) of 2 Hz. During the experiment, the brain electrical activity was recorded using electrodes chronically implanted on the dura mater. Two leads were used, frontal and parietal, on each hemisphere. After a 5-min control path, a 1-minute painful stimulus consisting of a mechanical clamp on the left hind paw was applied for three times, at an interval of two minutes. Cortical connectivity was analyzed in the right hemisphere using the median frequency variation (fronto-parietal index) and correlation coefficient. Signal acquisition was made using BIOPAC MP 150 system.

RESULTS: During painful stimulation, MEF increased ($MEF_{pain} = 3 * MEF_{control}$) in both leads, and frontal cortex activation time doubled (2 minutes) compared with the time of painful stimulation (1 minute) and greater than in the parietal cortex. Painful stimulation didn't modify fronto-parietal index and correlation coefficient.

CONCLUSIONS: Cortical connectivity did not change during painful stimulation at an anesthetic depth estimated by a MEF of 2 Hz, under chloral hydrate anesthesia, but MEF increased in both areas (frontal and parietal).

Keywords: anesthesia, nociception, cortical connectivity

Effects of levosimendan on diabetes-induced neuropathic pain in a mice model

Ömer Faruk Kalkan, Ahmet Ayar

Department of Physiology, Karadeniz Technical University, Trabzon, Turkey

OBJECTIVE: Previous experimental data indicate that ATP-sensitive potassium channel openers have promising effects on experimental diabetic neuropathy. We have investigated the possible effects of levosimendan, a calcium sanitizer agent with prominent K(ATP) channel opening activity, on painful diabetic neuropathy in a mice model.

METHODS: Diabetes was induced in adult male Balb/C mice by injection of streptozotocin [200 mg/kg intraperitoneally (i.p.)] and 4 weeks after induction of diabetes (serum glucose \geq 300 mg/dL) plantar test was performed and paw withdrawal latency was considered as pain threshold. Levosimendan (1 and 2 mg/kg body weight) was administered i.p. and plantar tests were repeated. Data were analyzed by Kruskal–Wallis one-way analysis of variance (ANOVA) followed by a pairwise comparison using a Dunnett's t-test on the ranked data.

RESULTS: The vehicle for levosimendan had no significant effect on pain threshold. Levosimendan (1, 2 mg/kg) significantly reduced the pain threshold values of diabetic mice. The effect was time dependent, and at about the same extent for the both doses tested. Normalized pain threshold values were reduced from 1 to 0.78 ($P<0.001$, $n=8$), 0.77 ($P<0.001$, $n=8$) and 0.77 ($P<0.001$, $n=8$) 10, 20 and 30 minutes after administration of 1 mg/kg levosimendan, respectively.

CONCLUSIONS: Results obtained from this in vivo behavioral test indicate that levosimendan has promising therapeutic potential for the treatment of diabetic neuropathic pain.

Keywords: diabetic neuropathy, levosimendan, K(ATP) channel

NMDA receptor antagonists modulate morphine analgesia and tolerance in rats

Ercan Özdemir¹, İhsan Bağcivan², Ahmet Altun², Nedim Durmuş², Sinan Gürsoy³

¹Departments of Physiology, Cumhuriyet University, Sivas, Turkey

²Departments of Pharmacology, Cumhuriyet University, Sivas, Turkey

³Departments of Anesthesiology and Reanimation, Cumhuriyet University, Sivas, Turkey

OBJECTIVE: The efficacy of opioids is limited in chronic pain treatment, as a result of development of opioid tolerance. The aim of this study was to investigate the role of N-methyl-D-aspartate (NMDA) receptor antagonists on the morphine analgesia and tolerance in rats.

METHODS: This study was applied on male Wistar albino rats (weighing 190±15 g). To constitute of morphine tolerance, animals received morphine (50 mg/kg; s.c.) once daily for 3 days. After last dose of morphine was injected on day 4, morphine tolerance was evaluated. The analgesic effects of ketamine, MK-801 (noncompetitive NMDA receptor antagonist), LY235959 (competitive NMDA receptor antagonist), cis-2,3-Piperidinedicarboxylic acid (PDA, NMDA receptor agonist), and morphine were considered at 30-min intervals (0, 30, 60, 90 and 120 min) by tail-flick and hot-plate analgesia tests (n=6 in each study group).

RESULTS: The results demonstrated that ketamine, MK-801, and LY235959 significantly attenuated the development of morphine tolerance ($p<0.05$), on the other hand, PDA increased the development of morphine tolerance but, the difference was not statistically significant ($p>0.05$).

CONCLUSIONS: In conclusion, obtained data indicated that NMDA receptor antagonists attenuated the development of morphine tolerance and have a significant role on the morphine analgesia and tolerance in rats.

Keywords: analgesia, ketamine, LY235959, MK-801, morphine, NMDA antagonist

Effects of alpha 2-adrenoceptor agonists dexmedetomidine and guanfacine on morphine analgesia and tolerance in rats

Sinan Gürsoy¹, Ercan Özdemir², İhsan Bağcivan³, Ahmet Altun³, Nedim Durmuş³

¹Departments of Anesthesiology and Reanimation, Cumhuriyet University, Sivas, Turkey

²Departments of Physiology, Cumhuriyet University, Sivas, Turkey

³Departments of Pharmacology, Cumhuriyet University, Sivas, Turkey

OBJECTIVE: Alpha 2 (α_2)-adrenoceptor agonists may be useful for their potential to increase or prolong opioid analgesia while attenuating the development of opioid tolerance. The purpose of this study was to investigate effects of dexmedetomidine and guanfacine (α_2 -adrenoceptor agonists) on morphine analgesia and tolerance in rats.

METHODS: Adult male Wistar albino rats weighing 195–205 g were used in these experiments. To constitute of morphine tolerance, animals received morphine (50 mg/kg; s.c.) once daily for 3 days. After last dose of morphine was injected on day 4, morphine tolerance was evaluated by the analgesia tests. The analgesic effects of dexmedetomidine (20 μ g/kg), guanfacine (0.5 mg/kg), MK-467 (0,25 mg/kg; α_2 -adrenoceptor antagonist) and morphine were considered at 30-min intervals (0, 30, 60, 90, and 120 min) by tail-flick and hot-plate analgesia tests.

RESULTS: Our findings indicate that dexmedetomidine and guanfacine significantly attenuated the expression of morphine tolerance (increased %MPE; $p < 0.05$). On the contrary, the data suggested that MK-467 significantly decreased morphine analgesia in the tail-flick and hot-plate tests (decreased %MPE; $p < 0.05$).

CONCLUSIONS: The interactions between noradrenergic and opioidergic mechanisms are complex. In addition, the localization of the receptor subtypes involved in the interactions of these systems with regard to antinociception and analgesic tolerance are still unclear. However, the obtained data suggested that the α_2 -adrenoceptor agonist dexmedetomidine and guanfacine increased antinociceptive effect of morphine and attenuated tolerance to morphine.

Keywords: dexmedetomidine, guanfacine, α_2 -adrenoceptor agonist, morphine, tolerance

Cocaine and Amphetamine Regulated Transcript (CART) Expression in Rat Frontal Cortex, Brain Stem, Pituitary Gland and Adrenal Gland and Its Regulation with Forced Swim Stress

Aysegul Keser¹, Oguz Gozen¹, Ersin Oguz Koylu¹, Mike Kuhar², Burcu Balkan²

¹Department of Physiology, Ege University, Izmir, Turkey

²Yerkes Regional Primate Center, Emory University, Atlanta, USA

OBJECTIVE: Cocaine and Amphetamine Regulated Transcript (CART) is synthesized in hypothalamo-pituitary-adrenal axis which is activated in stress response. CART increases synthesis of corticotropin releasing factor (CRF), adrenocorticotrophic hormone and corticosterone and the activity of the sympathetic nervous system. In this study, we aimed to show the expression of different CART peptide fragments in rat frontal cortex, brain stem, pituitary gland and adrenal gland and its regulation with acute stress.

METHODS: In the first step, male Sprague Dawley Rats were sacrificed and brains were removed. Frontal cortex, brain stem, pituitary gland and adrenal gland were dissected on ice. Total protein in each tissue sample was determined by the Nanodrop and CART peptide expression was investigated with Western Blotting.

In the second step, two groups were performed: Naïve and acute stress group which were forced to swim in 25°C water in a plexiglass cylinder for two consecutive days (n=6). CART was investigated with the same procedure.

RESULTS: We showed one CART peptide fragment at 5 kD in frontal cortex, three fragments at 8 kD, 5 kD and 4 kD in pons, four fragments at 8 kD, 7 kD, 5 kD and 4 kD in medulla, four fragments at 8 kD, 7 kD, 5 kD and 4 kD in pituitary and five fragments at 13 kD, 10 kD, 8 kD, 7 kD and 5 kD in adrenal gland.

CONCLUSIONS: Various CART fragments are expressed in large amounts in brain regions and endocrine glands related with stress response. In our next study, we will also investigate the regulation of CART peptide fragments by forced swim stress.

Keywords: Adrenal Gland, Brain Stem, CART, Frontal Cortex, Pituitary Gland, Stress, Western Blot,

Does ozone affect brain functions in diabetic rats?

Osman Genç¹, Gülten Erken², Ceylan Ayada¹, Haydar Ali Erken³, Gulsah Demirkaya Gundogdu¹, Hasan Doğan⁴

¹Department of Physiology, Pamukkale University, Denizli, Turkey

²Department of Physiology, Balıkesir University, Balıkesir, Turkey

³Balıkesir State Hospital, Balıkesir, Turkey

⁴Ozone Treatment Center, Denizli, Turkey

OBJECTIVE: The aim of this study was to compare the effects of insulin and medical ozone therapy on total EEG activity and EEG frequency bands in diabetic rats.

METHODS: Thirty female rats were divided into 5 groups, each consisting 6 rats: Control (K), diabetes (D), ozone (O), diabetes + insulin (DI), diabetes + ozone (DO). Diabetes was induced by a single ip injection of Streptozotocin. Ozone was applied by ip injection (1.1 mg/kg/d) to O and DO groups for 15 days. Insulin was administered as ip injection. The rats were anesthetized and the EEG recordings were collected. One Way ANOVA and Post Hoc Tukey tests were used, P values <0.05 were accepted as statistically significant.

RESULTS: Total EEG activity of D was increased compared to group K. While total activity of the DO group were similar to control values, total EEG activity of O group was decreased compared to group K. But, these alterations were not statistically significant. Total EEG activity of K, D, O and DO groups were increased compared to group DI. The frequency analysis did not reveal any significant alterations among the groups.

CONCLUSIONS: Diabetes and medical ozone therapy caused opposite effects on the EEG activity. These findings suggest that medical ozone treatment may decrease the negative effects of diabetes on brain function.

This study was supported by Pamukkale University Research Fund.

Keywords: Ozone, brain function, EEG activity

Medical Ozone Prevents The Harmful Effects Of Cadmium On Neurological Functions In Rats

Haydar Ali Erken¹, Gülten Erken², Omur Karaca³, Fatma Bahar Sunay⁴, Osman Genç⁵, Kamil Seyrek⁶, Hasan Akşit⁷, İlter Kuş³

¹Balikesir State Hospital, Balikesir

²Balikesir University, Faculty of Medicine, Department of Physiology, Balikesir

³Balikesir University, Faculty of Medicine, Department of Anatomy, Balikesir

⁴Balikesir University, Faculty of Medicine, Department of Histology and Embryology, Balikesir

⁵Dumlupinar University, Faculty of Medicine, Department of Physiology, Kutahya

⁶Balikesir University, Faculty of Medicine, Department of Biochemistry, Balikesir

⁷Balikesir University, Faculty of Veterinary Medicine, Department of Biochemistry, Balikesir

OBJECTIVE: It is well known that cadmium has harmful effect on neurological functions. The aim of this study was to investigate the protective effects of ozone on rats exposed to cadmium.

METHODS: A total of 28 Sprague Dawley male rats, were divided into 4 groups, each consisting 7 rats: Control (C), Cadmium (Cd), Cadmium+Oxygen (Cd+Ox), Cadmium+Ozone (Cd+Oz). Cadmium was given to all groups except the control for fifteen day (0.9mg/kg/day Cd, i.p, dissolved within 1ml SF). % 97 Oxygen was given to Cd+Ox group (4.5 ml/day, i.p.). Ozone was given to Cd+Oz group (1.1 mg/kg/day Ozone, i.p., 4.5 ml). At the end of the experimental period, EEG recordings in anesthetized rats were collected. Sciatic nerves of rats were removed and nerve conduction velocity (NCV) was measured. One Way ANOVA and Post Hoc Tukey tests were used for the differences between groups, p values <0.05 were accepted as statistically significant.

RESULTS: Delta activity was significantly increased in Cd group when compared to control; but the theta and alpha activities of Cd group were significantly decreased when compared to control. While delta activity was significantly decreased in Cd+Ox and Cd+Oz groups when compared to Cd group, theta and alpha activities of Cd+Ox and Cd+Oz groups were increased compared to Cd group. NCV values of Cd group were decreased when compared to C group; but NCV values were significantly increased in Cd+Oz group when compared to Cd group.

CONCLUSIONS: Our results demonstrated that medical ozone reduced the harmful effects of cadmium exposure on neurological functions.

Keywords: Cadmium, EEG, Nerve conduction velocity, Ozone, Rats

Improved Functional Recovery After Stroke Through Enhancement Of The Endogenous Neurogenesis In Aged Rats

Tudor Adrian Balseanu¹, Ana Maria Buga², Nicoleta Oprescu¹, Christine Poehlke¹, Josephine Kaiser¹, Raluca Vintilescu¹, Aurel Popa Wagner¹

¹Department of Neurology, EMAU University, Greifswald, Germany

²Department of Physiology, UMF Craiova, Craiova, Romania

OBJECTIVE: In adult rats, the endogenous neurogenesis is maintained in the subventricular zone and the dentate gyrus of the hippocampus and could be used to improve post-stroke outcome. Here we asked if stimulations of endogenous neurogenesis before or after stroke in aged rats, which are known to be more severely affected by stroke than young rats, may improve recuperation after stroke.

METHODS: Stroke was induced by middle cerebral artery occlusion (MCAO) in aged rats and neurogenesis was stimulated at different time points using neurogenesis enhancer, pentylentetrazole or electrical stimulation. After MCAO, rats were behaviorally tested for 7 weeks and global gene expression and immunohistochemical analyses of the periinfarcted region were done.

RESULTS: Our results indicate that stimulation of neurogenesis at 4 weeks before stroke does not improve post-stroke outcome. In contrast, stimulation of post-stroke neurogenesis is beneficial for behavioral recovery of aged rats. Global gene expression analysis has shown many new features of gene expression associated with aging and led to identification of over 400 new genes involved in stroke pathophysiology. Immunohistochemistry has revealed many new features related to the neurovascular unit in the aged post-stroke animals.

CONCLUSIONS: Stimulation of post-stroke neurogenesis is beneficial for behavioral recovery of aged rats.

Keywords: stroke, neurogenesis

Effects Of Chronic Ovariectomy On Learning And Locomotor Activity In Rats

Sehrazat Kavraal¹, Aise Seda Artis², Soner Bitiktas¹, Cem Suer², Derya Deniz Elalmis¹

¹Health Sciences Institute, Erciyes University Medical Faculty, Kayseri, Turkey

²Physiology Department, Erciyes University Medical Faculty, Kayseri, Turkey

OBJECTIVE: Estrogen may affect learning and memory through modulating the function of the basal cholinergic system. Ovariectomy has been shown to alter not only cholinergic but also glutamatergic activity. Estrogen increases the density of synapse in hippocampus and plays a role spatial memory. However the findings on how estrogens affect hippocampal functions are controversial. We aimed to test locomotor activity and learning in chronic ovariectomized rats.

METHODS: Experimental procedures were approved by Ethical Committee of Erciyes University. Thirty rats were randomly divided into ovariectomized and sham groups. After 6 weeks from ovariectomy open field and Y-maze test were performed to test locomotor activity and short-long term spatial learning, respectively.

RESULTS: The total number of line crosses in periphery of open field arena was higher in sham group than ovariectomized group ($p<0.03$). Numbers for grooming ($p<0.008$) and freezing reaction ($p<0.01$) were statistically higher in ovariectomized group than sham group. In Y maze, sham group preferred to visit novel arm more frequently than familiar arm ($p<0.05$). The ovariectomized rats did not show any preference between the arms ($p>0.05$) in the short-term memory task. In the long-term memory task, the ovariectomized and sham groups' animals did not show any preference between the arms. The ovariectomized rats spent less time in the novel arm than the familiar arm in the short-term ($p<0.05$) and long-term tasks ($p<0.05$).

CONCLUSIONS: Chronic ovariectomized rats showed reduced locomotor activity and impaired spatial memory in short- and long-term tasks, but not in working memory tasks for retaining spatial information.

Keywords: ovariectomy, open field test, Y-maze, locomotor activity, spatial learning

Effects Of 2-Oh-Dha In An Animal Model Of Alzheimer's Disease

Ruben Rial Planas, Xavier Busquets, Pablo Escribá, Antoni Gamundí, Pere Barceló, Maria Antonia Fiol Iunics, Universitat de les Illes Balears, Spain

OBJECTIVE: Membrane lipid dysfunction plays an important role in the etiology of Alzheimer's disease (AD) and a deficiency in Docosahexaenoic Acid (DHA) has been observed in AD human patients. This report aims to study the effects of a modified fatty acid 2OH-DHA on the working memory of 5XFAD a transgenic mouse model of AD.

METHODS: Wild mice B6JSL have been used as controls and two additional groups of 5XFAD mice have been studied (n=9 for each group). Three months old B6JSL and 5XFAD animals received during 12 week (5 days/week) a 5% intraesophagic ethanol solution (15 ml/kg). The 5XFAD experimental group received 2OH-DHA (15mg/kg dissolved in 5% ethanol). Then, all animals were fasted until reaching 80% of the ad libitum body weight and were submitted to 10 pre-training sessions in an eight arm radial maze. Then, the working memory was tested in 20 sessions with 4 baited and 4 non baited arms. Each session finished when the animals either succeeded in finding the baited arms or failed after 20 min of test. The total time for performance and the number of errors (failure, entrance in an unbaited arm or re-entrance in a visited arm) were computed as indexes of performance.

RESULTS: When compared with untreated 5XFAD controls, the treatment with 2OH-DHA caused a 15% reduction in time for performance. The number of errors also suffered a 25% reduction, reaching levels without difference with those observed in B6S JL wild controls.

CONCLUSIONS: The lipidic therapy improves the working memory in a mice model of AD and could be useful for the treatment of Alzheimer's Disease.

Keywords: alzheimer disease, transgenic mice, lipidic therapy, 2OH-DHA

Evaluation Of Cognitive Deficits In Patients With Parkin (Park2) Gene Mutations Using Auditory Event-Related Potentials

Atilla Uslu¹, Mehmet Ergen², Hasan Demirci³, Ebba Lohmann⁴, Hasmet Hanagasi⁴, Tamer Demiralp¹

¹Department of Physiology, Istanbul Faculty of Medicine, Istanbul University, 34093 Capa, Istanbul, Turkey

²Department of Physiology, School of Medicine, Acibadem University, 34384 Maltepe, Istanbul, Turkey

³Department of Neuroscience, Research Institute for Experimental Medicine, Istanbul University, 34093 Capa, Istanbul, Turkey

⁴Department of Neurology, Behavioral Neurology and Movement Disorders Unit, Istanbul Faculty of Medicine, Istanbul University, 34093 Capa, Istanbul, Turkey

OBJECTIVE: Studies on cognitive deficits in patients with parkin (PARK2) gene mutation are rare and report more slight changes in terms of cognitive deficits compared with other Parkinson's disease (PD) patients, probably because of the early-onset and slower progress of the disease in this group. The aim of the present study is to investigate the cognition in patients with parkin mutation more in depth using cognitive electrophysiological measures.

METHODS: The participants consisted of 25 healthy control volunteers, 65 non-demented PD patients. PD patients were divided into three groups as: PD patients with parkin mutation (PM-PD; n=15) and early onset (EO-PD; n=25) and late onset (LO-PD; n=25) idiopathic PD patients. Neuropsychological state of the subjects was evaluated by Mini-Mental State Examination (MMSE) and ERPs were recorded while the subjects performed an auditory oddball task.

RESULTS: We found MMSE scores of the all PD groups were significantly lower than those of the control group ($p<0.001$). P300 amplitudes in target-ERPs of the oddball test were significantly lower in all PD groups compared with the controls ($p<0.001$). However, P200 amplitudes of all PD groups were significantly larger than those of the control group ($p=0.042$). In addition, the latencies of both P200 and P300 potentials were significantly longer in all PD patients ($p=0.015$ and $p<0.001$, respectively). There was no significant difference among the PD groups.

CONCLUSIONS: Our results suggest that EO-PD patients with PM-PD show a similar delay in cognitive processing and a similar decrease in selective attention performance as in other PD groups.

Keywords: auditory oddball, event-related potentials, P200, P300, Parkinson's disease

Quantitative Evaluation Of Magnetic Resonance Images In Parkinson's Disease

Dursun Aygün¹, Bünyamin Şahin², Fikri Özdemir², Musa Kazım Onar¹

¹Ondokuz Mayıs University, Faculty of Medicine, Department of Neurology, Samsun

²Ondokuz Mayıs University, Faculty of Medicine, Department of Anatomy, Samsun

OBJECTIVE: Parkinson's disease (PD) is a neurodegeneratif disorder of central nervous system. It results from the death of dopaminergic cells in the substantia nigra, a region of the midbrain. However, there are limited studies evaluating the volume of brain regions and its relation to the PD's findings. In the present study we estimated the volume of hemispheres, cerebellum and brain stem and we evaluated the relation between the volume and features of the disease

METHODS: T1 weighted magnetic resonance images of the patients and clinical findings were obtained retrospectively. The volumes of right and left hemispheres, cerebellum and brain stem were estimated stereologically. The relation between the volume data and clinical findings were statistically analyzed

RESULTS: Our results showed that the volume of right and left hemispheres, cerebellum and brain stem were 548.6, 554.2, 143.6 and 24.2cm³, respectively. Patients stages was changing from 1.5 to 5 and most of them were in stage 2.5 (43.5%). There was correlation between the volume and stage of the disease ($r=0.441$, $p<0.05$). There were no correlation between the clinical signs and volumetric data ($p>0.05$).

CONCLUSIONS: Our data revealed the volume of brain stem was higher in the patients with high score of disease. We will discuss the finding on the basis of available literature. Additionally, stereological methods could be used for the evaluation of PD images.

Keywords: Parkinson disease, stereologic method

The Effects Of Docosahexaenoic Acid (Dha) On Inos And Bcl-2 Expression Levels In Bilaterally Mptp-Lesioned Rat Model Of Parkinson's Disease

Gulay Hacıoğlu¹, Yasemin Seval Celik², Gamze Tanrıöver², Ozlem Ozsoy¹, Esen Saka Topcuoğlu³, Sevin Balkan⁴, Aysel Agar¹

¹Department of Physiology, Faculty of Medicine, Akdeniz University, Antalya, Turkey

²Department of Histology and Embryology, Faculty of Medicine, Akdeniz University, Antalya, Turkey

³Department of Neurology, Faculty of Medicine, Hacettepe University, Ankara, Turkey

⁴Department of Neurology, Faculty of Medicine, Akdeniz University, Antalya, Turkey

OBJECTIVE: The aim of this study was to observe whether the neuroprotective effects of DHA in an experimental Parkinson model is related with alterations in iNOS and Bcl-2 expressions.

METHODS: Adult male Wistar rats were divided into 4 groups: Control; DHA-treated; MPTP-induced; MPTP-induced+DHA-treated. Motor activity was investigated by vertical pole test. The lesion was determined by immunohistochemical analysis for tyrosine hydroxylase (TH)-immunopositive cells in substantia nigra (SN). Immunoreactivities of iNOS and Bcl-2 in SN were evaluated by immunohistochemistry.

RESULTS: MPTP-induced animals exhibited decreased locomotor activity and motor coordination. Diminished symptoms and decreased dopaminergic neuron death were detected in the MPTP-induced+DHA-treated group compared to MPTP-induced group. iNOS immunoreactivity increased in MPTP-induced group compared to control and DHA, however it was decreased in the MPTP-induced+DHA-treated group compared to the MPTP-induced group. The staining intensity for Bcl-2 decreased in the MPTP-induced group compared to control, while it was stronger in the MPTP-induced+DHA-treated group compared to MPTP-induced group.

CONCLUSIONS: The alterations in the expression levels of iNOS and Bcl-2 would be possible factors in neuroprotective effects of DHA in experimental model of Parkinson's disease.

Keywords: Parkinson, MPTP, DHA, Bcl-2, iNOS, rat

The Interaction Between Carbenoxolone And Valproic Acid On Pentylenetetrazole Kindling Model Of Epilepsy In Rats

Fatih Sefil¹, Faruk Bağırıcı², Meryem Dilek Acar², Ramazan Kozan³

¹Department of Physiology, Faculty of Medicine, Mustafa Kemal University, 3100, Hatay

²Department of Physiology, Faculty of Medicine, Ondokuz Mayıs University, 55139, Samsun

³Department of Physiology, Faculty of Medicine, Bezmialem Vakif University, 34093, Istanbul, Turkey

OBJECTIVE: Gap junctions play an important role in the synchronized neuronal discharges. The main reason of the epileptic seizures is disruption of this synchronization. Therefore, the present study was designed to investigate the interaction between carbenoxolone (CBX), a gap junction blocker, and valproic acid on pentylenetetrazole (PTZ) kindling model of epilepsy in rats.

METHODS: Adult male Wistar albino rats were used in this study. In the first set of experiments, PTZ (35 mg/kg intraperitoneally, (i.p.) was administered to the rats to produce the kindling and then permanent screw electrodes were placed into the cranium of kindled rats to record EEG monitoring. In the second set of experiments, the interaction between CBX (40 mg/kg i.p.) and valproic acid (300 mg/kg, i.p.) was performed. While EEG recordings received from animals, behavioral scorings were done by an observer. The data analysis was performed using a one-way ANOVA with LSD post-hoc test.

RESULTS: The combination of CBX and valproic acid prevented generalized seizures and decreased seizure severity and score ($P < 0.01$). This combination also prevented myoclonic jerks by 83.3 % ($P < 0.01$).

CONCLUSIONS: The results of behavioral parameters and electrophysiological evidences show that CBX potentiates antiepileptic effects of valproic acid which is widely used antiepileptic drug. Thus, our results suggested that these two drugs can be used in combination for the treatment of epilepsy.

Keywords: Carbenoxolone; Gap junction; Epilepsy; Pentylenetetrazole kindling

Effect Of Levetiracetam And Carnosine Combination On The Experimental Model Of Penicillin Epilepsy

Faruk Bağırıcı¹, Aliye Erguvan Arık¹, Fatih Sefil²

¹Department of Physiology, Faculty of Medicine, Samsun, Turkey

²Department of Physiology, Faculty of Medicine, Hatay, Turkey

OBJECTIVE: Focal application of penicillin may cause seizures in rodents. Levetiracetam is a novel antiepileptic drug. It possesses anticonvulsant activity in animal models of epilepsy. Carnosine is a naturally occurring dipeptide in mammalian tissues. Animal studies have shown that carnosine can affect neurological function and can protect against penicillin-induced seizures in rats. In the present study we aimed to investigate the effects of carnosine and levetiracetam combination in penicillin epilepsy.

METHODS: Wistar albino rats weighing 200-250g were divided into four groups. Left somatomotor cortex of the animals was opened under urethane anesthesia Group I received 500 IU Penicillin G intracerebroventricularly. Group II, III and IV received levetiracetam (40 mg/kg), carnosine (500mg/kg) and levetiracetam-carnosine combination intraperitoneally 30 minutes before penicillin application. Electrocorticogram recording was taken for 180 minutes.

RESULTS: The data analysis was performed using One-way ANOVA and Post-Hoc Tukey test. Levetiracetam reduced spike frequencies in epileptic activity ($p<0.05$). Spike frequencies decreased ($p<0.05$) when carnosine was applied. Phenytoin and carnosine combination reduced spike frequencies too ($p<0.05$) but no significant difference was seen between the combination and the single use of the drugs.

CONCLUSIONS: According to these results, it was concluded that there was no synergetic interaction between carnosine and levetiracetam.

Keywords: Epilepsy, penicillin, rat, phenytoin, carnosine

Effect Of Phenytoin And Carnosine Combination On Experimental Model Of Penicillin Epilepsy

Aliye Erguvan Arık¹, Faruk Bağırıcı¹, Fatih Sefil², Cafer Marangoz¹

¹Department of Physiology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

²Department of Physiology, Faculty of Medicine, Mustafa Kemal University, Hatay, Turkey

OBJECTIVE: Topical application of penicillin is one of the methods to induce epileptic activity. Phenytoin is a commonly used antiepileptic drug. Carnosine is a biologically active dipeptide. Animal studies have shown that carnosine can protect against seizures in experimental models and can be used as an antiepileptic in future. We investigated the effects of phenytoin and carnosine combination on penicillin epilepsy in rats.

METHODS: Wistar albino rats weighing 200-250g were divided into four groups. Left somatomotor cortex of the animals was opened under urethane anesthesia. Group I received 500 IU Penicillin G intracerebroventricularly. Group II received phenytoin (60 mg/kg) intraperitoneally (i.p). 500 mg/kg carnosine was applied (i.p) to group III. Group IV received phenytoin and carnosine combination. Group II, III and IV received penicillin 30 minutes after drug applications. Electrocorticogram recording was taken for 180 minutes.

RESULTS: The data analysis was performed using One-way ANOVA and Post-Hoc Tukey test. Phenytoin and carnosine decreased spike frequencies in epileptic activity when they were used alone ($p < 0.05$). Phenytoin and carnosine combination reduced spike frequencies too ($p < 0.05$). But a significant difference was not seen between the combinations and the single use of these drugs.

CONCLUSIONS: We concluded that phenytoin and carnosine did not potentiate anticonvulsant effect of each other.

Keywords: Epilepsy, penicillin, rat, phenytoin, carnosine

Phenytoin Regulates Brain Development In The Offspring Of Epileptic Rats By Increasing The Level Of Brain-Derived Neurotrophic Factor

Handan Soysal¹, Yusuf Türköz², Nihat Ekinci³, Zümrüt Doğan³, Özden Kamışlı⁴, Nilay Güçlüer⁵

¹Medicine Faculty Departments of Anatomy, Adiyaman University, Adiyaman, Turkey

²Medicine Faculty Departments of Medical Biochemistry, Inonu University, Malatya, Turkey

³Medicine Faculty Departments of Medical Anatomy, Inonu University, Malatya, Turkey

⁴Medicine Faculty Departments of Neurology, Inonu University, Malatya, Turkey

⁵Medicine Faculty Departments of Microbiology, Inonu University, Malatya, Turkey

OBJECTIVE: We aimed to investigate the effects of phenytoin, frequently used antiepileptic, on brain development in offspring of pregnant rats with epilepsy.

METHODS: In this study, 127 new born rats were used to evaluate the levels of serum brain-derived neurotrophic factor (BDNF). Epileptic seizures were determined with EEG recordings. Each day of gestation, phenytoin (25 mg/kg) administered intraperitoneally to epileptic pregnant rats. After the birth, sera from 127 offspring rats were used for measurement of serum BDNF at the postnatal days 21 and 38. Serum BDNF concentrations were measured by ELISA method.

RESULTS: Serum BDNF levels decreased in epilepsy group at the postnatal day 21, compared to the control, but this decrease was not statistically significant ($p < 0.05$). Serum BDNF levels in epilepsy+phenytoin group were increased significantly at the postnatal day 21, compared to the epilepsy group ($p < 0.05$). But, no significant changes in serum BDNF levels of epilepsy + phenytoin group at the postnatal day 38 was found ($p < 0.05$).

CONCLUSIONS: Epilepsy affects brain development of the fetus in pregnant rats; therefore, anti-epileptic therapy should be continued during pregnancy. We conclude that phenytoin application in epileptic pregnant rats regulates the development of fetal brain by increasing the levels of BDNF.

Keywords: Epileptic rat, phenytoin, serum BDNF

The Role Of Olivocochlear System On Prevention Of Cochlear Damage Due To Noise, Using Antagonist Of Dopamin (Haloperidol) With Electrophysiologic Tests: An Experimental Study

Sevtap Devren¹, Recep Yagız¹, Erdogan Bulut², Abdullah Taş¹, Memduha Taş¹, Ahmet Rasim Karasalihoglu¹

¹Department of Otorhinolaryngology, Trakya University, Edirne, Turkey

²Department of Physiology, Trakya University, Edirne, Turkey

OBJECTIVE: In our study, the role of olivocochlear system prevention of cochlear damage due to noise, using antagonist of dopamin (haloperidol) were examined electrophysiologically.

METHODS: As a result of distortion product otoacoustic emission (DPOAE) measuring and auditory brainstem responses (ABR), 18 ears of 9 guinea pigs with detected normal hearing threshold and emission, have been taken into the scope of this study, and they were randomly divided into three groups. The first group of these three groups was planned as the experiment group, the second one as the working group, and the third group have been planned as the control group. The guinea pigs of the experiment group were administered only haloperidol (0.5 mg/kg), the other group was treated with haloperidol (0.5 mg/kg) and acoustic trauma (110 dB, 10 minutes, large band noise). The guinea pigs in the control group were exposed to acoustic trauma (110 dB, 10 minutes, large band noise) and received physiological saline. DPOAE and ABR measurements repeated after administration at the time of 30th, 60th, 90th and 120th minutes.

RESULTS: No statistically significant difference was observed when DPOAE (Signal/Noise ratio), ABR thresholds of hearing assessed in all groups prior to medicine and noise administration. In the experimental group, after administration there was no significant difference in DPOAE and ABR measurements.

CONCLUSIONS: In conclusion, a protective effect on the functioning of the olivo-cochlear system in noise induced cochlear damage is temporarily affected by the use of haloperidol.

Keywords: Otoacoustics emissions; efferent pathways; dopamine

Effects of different types of music on classical and nonlinear parameters of male temporal lobe EEG activity

Sinan Canan¹, Ismail Avcibas²

¹Yildirim Beyazit University School of Medicine Department of Physiology, Ankara, Turkey

²Turgut Ozal University Faculty of Engineering, Department of Electric and Electronic Engineering, Ankara, Turkey

OBJECTIVE: We aimed to investigate the EEG manifestations of concentrated music listening experience on four musical pieces belonging to four different musical genres: Turkish classical (Sufi), western classical (Vivaldi), rock (AC/DC) and pop (club beats) music.

METHODS: 21 right-handed male university students were selected for study. An audio file was prepared by attaching 3 minute-long segments of different musical pieces beginning with a three minutes of silence. Each subject was comfortably placed on a laboratory litter on supine position and EEG electrodes were attached over both temporal hemispheres at T4-T6 and T3-T5 points with a common earth electrode attached to the left earlobe. Each subject put on a pair of high-quality earphones for music presentation and a pulse transducer attached to their left hand middle finger. Before the actual audio file is presented, all subjects received a brief verbal suggestion to relax and concentrate on audio. Recording procedure started simultaneously with the audio presentation and continuous EEG and pulse recordings obtained using a digital data acquisition system, with a sampling rate of 1kHz. Recorded brain waves decomposed into delta, theta, alpha, beta and gamma frequencies and total power of each frequency were calculated together with the RMS values. In addition, false nearest neighbor fractions (FNFs) for different embedding dimensions were calculated and compared. FNFs are commonly used for finding the appropriate embedding dimension which can uncover useful information about the original unknown chaotic dynamics of the signal in phase space.

RESULTS: According to our findings, nonlinear analysis techniques revealed that there is a significant similarity between the silent period and the period in which the Turkish classical music is presented, in terms of nonlinear parameters of the brain signals. All other types of musical pieces were significantly different than silent and Turkish classical music period.

CONCLUSIONS: Our findings suggest that the nonlinear analysis methods may be a useful tool to better understand the complex procedure of music perception and interpretation.

Keywords: Music, male temporal lobe, EEG activity

Evoked Responses Of Trigeminal Mesencephalic Neurons To Artificial Whisking In The Rat

Ombretta Mameli¹, Gabriele Mulliri¹, Paolo Manca¹, Marcello Alessandro Caria¹, Pier Luigi De Riu¹, Stefania Stanzani², Antonella Russo², Rosalia Pellitteri³

¹Dept. Neuroscience: Human Physiology Division, Sassari University, Sassari, Italy

²Dept. Physiological Sciences, Catania University, Catania, Italy

³National Research Council, Inst. of Neuroscience, Catania Section, Catania, Italy

OBJECTIVE: In previous experiments we demonstrated that trigeminal mesencephalic neurons (TMne) are significantly activated by both spontaneous and mechanical displacement of the macrovibrissae in different directions. Furthermore, we showed that TMne are directly connected to the upper part of the ring sinus of the vibrissae follicle-sinus complex, by circumferential fine- and small-calibre fibres with lanceolate endings. The present study was aimed at analyzing the evoked responses of TMne to the artificial whisking.

METHODS: The artificial whisking was induced by the electrical stimulation of the peripheral stump of the facial nerve. The TMne responses were compared to those evoked in the whiskers neurons of the Gasser's ganglion (GG) following the same stimulation.

RESULTS: Results showed that trigeminal mesencephalic nucleus (TMnu) responded to the artificial whisking with evoked polyphasic potentials at 1.28 ± 0.03 ms latency. Excitatory responses of single TMne also appeared a short latency (2.3 ± 0.06 ms) and were usually followed by a delayed response at 5.2 ± 0.15 ms latency. Some neurons only responded to masseter stretch. At GG level, the artificial whisking induced monophasic evoked potential at shorter latency (0.87 ± 0.02 ms) and responses of single neurons at 2.2 ± 0.04 ms latency.

CONCLUSIONS: These electrophysiological findings seem to confirm the hypothesis that TMne relay sensory information to the brainstem trigeminal nuclei directly from macrovibrissae. Latencies of TMnu/TMne responses to artificial whisking is in fact consistent with: i) the electromechanical activation of pad muscles, induced by facial nerve stimulation, ii) the vibrissae deflection and iii) the activation of TMne axonal terminals around the upper level of ring sinus of the vibrissae follicle-sinus complex.

Keywords: trigeminal mesencephalic nucleus, evoked responses, artificial whisking, facial nerve stimulation, rat

Sensory Innervation Of The Whisker Pad By Mesencephalic Trigeminal Neurons

Ombretta Mameli¹, Stefania Stanzani², Antonella Russo², Rosalia Pellitteri³, Maria Palermo², Gabriele Mulliri¹, Paolo Manca¹, Marcello Alessandro Caria¹, Pier Luigi De Riu¹

¹Dept. of Neuroscience: Human Physiology Division, Sassari University, Italy

²Dept. of Physiological Sciences, Catania University, Italy

³National Research Council, Inst. of Neurological Sciences, Catania Section, Italy

OBJECTIVE: Recent results suggest that trigeminal mesencephalic nucleus (TMnu) also contains first-order neurons specialized in relaying spatial information, related to whisker location and their movement, to trigeminal-cortical pathways. Retrograde tracers revealed in fact neurons innervating i) the neuromuscular spindles of the masseter muscle, ii) the mystacial pad, as well as iii) neurons innervating both structures. Electrophysiological findings demonstrated that mesencephalic neurons (TMne) respond to spontaneous movements of the macrovibrissae. Here we analyze the peripheral distribution of TMne terminals to the whisker pad structures to locate their targets.

METHODS: Under aseptic conditions, in anaesthetized rat, the fluorescent carbocyanine dye Dil was unilaterally injected into the TMnu. After seven days, the animals were deeply anaesthetized and transcardially perfused. The brain and the mystacial pad were then removed, post-fixed and frozen sectioned. Brain sections were histologically processed, to assess the tracer injection site, and the fluorescence analysis. The ipsilateral whole pad was subdivided into four portions each containing two rows of vibrissae. Alternate sections parallel to the skin were mounted on gelatine-coated slides for fluorescence detection of anterograde Dil-labelled terminals in the pad structures.

RESULTS: Results showed that TMne directly innervate the macrovibrissae. Anterograde Dil-labelled terminals were in fact found around the upper level of the ring sinus of the vibrissae follicle-sinus complex. These were constituted of circumferential fine- and small-caliber fibers with lanceolate endings.

CONCLUSIONS: With concern to their functional roles, it is possible that TMne may convey spatial information related to the macrovibrissae location and displacement to the trigeminal brainstem nuclei.

Keywords: rat macrovibrissae; trigeminal mesencephalic nucleus; sensory innervation; proprioception; macrovibrissae location and displacement.

Effects Of Nitric Oxide On Anxiogenic-Like Behavior Via Simvastatin Administration In Rats

Mehmet Alkanat¹, Sukruhan Hasan Baytan²

¹Faculty of Health Science, Giresun University, Giresun, Turkey

²Faculty of Medicine, Department of Physiology, Karadeniz Technical University, Trabzon, Turkey

OBJECTIVE: As an important member of the statin family, simvastatin has been shown to increase nitric oxide (NO) levels in several studies. NO is a widespread and multifunctional biological messenger molecule in mammals. In this study, altered anxiety levels by simvastatin administration have been investigated to determine whether the NO mediated mechanisms are involved in this effect.

METHODS: Seven groups of animals, each consisting of 8 Sprague Dawley rats were tested by elevated plus maze (EPM) and open field margin time (OFMT) methods, to observe anxiety levels. When vehicle group was administered with phosphate buffered saline (PBS) alone, two groups were administered 20 or 40 mg/kg simvastatin + PBS and the other groups were administered simvastatin + L-NAME of 10 or 50 mg/kg. All groups were tested in the 1st, 4th, 7th, 10th, 15th, 21th and 28th days of vehicle or drug administration. The effects of different doses of simvastatin and L-NAME application on EPM and OFMT tests in rats were analyzed using two way analyzes of variances.

RESULTS: Results are shown that, 40mg/kg simvastatin only group EPM times spent in the close arms were significantly higher than the vehicle group ($p < 0.05$). Also, 20 and 40 mg/kg simvastatin only groups had higher OFMT when compared to vehicle group ($p < 0.05$). The higher observed time spent in closed arms in EPM and OFMT are considered as anxiogenic-like behavior.

CONCLUSIONS: The results suggested that anxiogenic like behavior caused by simvastatin administration was inhibited with L-NAME administration. Thus, these results indicate that anxiogenic like behavior caused by simvastatin administration is modulated by an NO-involved mechanism.

Keywords: Psychological behaviour, Nitric oxide, Elevated plus maze test, Open field test

Cholinergic, Substance P-Ergic And Nitrenergic Implications In Descending Reflex Responses Of Anal Canal In A Rat Model

Galya Tsvetanova Stavreva¹, Radomir Georgiev Radomirov²

¹Department of Experimental and Clinical Pharmacology, Medical University, Pleven

²Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia

OBJECTIVE: Investigation of activation-dependent descending reflex motility of anal canal.

METHODS: Mechanographic computerized on-line technique, partitioned organ bath, electrical stimulation (EFS, 0.8 ms; 40 V; 5 Hz) or balloon distension were used to evaluate motor responses of anal canal (AC) and recto-anal reflexes. Immunohistochemical and histochemical techniques were used to study the presence and distribution of acetylcholine, substance P and nitric oxide in neuronal structures of the myenteric plexus of the anal canal.

RESULTS: Frequency dependent local (14.9 ± 1.35 mN) and descending (5.3 ± 0.7 mN) contractions of AC, elicited by EFS applied to the AC or to the distal part of the rectum were registered and the amplitudes of local responses were more expressed ($p < 0.05$). The descending response of AC induced by balloon distension was a contraction (1.50 ± 0.18 mN) followed by relaxation (3.12 ± 0.34 mN). Atropine (3×10^{-7} M) suppressed EFS-elicited contractions of AC and a relaxation occurred. The distension-induced contraction was reduced while the relaxation was not altered. In the presence of atropine spantide (10^{-7} M) augmented contractile response of AC. NG-nitro-L-arginine (5×10^{-4} M) increased the excitatory responses, prevented the atropine-induced relaxation of the EFS-elicited response and inhibited the distension-induced relaxation. L-arginine (5×10^{-4} M) decreased the contractions and increased the relaxation. ChAT-, substance P- and NADPH-diaphorase-positive nerve fibers were found in myenteric ganglia of the anal canal.

CONCLUSIONS: The results suggest descending pathways involved in motor activity of anal canal comprising stimulatory cholinergic and tachykininergic, dependent on electrically-induced excitation and inhibitory nitrenergic, sensitive to distension of rectal wall.

Keywords: anal canal, descending reflex, atropine, L-arginine, NG-nitro-L-arginine, spantide

The Effects Of Regular Aerobic Exercise During Pregnancy On Maternal Deprivation And Spatial Learning And Memory

Ferihan Cetin¹, Seda Ozbal², Ayfer Dayi¹, Ilkay Aksu¹, Celal Gencoglu³, Aysegul Tas¹, Secil Ayca Cavus², Nazan Uysal Harzadin¹

¹Department of Physiology, Dokuz Eylul University, Izmir, Turkey.

²Department of Histology and Embriology, Dokuz Eylul University, Izmir, Turkey.

³School of Sports Sciences and Technology, Dokuz Eylul University, Izmir, Turkey.

OBJECTIVE: Aerobic exercise during pregnancy increases neurogenesis in pups. Maternal deprivation is a forceful stress which affects cognitive function, behaviour and endocrine system permanently during life time in mammals. The purpose of this study was to examine the effects of exercise during pregnancy on cognitive impairment and hippocampal cells of pups after maternal deprivation.

METHODS: Groups: exercised and sedentary mothers. Subgroups: maternal deprived males and females, control. Exercise: before pregnancy 5m/min- 10 min/day/5day. In pregnancy, 30 min/day, 5 days/week, 8m/min. Exercise speed was decreased 6m/min-last ten days. 24-hour maternal deprivation stress was applied to 18 days old pups. Performance of learning and memory tests of 26 days old pups were evaluated with Morris water tank.

RESULTS: Learning process is impaired in the maternal deprivation group. Exercise during pregnancy blocked the impairment in learning process. Maternal deprivation applied group spent less time in the target quadrant and more time in the opposite quadrant during the recall test. Exercise during pregnancy ameliorated these results. TUNEL+ cell number increased after maternal deprivation, but exercise during pregnancy reversed apoptosis normal. Cell numbers of hippocampal CA1 and CA3 and also in dentate gyrus region decreased in the maternal deprivation group and increased with exercise during pregnancy. In addition, during probe trial it is determined that very strong positive correlation between time spent in target quadrant and cell number of CA3 region and very strong negative correlation between time spent in the opposite quadrant and cell number of CA1 region.

CONCLUSIONS: These results suggest that regular aerobic exercise during pregnancy has protective effects on hippocampal cells and cognitive functions of pups after maternal deprivation.

Keywords: pregnancy, exercise, maternal deprivation

Functional Topography Of The Human Corpus Callosum As Depicted By Fmri And Dti Investigations

Mara Fabri¹, Gabriele Polonara², Giulia Mascioli², Andrea Ortenzi³, Ugo Salvolini², Tullio Manzoni¹

¹Dipartimento di Neuroscienze, sezione di Fisiologia e Storia della Medicina, Università Politecnica delle Marche, Ancona, Italy

²Dipartimento DISCO, sezione di Radiologia, Università Politecnica delle Marche, Ancona, Italy

³Clinica Neurologica, Ospedali Riuniti di Ancona, Ancona, Italy

OBJECTIVE: We tested the scope for describing the topography of human corpus callosum (CC) with functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI).

METHODS: We reviewed our recent published and unpublished fMRI data on the cortical representation of tactile, gustatory and visual sensitivity and motor activation obtained in 36 healthy volunteers and three partially callosotomized patients, two with anterior and one with posterior callosal resection. Anatomical correlates of functional activation were obtained from DTI and diffusion tensor tractography (DTT) data from 16 healthy subjects and the three patients.

RESULTS: In intact subjects taste stimuli activated anterior CC, motor tasks central CC, tactile stimuli central and posterior CC, and visual stimuli the splenium. DTT reconstruction of callosal fibers connecting activated gustatory, motor, somatosensory and visual cortices showed bundles crossing respectively through genu, anterior and posterior body, and splenium at sites harboring fMRI foci. In the patient with anterior callosotomy sparing only splenium a BOLD focus was observed after visual stimulation. In the one with anterior callosotomy also sparing posterior callosal body a focus was induced by tactile stimulation. In the patient with posterior callosotomy sparing only genu an activation focus was elicited by gustatory stimulation. In all patients, fibers connecting cortical areas activated by peripheral stimulation crossed the CC through sites harboring corresponding BOLD foci.

CONCLUSIONS: The functional topography of the CC described in present study agrees with previous reports.

Keywords: corpus callosum, interhemispheric transfer, fMRI, brain imaging.

Habituation Of Auditory Go-P3 And Nogo-P3 Potentials In Young Adults: Analysis Of Scalp Topography

Berrin Maraşlıgil, Figen Dağ, Tolgay Ergenoğlu

Department of Physiology, Medical Faculty, Mersin University, Mersin, Turkey

OBJECTIVE: Go-NoGo task has been widely used to evaluate response activation and inhibition in normal subjects as well as patients with neuro-psychiatric disorders. The aim of the present study was to investigate habituation of event related potential (ERP) responses to auditory Go and NoGo stimuli.

METHODS: ERPs were recorded with 30 electrodes using an auditory Go-NoGo paradigm from 38 healthy male volunteers (18-23 years). Go tones (1000 Hz) and NoGo tones (2000 Hz) with 50% probabilities were binaurally presented by headphones at 70 dB SPL. The amplitudes and latencies of the ERP responses in the first and the last half of the task were analyzed by ANOVA. Then, the interaction of the habituation with the scalp topography was tested using ANOVA after normalizing the data.

RESULTS: The NoGo-P3 potential amplitudes were significantly lower in the last half of the task compared to the first half of the task at all leads ($p < 0.01$) while Go-P3 potential amplitudes were not significantly different between the two periods ($p > 0.05$). Also, interaction of the two periods of the task and antero-posterior distribution of the NoGo-P3 potential amplitudes was significant: decrease of the amplitude of NoGo-P3 potential at the fronto-central areas was bigger than the parietal area ($p < 0.05$). After vector transformation, this significant interaction in the initial analysis turned out to be non-significant ($p > 0.05$), which shows that habituation of Go-P3 responses are not topographic specificity.

CONCLUSIONS: Our results indicate that the NoGo-P3 potential was habituated whereas the Go-P3 potential did not undergo any habitual changes at all recording electrode sites.

Keywords: Go-P3, Habituation, NoGo-P3, Topography

Biofeedback Method And Stress Tolerance Modulation In Students With Autonomic Nervous System Dysfunction

Daina Voita¹, Evita Valevica², Juris Porozovs¹, Toms Voits³

¹Riga Teacher Training and Educational Management Academy, Riga, Latvia

²Latvian University Institute of Cardiology, Riga, Latvia

³University of Sussex, Brighton, United Kingdom

OBJECTIVE: The aim of study is to elaborate the best biofeedback training method for students with autonomic nervous system dysfunction and decreased stress tolerance.

METHODS: Seventeen students (female, average age 21.3 years) with tension type headache attacks after stressful events were tested (group B). 66 age and gender matched healthy students were used as a control group (group A). Stress tolerance was detected using Vienna test systems Determination test (Schuhfried GmbH, Austria). Sympatho-parasympathetic balance was evaluated with Task Force Monitor device (CNS systems Medizintechnik, Austria). Biofeedback 2000 X-pert device (Schuhfried GmbH, Austria) was used for biofeedback trainings. Blood volume pulse amplitude training of a. temporalis, EMG training and skin temperature training were used with aim to decrease sympathetic activity in group B. Average training period was 8 weeks for each person. Training session lasted at least 20 min, performed 2 times weekly. Pain frequency and intensity were fixed before and after biofeedback training sessions.

RESULTS: Significantly decreased baroreflex sensitivity and decreased stress tolerance were detected in group B in comparison with group A. Pulse amplitude volume training for a. temporalis significantly decreased pain frequency. Pain intensity had tendency to decrease after biofeedback period. It is found that baroreflex sensitivity and stress tolerance had tendency to increase after biofeedback training.

CONCLUSIONS: The elaborated training model was proved to be optimal in order to re-establish symptho-vagal balance in students with decreased stress tolerance. Pulse volume amplitude training was found as the most suitable and effective method comparing with EMG and temperature trainings.

Keywords: autonomic nervous system, stress tolerance, biofeedback

Stress Reactivity And Motor Abilities Of Persons With Different Brain Hemisphere Dominance

Juris Porozovs¹, Daina Voita², Viktorija Perepjolkina², Gunita Praulite¹, Andris Spunde¹

¹Department of Informatics and Natural Science, Riga Teacher Training and Educational Management Academy, Riga, Latvia

²Department of Education and Social Psychology, Riga Teacher Training and Educational Management Academy, Riga, Latvia

OBJECTIVE: Hemispheric asymmetry evolved because of a left hemisphere speech processing specialization. Cortical asymmetries are well established in humans for language and motor regions and correlate with handedness. The most explicit specialization of brain hemisphere functions has persons with right hand dominance (right-handers).

METHODS: Thirty-two students of both genders (aged 19 -23) were involved in this study. All participants were asked to fill a questionnaire to detect their brain profile. For testing psycho-physiological indicators of students Vienna Test System was applied. Stress reactivity of students was detected using Determination Test (DT) S1 version. Hand motor capacity was detected using MLS (Motor performance series) tests.

RESULTS: No statistically significant differences of stress reactivity by DT test results between persons with different brain hemisphere dominance were observed. Although the results of investigation demonstrated that persons with unvoiced hemisphere dominance work faster: the total number of reactions for them was greater but they made more mistakes. Comparing hand motor capacity of right-handers and left-handers in ability to work with right hand no statistically significant differences were find, while regarding their ability to work with left hand left-handers remarkably surpass right-handers. Working with both hands simultaneously left-handers did not demonstrate remarkable differences in the number of taps performed with each hand, while right-handers demonstrate significant differences: with right hand they worked faster than with left hand.

CONCLUSIONS: Right-handers' brain functional asymmetry is more stable and the dominance of left hemisphere is more explicit than the dominance of right hemisphere of left-handers.

Keywords: Functional asymmetry of brain hemispheres, Vienna test System, stress reactivity, motor abilities, hand motor capacity, left-handers, right-handers.

Changes In Serum Beta Endorphin, Serotonin, Adrenaline, Noradrenaline And Dopamine Levels During Smoking Cessation By Electroacupuncture And Nicotine Patch

Mehmet Tuğrul Cabioğlu¹, Ruhuşen Kutlu², Canan Albayrak³, Kamile Marakoğlu⁴, Neyhan Ergene⁵

¹Department of Physiology, Medical Faculty of Başkent University, Ankara, Turkey

²Department of Pediatri, Selçuk University, Faculty of Meram Medical, Family Medicine, Konya, Turkey

³Department of Pediatric Hematology, Ondokuz Mayıs University, Faculty of Medical, Samsun, Turkey

⁴Department of Pediatri, Selçuk University, Faculty of Selçuklu Medical, Family Medicine, Konya, Turkey

⁵Department of Physiology, Selçuk University, Faculty of Meram Medical, Konya, Turkey

OBJECTIVE: The purpose of this study was to investigate the changes in serum beta endorphin, serotonin, adrenaline, noradrenaline and dopamine levels during smoking cessation with acupuncture and nicotine patch.

METHODS: This study consisted of 42 volunteer applying to quit smoking to the Outpatient Clinic of Family Medicine at Selcuk University, Meram Faculty of Medicine. Forty-two volunteers were divided in two groups; 1. Electroacupuncture (EA) application group (n=21) and training for smoking cessation, 2) Nicotine patch (NP) application group (n=21) and training for smoking cessation. Electroacupuncture sessions were performed once a day in the first three days and then once every other day for 30 days. The serum beta endorphin, serotonin, adrenaline, nor adrenaline, dopamine levels were measured before and after acupuncture and nicotine patch therapy.

RESULTS: : In the EA group, it was determined that beta endorphin (p=0.000), serotonin (p=0.009), adrenaline (p=0.007) and nor adrenaline (p=0.007) levels increased but dopamine (p=0.898) levels did not change. In the NP group, serum serotonin (p=0.045) levels increased but adrenaline (p=0.318), noradrenaline (p=0.099) and dopamine (p=0.655) levels did not change. There were increases in serum noradrenaline (p<0.000), dopamin (p<0.028) levels in EA compared with the NP group.

CONCLUSIONS: It is presumed that increased serum beta endorphin, serotonin, noradrenaline and adrenaline levels with EA application and similarly increased serotonin level following nicotin patch application facilitates to quit smoking for those trying to give up smoking by psychological support. As a result there was no statistically difference between the two groups of smoking cessation, therefore both electroacupuncture and nicotin patch applications are effective almost at the same rate.

Keywords: Adrenaline, Beta endorphin, Electroacupuncture, Noradrenaline, Serotonin, Smoking cessation

Encoding Of Vibrissae Movement Direction By Mesencephalic Trigeminal Neurons

Ombretta Mameli¹, Gabriele Mulliri¹, Paolo Manca¹, Marcello Alessandro Caria¹, Pier Luigi De Riu¹, Stefania Stanzani², Antonella Russo², Rosalia Pellitteri³

¹Dept. of Neuroscience, Human Physiology Division, University of Sassari, v.le S. Pietro 43/B, 07100 Sassari, Italy

²Dept. of Physiological Sciences, University of Catania, Catania Italy

³National Research Council, Inst. of Neuroscience, Catania Section, Catania, Italy

OBJECTIVE: It has been recently shown in rats that spontaneous movement of the macrovibrissae, in the air, induces significant changes of spontaneous trigeminal mesencephalic neurons (TMne) electrical activity. Here we report results concerning the TMne electrical activity recorded during mechanical deflection of the macrovibrissae.

METHODS: Mechanical deflection of the macrovibrissae was performed by a glass stick in forward/backward, backward/forward, down/up and up/down directions.

RESULTS: Different patterns of responses were observed in TMne, all characterized by the appearance of firing bursts and/or tonic increase of neuronal activity during vibrissae deflection in one or two specific directions. Preliminary results show that all neurons responded to down/up, while some to up/down directions.

CONCLUSIONS: Results demonstrated that the TMne are significantly activated also during their mechanical deflection in specific directions, thus it is possible that the TMne are able to encode spatial coordinates of the macrovibrissae. It is known that rats palpate the objects with their whiskers to recognize shapes, size and texture, information that is relayed to the trigeminal brainstem nuclei through the peripheral branch of the Gasser ganglion neurons. Since TMne are extensively connected to the trigeminal nuclei complex, it is possible that these latter identify and locate the objects in the environment by integrating touch information, received by the Gasser's neurons, with spatial information related to the vibrissae position, and detected by the TMne.

Keywords: Mesencephalic trigeminal nucleus, rat macrovibrissae, proprioception, encoding of movement

Does Imagination Affect The Electrical Activity Of The Brain?

Gökçer Eskikurt¹, Sernaz Demirel Temel², Tan Temel², Ümmühan Isoglu Alkac³

¹Institute of Experimental Medicine, Department of Neuroscience, Istanbul University, Istanbul, Turkey

²The Art and Design Faculty Department of Music and Performing Arts, YTU, Istanbul, Turkey

³Istanbul Faculty of Medicine, Department of Physiology, Istanbul University, Istanbul, Turkey

OBJECTIVE: Brain research on creativity has revealed valuable insights into brain correlates underlying mental imagination. The previous studies reveal evidence that highly creative individuals exhibited higher alpha activity during performance. The main aim of this study was to investigate the frequency properties of the spontaneous electrical activity of the brain (EEG) between two resting conditions in professional dancers who have attained a high level of expertise in modern dance.

METHODS: Ten healthy, right-handed professional dancers participated in this study (5 female, 5 male). Spontaneous EEG was recorded during the two different conditions: 5 min EEG sequences under resting conditions were recorded with eyes closed. And then after a task instruction the spontaneous EEG was recorded with eyes closed during 5 min. The order of task was fixed for each participant: while dancers were imagining about the dance style they want to perform mostly, their EEG were recorded.

RESULTS: The power of the beta frequency was higher in eyes closed condition than in the dance improvisation mentally condition for the frontal, central, parietal and occipital locations. This difference was statistically significant ($p < 0.05$). There were also differences for the Channel and Condition X Channel interaction significantly.

CONCLUSIONS: The present study is important to demonstrate the effects of the dance improvisation task mentally by electrophysiological properties of the brain in professional dancers.

Keywords: EEG, Dancers, Imagination, Frequency components

The Properties of the Auditory P300 Responses on Healthy Volunteers with Right- and Left-Handed

Gökçer Eskikurt¹, Ümmühan Isoglu Alkac²

¹Institute of Experimental Medicine, Department of Neuroscience, Istanbul University, Istanbul, Turkey

²Istanbul Faculty of Medicine, Department of Physiology, Istanbul University, Istanbul, Turkey

OBJECTIVE: The aim of the present study was to evaluate the responses to Auditory Oddball Paradigm in reference to hand preference.

METHODS: Twenty healthy volunteers (10 right-handed, 10 left-handed) participated in this study. Brain electrical activity (EEG) recorded by using WinEEG program (MITSAR) from 19 different locations. Volunteers were instructed to press a button every time when they hear the low frequency sound (target stimulus) and not to press button when they hear high frequency sound (standard stimulus). In average 330 stimuli were applied with percent 20 target stimulus (I.U. Ethics Commission File No: 2009/2648-43). Repeated measured ANOVA (SSPSS-PC 16.0) test is used for the statistical analysis.

RESULTS: The P300 latency of left-handed volunteers had longer than in right-handed volunteers [$F(1,9)=5,528$, $p=0.043$] by Auditory Oddball Paradigm, but there had no significant difference between left- and right-handed volunteers in amplitude of P300 response [$F(1,9)=0.037$, $p=0.852$]. There had no significant difference at the amplitude of P300 responses between right and left hemisphere [$F(1,9)=0.954$, $p=0.354$] and also at the values of latency [$F(1,9)=0.195$, $p=0.669$] of right-handed volunteers. And also there had no significant difference at the amplitudes [$F(1,9)=0.026$, $p=0.875$] or latencies [$F(1,9)=0.256$, $p=0.625$] of the P300 responses between hemispheres of left-handed volunteers.

CONCLUSIONS: The present study demonstrates the hemispheric properties of the cognitive functions between volunteers with right-handed and left-handed. This work was supported by Scientific Research Projects Coordination Unit of Istanbul University (Project # 4965).

Keywords: Auditory P300, Handedness, Dominant hemisphere

The Evaluation Of The Auditory Cognitive Function For The Professional Dancers And Sportsmen

Ümmühan Isoglu Alkac¹, Tan Temel², Gökçer Eskikurt³, Sernaz Demirel Temel², Ilker Yücesir⁴, Bülent Bayraktar⁵

¹Istanbul Faculty of Medicine, Department of Physiology, Istanbul University, Istanbul, Turkey

²The Art and Design Faculty Department of Music and Performing Arts, YTÜ, Istanbul, Turkey

³Institute of Experimental Medicine, Department of Neuroscience, Istanbul University, Istanbul, Turkey

⁴School of Physical Education and Sports, Istanbul University, Istanbul, Turkey

⁵Istanbul Faculty of Medicine, Department of Sports Medicine, Istanbul University, Istanbul, Turkey

OBJECTIVE: The main aim of this study was to investigate auditory cognitive responses of professional dancers and sportsmen.

METHODS: Ten healthy and right-handed professional dancers and 10 sportsmen participated in this study (5 female, 5 male). Electroencephalographic (EEG) activity was recorded from 19 sites with MITSAR. Since, the P300 component of the ERPs (Event Related Potentials) can be used to identify cognitive functions, Auditory Oddball Paradigm (AERPs; P300 Paradigm) applied to dancers and sportsmen. All statistical analyses were performed using SPSS-PC 16.0.

RESULTS: In AERPs paradigm, P300 responses of professional dancers had larger amplitude than of sportsmen especially for the Fz, F4 and C4 locations. The P300 responses of the sportsmen had larger amplitude than of dancers for the F3, C3, Cz, P3, Pz, P4, O1 and O2 locations. The Channel effect was significantly different between dancers and sportsmen [$F(18,198)=33,32$; $p<0.0001$]. There was no significant difference for the latency of P300 responses between the groups.

CONCLUSIONS: The cognitive responses of the dancers were higher in sportsmen for the anterior-right locations. On the other hand, the cognitive responses of sportsmen were higher in dancers for the anterior-left and posterior locations of brain. The present study demonstrates the electrophysiological differences of the auditory cognitive functions between dancers and sportsmen.

Keywords: Auditory Evoked Potentials, Dancers, Sportsmen, EEG

Eeg Power Spectra In Mental, Cold And Combined Stresses

Numan Ermutlu¹, Gökçer Eskikurt², Silvana Markovska Simoska³, Nada Pop Jordanova⁴, Ümmühan Isoglu Alkac⁵, Sacit Karamürsel⁵

¹Medical Faculty Department of Physiology, Istanbul Bilim University, Istanbul, Turkey

²Institute of Experimental Medicine, Department of Neuroscience, Istanbul University, Istanbul, Turkey

³Research Center for Energy, Informatics and Materials, Division of Neuroinformatics, Macedonian Academy of Sciences and Arts, Macedonia

⁴Faculty of Medicine, Department of Pediatrics, University of Skopje, Macedonia

⁵Istanbul Faculty of Medicine Department of Physiology, Istanbul University, Istanbul, Turkey

OBJECTIVE: Examination of EEG power bands during resting, cold stress (CS), mental stress (MS) and MA+CS conditions.

METHODS: Eleven channel EEG was recorded from 7 volunteered university students during resting, cold stress (CS) mental stress (MS) and MS+CS conditions. Blood pressures and heart rates of the participants were also recorded during aforementioned conditions. An arithmetical calculation task (Mental calculation) was used as Mental Stress. Cold stress was induced by immersing hands alternately into cold water (4°C). Paired t-tests and power spectra were analysed with repeated measures ANOVA with condition (4), channel (11) within subject factors. Reduced degrees of freedom (Greenhouse-Geisser) were used to counter violations of sphericity assumption where necessary.

RESULTS: There were significant differences in each power band for the Condition factor. delta: $F(1,7)=79,93$, $p<0.001$; theta: $F(1,7)=77,52$, $p<0.001$; alfa: $F(1,7)=17,75$; $p<0.001$; beta: $F(1,7)=118,37$, $p<0.001$. In pairwise comparisons LSD was used. Delta and theta powers were most apparently increased in the MS+CS ($p<0.001$). Alpha power was significantly decreased in the MS condition. Beta power was significantly increased in the MS and MS+CS. Channel and Channel X Condition interaction was not significant.

CONCLUSIONS: Our preliminary results suggest that during MS, there is a cortical activation and increased vigilance reflected by alpha decrease and beta increase. Whereas in double stress (MS+CS), presence of concomitant increases of delta, theta and beta bands, might suggest decrease of vigilance in the background of increased arousal.

Keywords: Mental arithmetic, cold stress, EEG power, vigilance

The Effect Of Low-Level Laser Therapy In Patients With Nerve Impairment Following The Treatment Of Bisphosphonate - A Case Report

Meltem Koray¹, Esra Somturk², Ilknur Özcan², Tanyeri Hakkı¹

¹Istanbul University, Faculty of Dentistry, Department of Oral Surgery and Oral Medicine

²Istanbul University, Faculty of Dentistry, Department of Oral and Maxillofacial Radiology

OBJECTIVE: Osteonecrosis of the jaw (ONJ) is a major complication associated with long-term use of bisphosphonates. Clinical signs and symptoms commonly reported include pain, swelling, the presence of pus, loose teeth, ill-fitting dentures, and paresthesias of the inferior alveolar nerve when affects the mandible. Low-level laser therapy (LLLT) with diode laser has been reported for biostimulation in the treatment of paresthesia. This paper reports the effects of LLLT in patients with nerve impairment following after therapy of bisphosphonates.

METHODS: We presented LLLT with diode laser to a 65-year-old woman with ONJ, who was treated with zoledronic acid for multiple bone metastasis after she had been diagnosed as multiple myeloma 7 years ago. Her complaints were paresthesia in her right lower lip. According to the intraoral and radiographic examinations and symptoms of the patient, ONJ was described as Stage 0 which includes patients exposed to bisphosphonate and who is present with non-specific symptoms or without clinical and radiographic abnormalities.

RESULTS: In addition to antibiotherapy LLLT with diode laser was applied two times a week for six months. Paresthesia was completely healed. She had no signs or symptoms of ONJ since then.

CONCLUSIONS: LLLT seemed to be effective in reducing nerve impairment following the treatment of bisphosphonates.

Keywords: Low-level laser therapy, biostimulation, paresthesia

Neuromuscular Investigations In Multiple Sclerosis

Catalin Bogdan¹, Neamtu Marius Cristian¹, Rusu Ligia², Avramescu Taina², Daniel Georgescu¹, Tudor Adrian Balseanu¹, Neamtu Oana Elena², Denisa Enescu Bieru², Romanescu Florin¹, Maria Iancau¹

¹University of Medicine and Pharmacy Craiova

²Universitatea din Craiova

OBJECTIVE: Multiple sclerosis (MS) is a chronic disease of the central nervous system, with a multifactorial etiology, characterized by inflammatory and degeneration processes with a multiphasic and multifocal evolution. The objective of our study was to identify predictable parameters when monitoring gait disorders that will appear in the evolution of multiple sclerosis (MS) patients, by using visual evoked potentials (VEP) and tensiomyography (TMG).

METHODS: We assessed 86 parameters, 36 parameters in VEP evaluation, 50 in TMG, both on a group of patients suffering from certain Ms (20 patients) and a control group. The MS lot was grouped in two subgroups: subgroup A, with clinically detectable gait disorders and subgroup B, without clinically detectable gait disorders. All groups were tested by neurophysiologic evaluation methods such as VEP, using a pattern reversal full field stimulation and TMG on quadriceps components, femoral biceps, gastrocnemian muscles and tibialis anterior.

RESULTS: We recorded high values of wave N75 latencies in subgroup A. The delays of the P100 wave were presented in both subgroups. In our study, the contraction time as TMG parameter – recorded higher values in posterior muscular group of the thigh and anterior muscular group of the shank. After analyzing muscular displacement and relaxation time, we observed a higher muscle tonus in all muscular groups we tested, especially in gastrocnemian. The ratio direct/converse correlation is high in VEP/TMG correlations.

CONCLUSIONS: We have used tensiomyography to explore MS patients, simultaneously completing an analysis by correlating the results of the investigations, to both create a new diagnosis algorithm and predict the evolution of balance and gait disorders at these patients.

Keywords: Multiple sclerosis, Tensiomyography, Visual evoked potentials

Neuroprotective Efficacy Of The Peroxisome Proliferator Activated Receptor Gamma Ligand In Chronic Cerebral Hypoperfusion

Hale Sayan Ozaçmak¹, Veysel Haktan Özaçmak¹, Figen Barut², Ewa Jakubowska Dogru³

¹Department of Physiology

²Pathology, Zonguldak Karaelmas University, Zonguldak Turkey

³Department of Biology, Middle East Technical University, Ankara Turkey

OBJECTIVE: Chronic cerebral hypoperfusion can cause learning and memory impairment and neuronal damage resembling the effects observed in vascular dementia. The present study was designed to evaluate possible neuroprotective effects of rosiglitazone, a PPAR-gamma agonist, in rat model of chronic cerebral hypoperfusion.

METHODS: Cerebral hypoperfusion was induced by permanent bilateral occlusion of the common carotid arteries. Oral administration of rosiglitazone (1.5, 3, and 6 mg/kg/day) or vehicle was carried out for 5 weeks, starting one week before the surgery. Cognitive performance was assessed using the Morris water maze. The density of the OX-42-labeled microglial activation and hippocampal neuronal death were estimated. Synaptogenesis was also evaluated by the measurement of synaptophysin, the pre-synaptic vesicular protein, and level via western blotting technique.

RESULTS: Cerebral hypoperfusion for 30 days induced a significant cognitive impairment along with hyperactivation of microglial and astroglial cells, hippocampal neuronal loss, and reduction of synaptophysin level. The escape latencies for both 3 and 6 mg/kg of rosiglitazone-treated groups were significantly shorter than in the ischemia control group ($P<0.05$). For the group treated with 3 mg/kg of rosiglitazone, the number of OX-42 positive cells significantly decreased, as compared with two other treatment groups ($P<0.05$). Compared to the sham-operated group, the amounts of synaptophysin protein in the ischemia, 1.5 mg/kg, and 3 mg/kg rosiglitazone-treated groups, were statistically lower ($P<0.05$), whereas in the group treated with 6 mg/kg of rosiglitazone, no significant difference was noted.

CONCLUSIONS: Our results suggest that the chronic administration of rosiglitazone significantly prevents chronic cerebral hypoperfusion-induced brain damage, at least, partly through suppressing glial activation and preserving synaptic plasticity.

Keywords: Chronic cerebral hypoperfusion, rosiglitazone, OX-42, synaptophysin

Bright Light Therapy Improves The Sleep And Circadian Rhythms In Institutionalized Elderly

Cristina Nicolau¹, Marta Mouzo¹, Maria Del Mar Fiol¹, Antonio Martínez Nicolás², Juan Antonio Sarabia³, María Sanchez Campillo³

¹Iunics, Universitat de les Illes Balears, Spain

²Depto de Fisiologia, Universidad de Murcia, Spain

³Cronobiotech, R&D Dept. CEEIM, Spain

OBJECTIVE: Aged individuals show disturbances in sleep and general activity. These changes have been attributed to disruptions in the circadian clock caused by dampening of the light exposure in institutionalized patients. This report aims at recording the activity and body temperature to assess sleep disturbances in aged patients with mild to severe dementia after exposure to bright light.

METHODS: Activity, body position, light exposure and wrist temperature has been continuously monitorized during three weeks in 11 elderly (mean age 75) institutionalized subjects with Alzheimer dementia, mild cognitive deficits and sleep disturbances using Hobo sensors for light exposure, activity and body position. Ibuttons (Maxim ®) were also used to record wrist temperature (Cronobiotech, R&D). The first week was used as baseline. During the second week the subjects were submitted to bright light treatment (10000 lux, 90 min daily) and the third week to observe the eventual carry over effects.

RESULTS: As a general result, sleep and circadian rhythms of the subjects were improved after exposure to bright light, increasing the amplitude of the temperature and activity daily rhythms and causing a 90 min phase delay in activity.

CONCLUSIONS: The bright light therapy caused significant improvements in indicators of sleep and activity circadian rhythms of institutionalized elderly subjects.

Keywords: elderly, bright light, sleep, circadian rhythms

An Examination Of The Reliability Of Gundogan Method Used In Determining The Dominant Eye With Tno Test Results

Belkıs Koçtekin¹, Nimet Ünay Gündoğan², Ayşe Gül Koçak Altıntaş³

¹Department of Physiology, Antalya Education and Research Hospital, Antalya, Turkey

²Department of Physiology, Baskent University, Ankara, Turkey

³Ulucanlar Education and Research Hospital Clinical of Ophtalmology, Ankara, Turkey

OBJECTIVE: The aim of this study is to analyse the reliability of Gundogan Method, which is used in determining the dominant eye (DE), by comparing TNO Test results, regarded as a standard in determining binocularity.

METHODS: Fifty-two students from higher school have attended in this study. These students aged between 19 and 29 have perfect visuality and they have no congenital color vision defect. Gundogan Method has been used in determining their DE. Subsequently, TNO test has been applied to all the students. Data set has been examined using SPSS programme.

RESULTS: Gundogan Method has established that right eyes of 7 female students (36.8%), left eyes of 12 female students (63.2%), right eyes of 15 male students (45.45%), left eyes of 18 male students (54.55%) are DE. When TNO test results have been examined, it is stated that 2 of 52 students have no binocularity and that these students only use their left eyes to see. It has been seen by using Gundogan Method that DEs of the same students are their left eyes.

CONCLUSIONS: In addition to being the most reliable measure which shows the functional asymmetry of brain, DE is the eye that is used to see, and it has been stated that it is dominant in various visual functions. It is crucial to determine the right eye as the DE. DE which has been determined by Gundogan Method and DE which has been found in the students using one eye to see by TNO test are the same. It is hoped that comprehensive research will throw light on the issue in the future.

Keywords: Dominant eye, Gundogan Method, TNO Test

Associations Among Ratio Of Digit Lengths (2d:4d), Hand Preferences, Nonverbal Intelligence, Visual, Auditory And Verbal Ability, Motor Skill And Cerebral Lateralization In Healthy Persons

Nuray Oztasan¹, Necip Kutlu², Zuhale Can¹

¹Department of Physiology, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey

²Department of Physiology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey

OBJECTIVE: The associations among the parameters of hand, foot and eye preferences, ratio of digit lengths (2D:4D), nonverbal intelligence (IQ), hand motor skill in man and women were aimed to investigate.

METHODS: Hand preference using 'Edinburg Hand Preference questionnaire', IQ point using 'Cattell's Culture Free Intelligence Test' and motor skill using 'Nail Movement Test'. For parameter of eye preference, the eye used for key hole were questioned, for foot preference the foot used for kicking the ball, as well as the presence of someone in the family who kicks the ball by left foot. Respective finger length from anatomic boundaries was measured using digital compass caliper and 2D:4D were calculated (at 439 volunteer participants).

RESULTS: A meaningful correlation was determined between right and left hand digit ratios of participants without any gender preference. The value for motor skill was higher in women in comparison to men. In women, a positive correlation was found between right hand ratio and IQ point. On the other hand, in men, a positive correlation was found between left hand ratio and IQ point. A positive correlation between lateralization coefficient and motor skill values was also been shown in men.

CONCLUSIONS: Hand motor skill was higher in women than the one in men. Digit ratios were found higher in the ones having higher IQ points. This was particularly valid for different digit ratios depending on the gender. Altogether, in light of this study, it was concluded that the motor skills were higher in men who have more lateralization.

Keywords: Digit length ratio, nonverbal intelligence, motor skill, hand, foot and eye preferences.

The role of melatonin MT1 receptor on the neuroprotective effects of estrogen after traumatic brain injury in rats

Nader Shahrokhi Sardoo¹, Mohammad Khaksari², Nezhat Adeli¹, Shahla Nourizad¹, Mohammad Shabani²

¹Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, Iran

²Physiology Research Center Kerman University of Medical Sciences, Kerman, Iran

OBJECTIVE: The possible role of melatonin on the estrogen neuroprotection effects of following traumatic brain injury (TBI) is not known. The objective of this study was to assess the possible role of the MT1 melatonin receptor on the effects of estrogen following TBI.

METHODS: Diffuse TBI was induced by Marmarou method in ovariectomized (OVX) female rats, which were divided into 4 groups with each including three subgroups; OVX+TBI; ovx+ E2; OVX+Veh+E2; and OVX + Luzindole + E2. Drugs injections were given 30 minutes after TBI. Drugs and vehicles were injected in a volume of 0.33 ml/rat by intraperitoneal (ip) route. Twenty-four hours after TBI, brain water content was measured, while brain Evans blue content was determined 5 h after TBI. intracranial pressure (ICP) was measured in spinal cord and cerebral perfusion pressure (CPP) calculated by subtracting the mean arterial pressure (AMP) from ICP in time interval one hour before trauma induction and at 0, 1, 4, 24 hours minutes after TBI.

RESULTS: The results showed that after TBI, brain water content in OVX + Luzindole + E2; significantly increased compared OVX+Veh+E2 group ($p > 0.01$). Brain Evans blue content in OVX + Luzindole + E2; group significantly increased compared to other groups ($p > 0.001$). CPP 1 hours after in OVX + Luzindole + E2 group significantly increased compare to OVX+Veh+E2 ($p < 0.01$).

CONCLUSIONS: We conclude that melatonin MT1 receptor inhibition prevented from estrogen's protective effects on brain water content and blood-brain barrier; and CPP after TBI.

Keywords: Estrogen, melatonin MT1 receptor, ICP, CPP, Brain edema, TBI

The Effect of Fenugreek (*Trigonella foenum graecum*) Seeds on Thyroid Gland Function in Male Wistar Rats

Ahmed Kamal Muhammed

Department of Biology, Faculty of Science, Ankara University, Ankara, Turkey

OBJECTIVE: Fenugreek seeds (*Trigonella foenum-graecum* L.) are assumed to have restorative and nutritive properties. The present study was undertaken to investigate the effect of aqueous extract of fenugreek seeds on thyroid gland function.

METHODS: Male Wistar rats (n= 40) were divided into four groups of ten rats each as follows; Group I: control, Group II: 0.4, Group III: 0.8, and Group IV: 1.6 g/kg body weight of fenugreek seeds extract. Extract was administered daily and orally for 15 days.

RESULTS: There was a gain in body weight and thyroid gland weight in the treated groups as compared with the control. Furthermore results of treated groups showed a significant decrease ($P < 0.05$) in serum total and free triiodothyronine (T3) and tetraiodothyronine (thyroxin, T4) levels. However serum thyroid stimulating hormone (TSH) levels were increased. On the other hand, there was significant decrease of total T3 and T4 in thyroid gland tissue. In the liver tissue, the level of T3 decreased while T4 increased which may indicate an inhibitory action on the conversion of T4 to T3 as compared with the control group.

CONCLUSIONS: Treatment with fenugreek seeds extract caused significant decreases in serum total and free thyroid hormones T3 and T4, which were also decreased in the thyroid tissue, except for T4 that was increased in the liver tissue. The effect of extract was dose dependent.

Keywords: *Trigonella foenum-graecum*, Rat, Triiodothyronine, thyroxin, TSH

The Growth Hormone Receptor Polymorphism in Patients with Acromegaly: relationship to BMI and glucose metabolism

Sebahat Turgut¹, Fulya Akin², Ceylan Ayada¹, Şenay Topsakal², Emrah Yerlikaya², Günfer Turgut¹

¹Department of Physiology, Faculty of Medicine, University of Pamukkale, Denizli, Turkey

²Division of Endocrinology, Department of Internal Medicine, Faculty of Medicine, University of Pamukkale, Denizli, Turkey

OBJECTIVE: The aim of this study was to investigate the association between the frequencies of Growth Hormone receptor (d3GHR) gene polymorphisms and some clinical parameters of acromegalic patients.

METHODS: A total of 35 acromegalic patients were enrolled to the study. The d3GHR polymorphism was identified by using polymerase chain reaction from peripheral blood samples. The levels of systolic and diastolic blood pressure, BMI, fasting plasma glucose (FPG), Fasting insulin, HOMA-IR, IGF-I, GH, IGFBP3, triglyceride, HDL and LDL cholesterol concentrations were evaluated.

RESULTS: The frequencies of d3GHR genotypes were found as follows; 5 (14.3%) subjects had d3/d3, 11 (31.4%) had d3/fl and 19 (54.3%) had fl/fl in patients. The prevalence of the d3 and fl alleles was %30 and %70, respectively. Systolic blood pressure, fasting insulin and HOMA-IR was found significantly increased in homozygote d3GHR genotype group compared to d3/fl subjects ($p < 0.05$). In addition, BMI was observed significantly different among three genotypes ($p = 0.007$) and in the subjects with d3/d3 genotype, BMI was found significantly higher than d3/fl and fl/fl genotypes groups. As well as, no significant difference was found between the d3 and fl alleles group in terms of the clinical parameters except for BMI ($p = 0.002$).

CONCLUSIONS: It can be said that the d3GHR gene polymorphism may affect BMI, systolic blood pressure and insulin regulation. At the same time, we can say homozygote d3GHR genotype and d3 allele carriers may have more risk than other genotypes for high BMI.

Keywords: Acromegaly, d3GHR polymorphism, IGF-I, BMI, insulin

Metabolism of thyroid hormones in murine white adipose tissue: Potential involvement of leptin

Stanislav Pavelka¹, Zuzana Macek Jílková², Jan Kopecky²

¹Department of Radiometry, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and Central-European Technology Institute, Masaryk University, Brno

²Department of Adipose Tissue Biology, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

OBJECTIVE: Our aims were to measure possible changes in activities of thyroid hormones (TH)-metabolizing enzymes in white adipose tissue (WAT) and to describe the role of metabolic conversions of TH in WAT during obesogenic treatment, caloric restriction and in response to leptin in mice.

METHODS: Male C57BL/6J mice were subjected to three above mentioned treatments. Subcutaneous and epididymal WAT and interscapular brown fat (BAT) depots were dissected and used for morphometric and enzymatic analyses. Plasma levels of leptin, as well as total and free thyroxine (T4) and triiodothyronine (T3) concentrations were determined using RIA kits. Enzyme activities of iodothyronine deiodinases of the types 1 (D1) and 2 (D2) in WAT and BAT and in the liver were measured with the aid of our newly developed radiometric enzyme assays.

RESULTS: D1 activity in WAT was stimulated by a high-fat-diet feeding, which also increased plasma levels of leptin. However, D1 or D2 activities in BAT did not change. Caloric restriction decreased D1 activity in WAT, but not in the liver, and reduced leptin levels. In return, leptin injections increased D1 activity in WAT. In summary, our results demonstrate changes in D1 activity in WAT under the conditions of changing adiposity, and a stimulatory effect of leptin on D1 activity in white adipose tissue.

CONCLUSIONS: Attained results suggest a functional role for D1 in WAT, with D1 possibly being involved in the control of adipose tissue metabolism. Supported by the Academy of Sciences of the Czech Republic (Project No. AV0Z50110509), Ministry of Education of CR (Project No. MSM0021622413) and Czech Science Foundation GA CR (Grant No. 304/08/0256).

Keywords: Adipose tissue, Leptin, Metabolism, Obesity, Thyroid hormone

Effect of Growth Hormone Deficiency on Attention in adults patient

Setenay Cuğ¹, Nazan Dolu¹, Fahri Bayram², Yasin Şimşek²

¹Erciyes University Faculty of Medicine, Physiology EU, Kayseri

²Erciyes University Faculty of Medicine, Endocrinology EU, Kayseri

OBJECTIVE: Children diagnosed with growth hormone deficiency (GHD) display a normal intelligence quotient (IQ) distribution, but they may display behavioral problems consistent with attention-deficit disorder. In this study, we investigated effects of GH deficiency on attention with electrodermal activity (EDA) and continuous attention test in adult patients. Electrodermal activity (EDA) is thought to reflect sympathetic skin responses and attention to new information in the environment. We also examined autonomic functions.

METHODS: We studied 8 healthy subjects and 8 patients with GHD. EDA (Skin Conductance Level, SCL), heart rate and respiratory frequency were recorded with MP30 system. While tonic records of EDA (no stimuli) were taken during 120 seconds, phasic records of EDA were recorded together with 15 auditory stimuli (1000 Hz, 90 dB, 1 sec duration, to attract their attention). The Spielberger Anxiety Scale was also performed.

RESULTS: In patients, the left-hand tonic SCL ($p < 0.01$) was significantly lower than the control group. Phasic SCL was maximum response among first 3 auditory stimuli. The left-hand phasic SCL was also significantly lower than the control group ($p < 0.04$) There were no significant differences for The Spielberger Anxiety Scales, continuous attention test and autonomic functions between groups.

CONCLUSIONS: The patients with GHD showed diminished EDA parameter and so diminished attention level. Activation of alpha 2-adrenoceptors in the hypothalamus stimulates GH release, probably through stimulation of GHRH. Activation of beta-receptors inhibits GH release through stimulation of hypothalamic SS function. It is thought that diminished EDA responses and attention level in patients with GHD may result from differences in catecholaminergic system via beta-receptors.

Keywords: Growth hormone deficiency, Electrodermal activity, Cognitive function, Attention

Association of leptin gene -2548 G/A polymorphism with plasma levels in Turkish patients with obesity

Deniz Say Şahin¹, Cemil Tümer¹, Cemil Demir², Edip Uçar³, Muhammet Murat Çelik³, Ramazan Güneşaçar⁴

¹Mustafa Kemal University Faculty of Medicine Department of Physiology

²Mustafa Kemal University Faculty of Medicine Department of Central Laboratory

³Mustafa Kemal University Faculty of Medicine Department of Internal Medicine

⁴Mustafa Kemal University Faculty of Medicine Department of Medical Biology and Genetics

OBJECTIVE: Leptin is a product of the obesity gene which is secreted by mostly adipose tissue, operates by inhibiting food intake and stimulating energy expenditure. The gene encoding leptin (LEP) is located in human beings on long arm of chromosome 7 at band q31.3 and is organized into three exons that are separated by two introns. Leptin may play an important role in the pathogenesis of obesity. In the present study, we aimed to investigate the association of LEP -2548 G/A polymorphism with plasma leptin levels in Turkish obese patients.

METHODS: Sixty obese patients and 60 healthy controls were included in this study. Plasma leptin levels were determined using ELISA technique. Leptin gene promoter -2548G/A genotype was determined by polymerase chain reaction followed by a digestion with the Hha I restriction enzyme.

RESULTS: Plasma leptin levels were significantly higher in obese patients ($p < 0.0001$). In the obese patients, AA and GA genotype frequencies were higher than in healthy controls ($p < 0.016$ for AA genotype; $p < 0.011$ for GA genotype). A allele frequency was also found to be higher in obese than those of healthy subjects ($p = 0.0001$). In the entire study sample, LEP -2548 A allele carriers (AA+GA) had significantly higher levels of plasma leptin than subjects with GG genotype ($p < 0.0001$). Obese patients carrying the A allele exhibited higher leptin level than homozygous for G allele ($p < 0.0001$).

CONCLUSIONS: Present results showed that LEP -2548 GA genotype or A allele is associated with higher plasma leptin levels and this variant may contribute to the development of obesity in Turkish population.

Keywords: Obesity, Leptin, leptin gene polymorphism, PCR-RFLP

Effect of vildagliptin administration on islet cells viability and insulin secretion

Gulbahar Boyuk¹, Sule Coskun Cevher², Zeynep Ginis¹, Ahmet Yesilyurt¹, Gulfer Tabur¹, Ersin Fadillioglu³, Tuncay Delibasi¹

¹Ankara Diskapi Training and Research Hospital, Translational Medicine Center, Ankara, Turkey

²Department of Biology, Faculty of Sciences and Art, Gazi University, Ankara, Turkey

³Department of Physiology, School of Medicine, Hacettepe University, Ankara, Turkey

OBJECTIVE: Islet cells transplantation is one of the cure options on treatment of diabetes mellitus type 1. However, viability of isolated islet is important for successful transplantation. Vildagliptin increases insulin secretion and, inhibits glucagon secretion via increasing the level of GLP-1. In this study, we investigated the effect of vildagliptin on islet cells viability and insulin secretion capacity.

METHODS: Islet cells were isolated after rats were given vildagliptin orally 20 and 60 mg/kg/day for every 12 hours and 60 and 100 mg/kg/day for every 24 hours during 4 days. After isolation, islets were kept in medium containing 0 μ l H₂O₂ or 300 μ l H₂O₂ at +4 ° C for 15 minutes. Islet cells viability was examined with fluorescein diacetate and propidium iodide mixture by using a fluorescence microscope. The rest of cells were stored for glucose stimulation test.

RESULTS: The viability of islet cells in control group were 90.9 % and 81.1% in medium with 0 and 300 μ l H₂O₂, respectively. The viability of islet cells with 0 and 300 μ l H₂O₂ were 86.3 % and 77.3 % respectively, for two times 20 mg vildagliptin treated group, 85.2 % and 81.7 % respectively, for two times 60 mg treated group, 85.9 % and 80.2 % respectively, for 60 mg treated group and 92.6 % and 88.5 % respectively, for 100 mg group. The islet cells of control and 100 mg vildagliptin groups secreted insulin successfully within high glucose containing medium (P<0.05).

CONCLUSIONS: Even a short term vildagliptin administration to the donor before islet isolation process might improve islet cells viability and insulin secretion capacity.

Keywords: pancreatic islet cell isolation, vildagliptin

Insulin Regulates Plasma Ghrelin Concentrations in Streptozotocin-Induced Diabetic Rats

Basra Deniz Obay¹, Hakkı Murat Bilgin¹, Ezel Tasdemir², Abdurrahman Sermet¹, Cemil Tümer³, Yüksel Koçyiğit¹

¹Department of Physiology, Dicle University Faculty of Medicine, Diyarbakır, Turkey

²Developmental Medical Center, Diyarbakır, Turkey

³Department of Physiology, Mustafa Kemal University Faculty of Medicine, Hatay, Turkey

OBJECTIVE: Ghrelin, an orexigenic peptide produced in the stomach is increased in streptozotocin (STZ)-induced diabetic rats. In rodents, intracerebroventricular or peripheral administration of ghrelin stimulated food intake and body weight gain. This study clarifies the regulation of ghrelin by insulin in STZ- induced diabetic rats.

METHODS: Adult male Wistar rats were divided into control and three experimental groups as each group had 7 rats (n=28). To investigate the role of ghrelin in the hyperphagic response to uncontrolled diabetes, rats in each group were studied for 7 days. Rats were injected once daily for 7 days with either PBS or insulin subcutaneous injection (5-7 U). Body weight and food intake were measured before and after the experimental procedure. Plasma insulin, ghrelin and glucose concentrations were also measured.

RESULTS: Streptozotocin (STZ)-induced diabetic rats were markedly hyperphagic. This hyperphagia was accompanied by hyperglycemia and hypoinsulinemia. Treatment of diabetic rats with insulin reversed these changes. STZ- diabetic rats had higher plasma ghrelin concentrations than control rats. Changes in ghrelin levels were attenuated by the subcutaneous injection of insulin (5-7 U over 7 days). Insulin treatment also partially reversed hyperphagia observed in STZ- induced diabetic rats and there was a decrease in plasma ghrelin concentrations compared with STZ-INS pair fed rat.

CONCLUSIONS: The results indicate that insulin treatment reverses elevated plasma ghrelin concentrations in STZ- induced diabetic rats. So this shows the pathophysiological significance of ghrelin in diabetes.

Keywords: Ghrelin, streptozotocin, diabetic, rat, insulin

The Role of Growth Hormone In Regulation of Ghrelin Plasma Concentration and its Secretion

Hakkı Murat Bilgin¹, Basra Deniz Obay¹, Ezel Tasdemir², Abdurrahman Sermet¹, Cemil Tümer³, Yüksel Koçyiğit¹

¹Department of Physiology, Dicle University Faculty of Medicine, Diyarbakır, Turkey

²Developmental Medical Center, Diyarbakır, Turkey

³Department of Physiology, Mustafa Kemal University Faculty of Medicine, Hatay, Turkey

OBJECTIVE: Ghrelin is a novel growth hormone (GH) releaser acylated peptide that has recently been purified from stomach, and which potently binds to the GH secretagogue receptor. In rats, fasting leads to elevated serum GH concentrations. Age-related decreases in energy expenditure have been associated with the loss of skeletal muscle and decline of food intake, possibly through a mechanism involving changes of growth hormone (GH) secretion and feeding behavior. Ghrelin releases GH in vitro and in vivo in animal models, however its actions, potency and specificity in humans are unknown.

METHODS: Here, we investigate the relationship between age-related decline of growth hormone secretion and/or food intake and ghrelin function. Ghrelin (10 nmol/kg body weight) was administered intravenously to male 3-, 12-, 24- and 27-month-old Wistar adult rats, after which growth hormone concentrations and 2 h food intake were measured.

RESULTS: An intravenous administration of ghrelin to rats increased food intake in all generations. In addition, to orexigenic effect by ghrelin, intravenous administration of ghrelin elicited a marked increase in plasma GH levels, with the peak occurring 15 min after administration.

CONCLUSIONS: These changes in serum ghrelin concentrations during fasting were followed by similar, profound changes in serum GH levels. These data indicate that ghrelin is the main driving force behind the enhanced GH secretion during fasting.

Keywords: Ghrelin, growth hormone, food intake

Somatotropin, somatostatin and prolactin effects on fibrinopeptide A, prothrombin fragment 1+2, fibronectin and heparin cofactor II in rats

Yuri Penkov Nyagolov¹, Nadezhda Ivanova Doncheva², Negrin Nestorov Negrev³

¹Department of Physiology, Medical University, Sofia, Bulgaria

²Department of Clinical Laboratory, Medical Institute, Ministry of Interior, Sofia, Bulgaria

³Department of Physiology, Medical University, Varna, Bulgaria

OBJECTIVE: Multiple clinical surveys on somatotropin and prolactin hormonal disorders provide evidence of modulated hemostasis. However, the precise role of those hormones in hemocoagulation is not yet clearly defined. The present study was designed to estimate the effects of somatotropin, somatostatin and prolactin on two indicators of activated blood coagulation: fibrinopeptide A, prothrombin fragment 1+2; and two hemostasis inhibitors: fibronectin and heparin cofactor II in rats.

METHODS: Male Wistar rats (n=13) were treated in three consecutive days s.c. with somatotropin (0.2 mg/kg body mass); somatostatin (0.1 mg/kg); somatostatin applied 3 h before somatotropin in the same doses and scheme; prolactin (2 x 0.1 mg/kg) applied i.p. Fibrinopeptide A, prothrombin fragment 1+2, and fibronectin were determined by ELISA methods; heparin cofactor II – by a chromogenic kinetic method.

RESULTS: Somatotropin application reduced significantly ($p < 0.001$) fibrinopeptide A, and prothrombin fragment 1+2 and elevated ($p < 0.001$) fibronectin and heparin cofactor II. Somatostatin applied 3 h before somatotropin and prolactin alone increased significantly fibrinopeptide A, and prothrombin fragment 1+2 in plasma and decreased ($p < 0.001$) fibronectin and heparin cofactor II.

CONCLUSIONS: The results indicate the hormones examined in this study are involved in regulation of blood coagulation in the rat as: 1.) somatotropin suppresses hemocoagulation by decreasing thrombin and fibrinogen formation and activates anticoagulation via fibronectin and heparin cofactor II; 2.) somatostatin and prolactin increase the two markers of activated hemocoagulation fibrinopeptide A, and prothrombin fragment 1+2 and decrease the two anticoagulation factors; 3.) somatostatin applied in combination with somatotropin abolishes the effect of the latter and exerts its own effect which may be an evidence of endogenous somatotropin suppression.

Keywords: somatotropin, somatostatin, prolactin, prothrombin fragment 1+2, fibrinopeptide A, fibronectin, heparin cofactor II

Screening study of melatonin effects on basic integral parameters of blood coagulation (PT, APTT, TT, ECLT)

Irina Ilieva Pashalieva¹, Lyubka Yurieva Decheva¹, Emiliya Georgieva Stancheva¹, Yuri Penkov Nyagolov², Negrin Nestorov Negrev¹

¹Department of Physiology, Medical University, Varna, Bulgaria

²Department of Physiology, Medical University, Sofia, Bulgaria

OBJECTIVE: Though melatonin is a hormone with multiple biological effects, its particular role in hemostasis is not yet thoroughly elucidated. The present study has been designed to provide screening data of the effects of melatonin on the basic integral parameters of hemostasis: prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT) and euglobulin clot lysis time (ECLT) in rats.

METHODS: The studies were performed on 26 male Wistar rats exposed to 12/12 h natural light/dark cycle. Thirteen rats were treated with Melatonin in a daily dose 0.2 mg/kg body mass applied twice daily s.c. for three days. The rest of the animals (control group) were treated with the vehicle by the same scheme and volume. The parameters tested were determined using standard laboratory kinetic tests on a Stago analyzer.

RESULTS: PT, APTT, and TT were significantly shortened ($p < 0.001$), while ECLT was elongated ($p < 0.001$) by melatonin application.

CONCLUSIONS: The results indicate that melatonin inflicts substantial shifts in hemostasis by activating coagulation via both intrinsic and extrinsic pathways, and additionally enhances transformation of fibrinogen to fibrin. The elongated ECLT may be interpreted as an effect of the hormone on plasma plasminogen activators. The data suggest melatonin is involved in hemostasis regulation by generating a tendency of hypercoagulability and reduced plasma fibrinolytic activity.

Keywords: melatonin, hemocoagulation, prothrombin time, aPTT, thrombin time, euglobulin lysis time

Serum Melatonin and Leptin Profiles of Japanese Quail

Gözde Gülşin¹, Nursel Hasanoğlu¹, Baver Coşkun², Türker Savaş², Bülent Gündüz¹

¹Department of Biology, Faculty of Arts and Sciences, Canakkale Onsekiz Mart University, Canakkale, 17100

²Department of Animal Sciences, Faculty of Agriculture, Canakkale Onsekiz Mart University, Canakkale, 17100

OBJECTIVE: Leptin, a hormone produced mainly by adipose tissue, regulates food intake and energy expenditure. It functions also as a metabolic and neuroendocrine hormone. In mammals, leptin shows a diurnal rhythm. Circadian rhythm governs cycles in rest-activity, food intake and metabolism. Such rhythms are mediated and imposed on the organism through the circadian system consisting of the hypothalamus and pineal gland. The main signal from this complex is hormone melatonin. The present study examined daily fluctuations in serum levels of melatonin and leptin.

METHODS: We investigated a daily pattern of melatonin and leptin levels in serum of Japanese quail kept in light:dark cycle 16:8 h. Blood samples were taken from the wing jugular vein at 12:00, 16:00, 20:00, 00:00, 04:00 or 08:00 h throughout a 24-h period. Blood in dark period was taken under dim red light. Serum aliquots were aspirated and frozen at -20 °C. Hormones were measured by commercial ELISA kits.

RESULTS: Significant daily patterns in concentration of melatonin and leptin were recorded. As in other species melatonin levels were low during the day (below 60 pg/ml) and high at night (280 pg/ml). Leptin showed a clear daily rhythm as being low during the day (below 7 ng/ml) and high at night (28 ng/ml)

CONCLUSIONS: The results show that in addition to melatonin, there is also a rhythmic change in leptin hormone, but melatonin/photoperiod may not contribute directly to the difference of leptin levels. Leptin might be controlled by different circadian oscillators or of the same set of circadian oscillators, but they are not closely coupled at least, in Japanese quail.

Keywords: Melatonin, Leptin, Photoperiod, Japanese quail

Lymphocyte fluidity and membran protein content in dogs with diabetes mellitus

Ayşe Şule Tamer¹, Alev Akdogan², Isil Albeniz³, Kivanc Cefle⁴, Cahide Gokkusu⁵

¹Department of Physiology, Medical Faculty of Istanbul, Istanbul University, Istanbul, Turkey

²Department of Internal Diseases, Veterinary Faculty of Istanbul University, Istanbul, Turkey

³Department of Biophysics, Medical Faculty of Istanbul, Istanbul University, Istanbul, Turkey

⁴Department of Internal Medicine, Medical Faculty of Istanbul, Istanbul University, Istanbul, Turkey

⁵Department of Biochemistry, Medical Faculty of Istanbul, Istanbul University, Istanbul, Turkey

OBJECTIVE: Leukocyte fluidity plays an important role in microcirculation and therefore in the maintenance of immune function. Functional alterations of polymorphnuclear leukocytes and lymphocytes subgroups have been implicated in the pathogenesis of immune dysfunction associated with diabetes mellitus (DM). Data regarding rheological properties of lymphocytes in dogs with DM is very limited. In the present study, we aimed to assess the rheological properties of lymphocytes and their membrane protein content in dogs with DM.

METHODS: Venous blood samples were obtained from dogs with DM (n=10) and healthy dogs (n=10). Lymphocyte deformability was assessed with the microfiltration technique by measuring cell rigidity against pressure. Membrane proteins were evaluated with the SDS-PAGE polyacrilamide gel electrophoresis technique. Statistical analysis were performed by Student-t test using the SPSS statistical package program

RESULTS: The deformability of peripheral blood lymphocytes were found to be significantly decreased in the study group compared to controls ($p < 0.001$). Membrane proteins were not different between the two groups.

CONCLUSIONS: It has been well established that DM is associated with impairment of microcirculation and immune dysfunction. As the findings of the present study suggest, although the membrane protein content seems to be unaltered, decreased lymphocyte deformability may be involved in such circulatory disturbances and possibly in the propensity to infections.

Keywords: Lymphocyte deformability. diabetes mellitus, membrane proteins, dog

Kisspeptin has differential effects on plasma neuropeptide Y and peptide YY concentrations in young male rats

Sinan Canpolat¹, Ahmet Alver², Ahmet Mentese², Sinan Saral¹

¹Department of Physiology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

²Department of Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

OBJECTIVE: Neuropeptide Y (NPY) and peptide YY (PYY) are important neuromediators related to feeding behaviour in mammals. New peptide hormone kisspeptin has an essential role for the onset of puberty and gonadal function by stimulating the secretion of gonadotropin-releasing hormone. There are very limited studies regarding effect of kisspeptin on blood NPY and PYY concentrations. We have therefore investigated effects of kisspeptin on plasma levels of NPY and PYY in relation with feeding behaviour.

METHODS: Young male Sprague-Dawley (7 weeks old) rats were used in this study. Kisspeptin (20nmol/kg/day) was intraperitoneally administered for 12 days (n=8). Control animals received vehicle alone (n=8). Animals were decapitated one hour later after the last kisspeptin administration on the 12th day of experiments and blood samples were collected. Plasma concentrations of NPY and PYY were analysed by ELISA. Mann-Whitney U Test was used for statistical evaluations.

RESULTS: Kisspeptin treatment increased plasma NPY level ($P<0.01$). Plasma PYY concentration was decreased in kisspeptin treated group compared to control ($p<0.05$).

CONCLUSIONS: Results from this study demonstrate for the first time that exogenous kisspeptin administration decrease plasma PYY level. The present findings indicate that kisspeptin may have an orexigenic effect in the post-pubertal young male rat by increasing NPY and decreasing PYY levels.

Keywords: Kisspeptin, Neuropeptide Y, Peptide YY

Determination of Thyroid hormones and Prolactin Levels in Fertile and Infertile Women

Arzu Şahin¹, Feyyaz Önder²

¹Department of Health School, Artvin Çoruh University, Artvin, Turkey

²Department of Physiology, Akdeniz University, Antalya, Turkey

OBJECTIVE: This study is performed to determine the role of thyroid hormones in women with regard to infertility.

METHODS: This study was carried out in fertile (n=44) and infertile (n=40) women (15 to 45-year-old, mean age= 32.6±0.69) who consulted to different maternity and gynecology outpatients in Kars Maternity Hospital and Artvin State Hospitals. Women were divided into three groups as primary fertile (n=30), secondary infertile (n=10) and fertile (n=44). Serum hormone levels were determined in blood samples collected during early follicular phase by Microparticulary Enzyme Immunoassay (MEIA).

RESULTS: Serum TSH levels were 1.7±0.4, 1.6±0.2, 0.8±0.2 µIU/ml for fertile, primary infertile and secondary infertile groups, respectively (p>0.05). FT3 levels were 2.36± 0.06, 2.46±0.09, 2.35±0.2 pg/ml while FT4 levels 0.98±0.02, 0.94±0.04, 1.0±0.03 ng/dl for fertile, primary infertile and secondary infertile groups, respectively (p>0.05 for both FT3 and FT4). There was a significant negative relationship between TSH and FT4 (p=0.012, r=-0.275) and a significant positive relationship between FT3 and FT4 (p=0.002, r= 0.330). Prolactin levels also did not differ between the groups and they were 16.7±2.6, 21.1±2.1, 16±1.9 ng/ml for fertile, primary infertile and secondary infertile groups, respectively (p>0.05).

CONCLUSIONS: In conclusion, there were no differences between the groups in terms of TSH, FT3, FT4 and prolactin levels. The hormone levels were generally within normal range, therefore a relationship between these parameters and infertility was not observed. A more comprehensive study might be needed to be carried out with patients having hypothyroidism or hyperthyroidism.

Keywords: infertility, prolactin, Thyroid hormones, woman.

The Effects of Intra-gastric Prosthesis Placement and Roux-En-Y Gastric Bypass Surgery on Postprandial Plasma Levels of Ghrelin, Leptin and Obestatin

Gülsün Memi¹, Özge Dağdeviren², Zarife Nigâr Özdemir¹, Cumhuriyet Yeğen³, Berrak Ç. Yeğen¹, Yunus Yavuz⁴

¹Department of Physiology, Marmara University School of Medicine

²Department of Biochemistry, Marmara University School of Pharmacy

³Department of General Surgery, Marmara University School of Medicine

⁴Department of General Surgery, Bariatric Surgery Unit, Istanbul Bilim University School of Medicine

OBJECTIVE: Intra-gastric devices and jejunoileal bypass are applied in obese subjects to promote weight loss. Although the mechanisms are not well-known, they simply act by reducing the amount of food needed to induce satiety. The aim was to investigate the effect of surgical procedures on plasma levels of satiety-related hormones following a liquid or a solid meal.

METHODS: Male Sprague Dawley rats had a Roux-en-Y gastric bypass or a gastric prosthesis was sutured into fundus, while control rats had sham-surgery. In other rats, following gastric surgery, capsaicin was applied perineurally on the vagus. Three to four weeks after the surgery, either pre-weighed chow or a 3-ml non-nutrient meal was given. Thirty min after the liquid test meal and 8-h after the solid meal, rats were decapitated and trunk blood was collected for the measurement of plasma ghrelin, leptin and obestatin levels by ELISA. Values were compared by ANOVA

RESULTS: In the rats with prosthesis, postprandial plasma ghrelin and obestatin levels following liquid meal were significantly lower ($p<0.05$) than those of the control rats, while in the bypass group ghrelin and obestatin levels were elevated with a concomitant reduction in leptin ($p<0.01$). Following solid meal, lower obestatin and ghrelin levels and higher leptin levels were observed in rats with prosthesis as compared to control rats ($p<0.05$), but in the capsaicin-treated group plasma hormone levels were not significantly different from the control values.

CONCLUSIONS: The present results suggest that weight-reducing effects of gastric prosthesis and bypass include the control of postprandial secretion of ghrelin, obestatin and leptin.

Keywords: obesity, bariatric surgery, satiety-related hormones,

Patterns of calcium dynamics in islets from pancreas tissue slices

Andraz Stozer, Jurij Dolenšek, Dean Korošak, Marjan Slak Rupnik
Institute of Physiology, Faculty of Medicine, University of Maribor, Slovenia

OBJECTIVE: In secretory cells, changes in intracellular calcium concentration $[Ca^{2+}]_i$ couple cell activation with hormone secretion.

METHODS: By employing confocal microscopy, we were able to resolve dynamics of $[Ca^{2+}]_i$ in individual islet cells within the pancreas tissue slices as a function of time and space.

RESULTS: Activity patterns of a large number of individual cells per islet, subregions of the islet, as well as the whole islet were analyzed following administration of various physiological stimuli and modulators of calcium membrane transport. Patterns of $[Ca^{2+}]_i$ oscillations in response to glucose have been heterogeneous. Increasing concentrations of glucose progressively recruited individual cells and later groups of cells, with progressively increasing frequency and synchronicity. However, the time in which each group of cells oscillated as a functional cluster appeared to be limited and the clusters changed constantly in terms of their position inside the islet and the number of cells they included. Synchronous activity was strongly influenced by extracellularly applied Ba^{2+} and Cd^{2+} , indicating that extracellular Ca^{2+} contributed to oscillatory activity.

CONCLUSIONS: In the present work, the properties of experimentally obtained islet cell networks were also compared with the theoretical network model of spatially embedded heterogeneous cells to gain new insights into the relationship between structure and function of the tissue.

Keywords: pancreas tissue slices, Ca^{2+} oscillations, network model

Effect of Growth Hormone treatment on pancreas oxidative stress, apoptosis and inflammation related to aging in SAMP8 mice

Sara Cuesta¹, Roman Kireev¹, Cruz Garcia², Elena Vara², Jesus A. F. Tresguerres¹

¹Department Physiology, Medical School, University Complutense of Madrid, Madrid, Spain

²Dept of Biochemistry and Molecular Biology, Medical School, University Complutense of Madrid, Madrid, Spain

OBJECTIVE: The aim of this study was to examine the regulation of oxidative stress, apoptosis and inflammation associated with aging on pancreas of old mice and how Growth Hormone (GH) treatment could affect this process.

METHODS: Male senescence-accelerated SAMP8 and SAMR1 mice of 2 (young) and 10 months (old) of age were used (n = 40). Animals were divided into five experimental groups (1, 2) SAMP8/R1 young, (3, 4) SAMP8/R1 old, (5) SAMP8 old treated with GH. Physiologically equivalent doses of GH were administered for one month (2mg s.c./kg/day) and parameters were analysed by RT-PCR, Western Blot and ELISA.

RESULTS: Aging was associated with increased inflammation and oxidative stress (increased TNF- α , IL- β , IL-6, MCP1, HO-1, NO x). The ratio anti/pro apoptotic mRNA expression (Bcl-2/BAX+BAD) was decreased during aging in SAMP8 mice. With aging, protein expression of NF κ B p52-100 was increased and I κ B beta was decreased in pancreas of SAMP8 mice. GH treatment lowered the expression of inflammatory, oxidative stress and apoptotic markers in SAMP8 mice. Furthermore GH was able to reduce the increase observed in old SAMP8 mice in HOMA-IR index. No statistical differences were found in SAMR1 young and old animals in these parameters.

CONCLUSIONS: These results indicate that aging is associated with significant alterations in the relative expression of pancreatic proteins and genes involved in inflammation, oxidative stress and apoptosis. These results showed that GH treatment was able to improve pancreatic function reducing the markers of these processes.

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Keywords: Aging, Inflammation, oxidative stress, apoptosis, pancreas, senescence accelerated mice.

The effects of melatonin on the increased hsp70 immunoreaction in the liver induced by experimental CCl₄ toxicity

Omur Karaca¹, Hıdır Pekmez², Murat Abdülğani Kuş³, Nusret Akpolat⁴, Murat Ögetürk², İlter Kuş¹

¹Department of Anatomy, Balıkesir University, Balıkesir, Turkey

²Department of Anatomy, Fırat University, Elazığ, Turkey

³Health college, Mehmet Akif University, Burdur, Turkey

⁴Department of Anatomy, İnönü University, Malatya, Turkey

OBJECTIVE: Carbon tetrachloride (CCl₄) is a volatile organic chemical, which causes tissue damage, especially to the liver and kidney. The potential for protective effects of melatonin on carbon tetrachloride-induced acute liver injury in rats was investigated in this study.

METHODS: Twenty-four male Wistar-Albino rats were randomly divided into three equal groups: Control, CCl₄ and CCl₄ plus melatonin (CCl₄+Melatonin). Rats in CCl₄ group were injected subcutaneously with CCl₄ 0.5 ml/kg in olive oil while rats in CCl₄+Melatonin group were injected subcutaneously with CCl₄ (0.5 ml/kg) plus melatonin (25 mg/kg) every other day for one month. Control rats were injected olive oil. Liver tissues were stained with heat shock protein 70 (Hsp70) immunohistochemically performed.

RESULTS: A dense staining indicating Hsp70 immunoreaction was observed in the liver depending on the toxicity of CCl₄. The liver tissue sections of rats injected with melatonin and CCl₄, the HSP70 staining was detected minimal.

CONCLUSIONS: Results of the present study have shown that CCl₄ induced tissue damage in the liver is prevented by melatonin administration.

Keywords: Carbon tetrachloride, melatonin, liver, Hsp70.

The effect of kisspeptin on ghrelin and body weight in young male rat

Mehmet Aydin¹, Fatih Sefil¹, Sedat Yildiz², Bayram Yilmaz³

¹Department of Physiology, Medical Faculty, Mustafa Kemal University

²Department of Physiology, Medical Faculty, Inonu University

³Department of Physiology, Medical Faculty, Yeditepe University

OBJECTIVE: Kisspeptin is a recently discovered hypothalamic peptide which plays an important role in the central control of reproductive functions. We have investigated direct and indirect effects of kisspeptin on the serum ghrelin and follicle-Stimulating Hormone (FSH) levels, and body and testicular weight in young male rats.

METHODS: Twenty-four male Wistar rats (6 weeks of age) were divided into four groups (n=6/group). First group served as control and received saline. Kisspeptin-10 was administered to the animals in the second group (20nmol/rat/day) for a period of seven days. Rats were given only one dose gosereline (0.9mg/rat), a GnRH agonist in the third group. The last group received kisspeptin-10 with gosereline. Serum ghrelin and FSH levels were determined by RIA and ELISA, respectively.

RESULTS: Serum ghrelin levels were increased in the gosereline groups in comparison with the other three groups (p<0.01). Serum FSH levels were lower in the groups treated with gosereline group than in both control and kisspeptin (p<0.01 and p<0.05). Kisspeptin-alone application, on the fourth day, reduced body weight. Testicular weights were lower in the groups treated with gosereline (p<0.01). Positive correlations were found between ghrelin levels and body weight, between testicular weight and FSH levels (p<0.01).

CONCLUSIONS: : In conclusion, our findings suggest that kisspeptin may directly have a suppressive effect on ghrelin and body weight. Kisspeptin, this way, may have a modulatory effect on energy balance.

Keywords: Kisspeptin, ghrelin, gosereline, FSH, young male rats.

The Link Between Chronic Kidney Disease and Subclinical Hypothyroidism in Patients with Type 2 Diabetes Mellitus

Rucsandra Danciulescu Miulescu¹, Anca Lelia Riza², Ana Maria Tinu¹, Vasilica Cristescu³, Suzana Danoiu²

¹UMF Carol Davila Bucharest

²UMF Craiova

³Institute Prof. N. Paulescu Bucharest

OBJECTIVE: The aims of the present study were to investigate the prevalence of subclinical hypothyroidism in patients with type 2 diabetes mellitus and chronic kidney disease.

METHODS: Sample: 34 patients with type 2 diabetes and chronic kidney disease (glomerular filtration rate between 60-89 ml/minute per 1.73 m²). The subjects of study group were between 55 and 62 years. To diagnose subclinical hypothyroidism, free thyroxine (free T₄) and TSH were determined. TSH was measured using a chemiluminescent immunometric assay with a normal range 0.10 - 4.50 mU/l, free T₄ with a direct, monoclonal antibody assay were measured with a normal range of 0.7 to 1.7 ng/dl. Results were compared with measurements in 32 patients with type 2 diabetes mellitus but without chronic kidney disease.

RESULTS: The subjects of study group: 22 women (64.70%) and 12 men (35.29%) were between 55 and 62 years mean age SD 56.20 ± 3.28 years. Subclinical hypothyroidism occurred in 7 (20.58%) participants. In the patients of study group TSH were significantly correlated with age (r=0.872, p<0.001) and decreased of glomerular filtration rate (r=0.788, p<0.001). The prevalence of subclinical hypothyroidism in patients with type 2 diabetes mellitus but without chronic kidney disease was 6.25% (2 patients -2 women).

CONCLUSIONS: The results suggest that subclinical hypothyroidism is a relatively common condition among diabetic patients with chronic kidney disease. The potential causal mechanisms linking between subclinical primary hypothyroidism and chronic kidney disease is not fully understood. The measurement of serum TSH levels should be included in the screening of patients with type 2 diabetes mellitus and chronic kidney disease.

Keywords: subclinical hypothyroidism, chronic kidney disease, thyroid-stimulating hormone

Effect of Melatonin Administration on Lipid Peroxidation in Testis Tissue of Diabetic Rats Subjected to Acute Swimming Exercise

Mursel Bicer¹, Abdulkerim Kasim Baltaci², Rasim Mogulkoc², Mustafa Akil³, Mine Yilmaz²

¹School of Physical Education and Sports Selcuk University, Konya-Turkey

²Selcuk University, Selcuklu Medical School, Department of Physiology, Konya-Turkey

³Karabuk University, Hasan Dogan High School of Physical Education and Sports, Karabuk-Turkey

OBJECTIVE: The objective of this study was to examine the effect of melatonin administration on lipid peroxidation in the testis tissue of diabetic rats subjected to acute swimming exercise.

METHODS: The study included 80 adult male Sprague-Dawley rats which were divided into 8 groups: Group 1, general control; Group 2, melatonin-administered control; Group 3, melatonin-administered diabetic control; Group 4, swimming control; Group 5, melatonin-administered swimming; Group 6, melatonin-administered diabetic swimming; Group 7, diabetic swimming; Group 8, diabetic control group. In order to induce diabetes, the experimental animals were injected with 40 mg/kg intraperitoneal (ip) streptozotocin (STZ) "Sigma, S-0130". The injections were repeated at the same dose after 24 hours. The animals whose blood glucose was found 300 mg/dl and above 6 days after the last injection were accepted as diabetic. These animals were administered 3 mg/kg/day ip melatonin for 4 weeks. Levels of MDA and GSH were determined (by TBARS and ELLMANN methods, respectively) in the testis tissue of the rats decapitated at the end of the 4-week study.

RESULTS: The highest MDA values in the testis tissue were found in groups 7 and 8 ($p < 0.001$). Testis MDA values in groups 4 and 6 were lower than the levels in group 7 and 8, but higher than those in other groups ($p < 0.001$). Group 5 had the highest testis GSH values ($p < 0.001$). Group 6 had testis GSH values lower than group 5, but higher than all other groups ($p < 0.001$). The lowest testis GSH levels were found in groups 7 and 8 ($p < 0.001$).

CONCLUSIONS: Results obtained from the study indicate that melatonin administration can prevent the increase in free radical production and the inhibition of the antioxidant activity resulting from diabetes and acute exercise.

Keywords: Diabetes, exercise, lipid peroxidation, melatonin

Effect of Pinealectomy and Melatonin Administration on the Levels of Various Elements in the Kidney and Testis Tissues of Rats

Dondu Koykun¹, Abdulkerim Kasim Baltaci¹, Fatma Burcu Seker², Rasim Mogulkoc¹, Mine Yilmaz¹, Bayram Yilmaz²

¹Selcuk University, Selcuklu Medical School, Department of Physiology, Konya-Turkey

²Yeditepe University, Medical School, Department of Physiology, Istanbul, Turkey

OBJECTIVE: The aim of the present study is to explore how pinealectomy and melatonin administration affects elements (molybdenum, cadmium, chrome, boron, manganese, magnesium, copper, iron and zinc) in the testis and kidney tissues of rats.

METHODS: This study included 24 adult male Sprague-Dawley type rats, which were allocated to 4 groups: Group 1, control; Group 2, melatonin-administered control; Group 3, pinealectomy (Px); Group 4, pinealectomized, melatonin-administered group. Pineal glands of the animals in groups 3 and 4 were removed under general anesthesia. The animals in groups 2 and 4 were administered 3 mg/kg ip melatonin for 4 weeks. The protocol of the study was approved by the local ethics committee and carried out at the Experimental Research Center of Yeditepe University. At the end of the study, the animals were decapitated. Kidney and testis tissue samples were obtained and analyzed for determination of cobalt, molybdenum, cadmium, chrome, nickel, boron, manganese, magnesium, lead, phosphorus, calcium, copper, iron, selenium and zinc levels using atomic emission (mg/L).

RESULTS: Levels of molybdenum, cadmium, chrome, boron, manganese, magnesium, copper, iron and zinc in the kidney tissue of the pinealectomy group (group 3) were found significantly elevated ($p < 0.001$). Similarly, pinealectomy (group 3) significantly increased cobalt, molybdenum, nickel, boron, manganese, magnesium, phosphorus and selenium levels and significantly reduced zinc levels in the testis tissue ($p < 0.001$). Melatonin administration (group 4) to pinealectomized animals restored the parameters under investigation almost to control values.

CONCLUSIONS: Results of the study show that pinealectomy significantly changes the element metabolism in kidney and testis tissue, while melatonin administration to pinealectomized animals partly prevents these changes.

Keywords: Pinealectomy, melatonin administration, testis and kidney, elements

Effect of Pinealectomy and Melatonin Administration on Leptin and NPY Secretion in Rats with Induced Hypothyroidism

Abdulkerim Kasim Baltaci, Rasim Mogulkoc

Selcuk University, Selcuklu Medical School, Department of Physiology, Konya-Turkey

OBJECTIVE: Although there is an abundance of reports on the relationship between the pineal gland and thyroid hormones, there is little information in the literature about the role of the melatonin in thyroid dysfunction. The present study aims to examine the effect of pinealectomy and/or melatonin administration on leptin and NPY secretion in hypothyroidism induced rats.

METHODS: The study included 50 adult male Sprague-Dawley rats which were allocated into 5 groups: Group 1, control; group 2, sham-hypothyroidism; group 3, hypothyroidism (group which was injected 10 mg/kg/day ip PTU for 4 weeks); group 4, hypothyroidism + pinealectomy group (the group in which the pineal gland was removed under general anesthesia and which was injected 10 mg/kg/day ip PTU for 4 weeks); and group 5, hypothyroidism + melatonin administered group (the group which was injected 10 mg/kg/day ip PTU and 3 mg/kg/day melatonin for 4 weeks). At the end of the 4-week procedures, the rats were decapitated and blood samples were obtained. Plasma levels of melatonin, leptin and NPY were determined by RIA.

RESULTS: The highest melatonin levels were obtained in the melatonin-administered group 5 and the lowest levels were found in the pinealectomy group (group 4) in the study ($p < 0.001$). Melatonin levels in the hypothyroidism group (group 3) were higher than those in group 4, but significantly lower than the levels in all other groups ($p < 0.001$). Experimental hypothyroidism resulted in an increase in leptin and neuropeptide-Y levels in all groups (groups 3, 4 and 5).

CONCLUSIONS: Results of the present study indicate that hypothyroidism causes significant inhibition in melatonin levels and a remarkable elevation in leptin and NPY levels. We conclude that the increase in leptin and NPY levels is a result of hypothyroidism, rather than from pinealectomy and/or melatonin administration.

Keywords: Hypothyroidism, pinealectomy, melatonin, leptin, NPY.

Effect of Zinc Deficiency and Supplementation on the Secretion of Melatonin, Leptin and NPY in Experimental Hyperthyroidism

Abdulkerim Kasim Baltaci, Rasim Mogulkoc

Selcuk University, Selcuklu Medical School, Department of Physiology, Konya-Turkey

OBJECTIVE: The present study aims to examine the effect of zinc supplementation and zinc deficiency on the secretion of leptin and NPY in experimental hyperthyroidism in rats.

METHODS: This study included 50 adult male Sprague-Dawley rats which were divided into 5 groups: Group 1, general control; group 2, sham-hyperthyroidism; group 3, hyperthyroidism (the group which was injected 0.3 mg/kg/day ip thyroxine for 4 weeks); group 4, hypothyroidism and zinc-deficient group (the group which was injected 0.3 mg/kg/day ip thyroxine and fed on a zinc-deficient diet "0.65 ppm/zinc/g/diet" for 4 weeks); and group 5, hyperthyroidism + zinc-supplemented group (the group which was administered 0.3 mg/kg/day ip thyroxine and 3 mg/kg/day zinc sulfate for 4 weeks). At the end of the 4-week procedures, all the animals were decapitated and their blood samples were analyzed to determine plasma melatonin, leptin and NPY levels by RIA.

RESULTS: The highest melatonin levels in the study were found in the hyperthyroidism groups (groups 3, 4 and 5) ($p < 0.001$). Leptin and NPY levels in the hyperthyroidism (group 3) and zinc-deficient hyperthyroidism (group 4) groups were higher than those in all other groups ($p < 0.001$).

CONCLUSIONS: Results of the study indicate that hyperthyroidism brings about important alterations in the secretion of leptin and NPY, while zinc supplementation prevents these changes.

Keywords: Hyperthyroidism, zinc, melatonin, leptin, NPY.

A preliminary study: Caffeic acid phenethyl ester containing culture medium may preserve pancreas islets from early apoptosis and loss of function

Şefika Nur Kurt¹, Ersin Fadillioglu¹, Sercan Mercan², Zeynep Ginis³, Tuncay Delibasi⁴

¹Department of Physiology, Hacettepe University, Faculty of Medicine, Ankara, Turkey

²Pancreas Islet Research Center, Ankara Diskapi Yildirim Beyazıt Training and Research Hospital, Ankara, Turkey

³Department of Clinical Biochemistry, Ankara Diskapi Yildirim Beyazıt Training and Research Hospital, Ankara, Turkey

⁴Department of Endocrinology and Metabolism, Ankara Diskapi Yildirim Beyazıt Training and Research Hospital, Ankara, Turkey

OBJECTIVE: Pancreatic islet transplantation is an emerging therapy for diabetes mellitus. The effective use of limited number of available islets is required for successful islet transplantation. However, there are some problems that are usually related with high apoptotic changes in the islets during in vitro culture period. To improve successful culture without loss of islet cell and their function, we planned to add caffeic acid phenethyl ester (CAPE), have anti-inflammatory and anti-oxidant properties, to culture medium.

METHODS: Male Wistar Albino rats were used to isolate islets. The pancreas was digested with collagenase V and the purification was performed with ficoll-1077. Following isolation, islets were cultured in three different mediums for 24 hours. The equal IEQ numbers of islets were cultured with three different mediums. The mediums were RPMI-1640/FBS without CAPE or DMSO, with %0.1 DMSO and with 10 µM CAPE. The viability was examined with florescein diacetate (FDA) and propidium iodine (PI). Insulin secretion was assessed by stimulation with low and high glucose containing medium.

RESULTS: The islets showed more than 90% purity in CAPE medium. The viability of CAPE treated islets (73.3%) was greater than the controls (64.3%). The insulin concentrations in low glucose were 0.77, 0.73 and 3.08 ng/IEQ in control, DMSO and CAPE mediums, respectively. The high glucose induced insulin secretion with concentration of 2.38, 1.74 and 6.50 ng/IEQ in control, DMSO and CAPE mediums, respectively. The CAPE treated islets showed higher insulin secretion capability.

CONCLUSIONS: It has been concluded that CAPE containing medium may protect islets from apoptosis and function loss before transplantation procedure.

Keywords: islet cell, culture, CAPE

In vivo effects of leptin on lymphocyte subpopulations in mice

Aykut Göktürk Üner¹, Nesrin Sulu²

¹Department of Physiology, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın, Turkey

²Department of Physiology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

OBJECTIVE: Leptin, a hormone-cytokine mainly produced by the adipose tissue, has pleiotropic effects on many biological systems. The aim of this study was to investigate the effects of peripheral leptin on lymphocyte subpopulation.

METHODS: Initially forty male Swiss albino mice were divided into five groups. Mice in group I (Control) were given serum physiologic (SP) and group L100, group L250, group L500, and group L1000 were given 100, 250, 500 and 1000 µg/kg/day recombinant mouse leptin, respectively. Leptin or SP was injected subcutaneously for the next 6 days. Daily food/water intake was recorded for each group. At the end of the study, whole blood samples (500 µl) were obtained via intracardiac puncture in anesthetized mice after 15 hours from the last injections. Leptin levels and lymphocyte subpopulations in blood samples were analyzed.

RESULTS: No in vivo dose-dependent effect of leptin was seen on lymphocyte subpopulation count in mice. Treatment of mice with high-dose leptin led to increase only CD4+ cells ($P < 0.05$). In addition, high-dose leptin slightly increased CD3+ cells but this was not statistically confirmed ($P = 0.08$). Notably, it was found that leptin caused insignificant changes on body weight and food intake in normal body weight mice.

CONCLUSIONS: The data support that high-dose leptin has proliferative effect on CD4+ cells in vivo.

Keywords: CD4+ cell, dose, food intake, in vivo, leptin, lymphocyte subpopulations

The Evaluation of the Levels of Blood Parameters with respect to Premenstrual Syndrome During the Early Follicular and Late Luteal Phases in Adolescent Girls

Hatice Yorulmaz¹, Burcu Bal², Tuğba Babacan², Hasibe Sena Tekin², Özge Acartürk²

¹Department of Physiotherapy and Rehabilitation, Haliç University, Istanbul, Turkey

²School of Nursing, Haliç University, Istanbul, Turkey

OBJECTIVE: aimed to evaluate the several blood parameters with respect to premenstrual syndrome during follicular and luteal phase.

METHODS: In this study, adolescent girls ranged between 18-25 years were tested blood biochemical parameters in early follicular and late luteal phases. Their symptoms and complaints associated with Premenstrual Syndrome were assessed using Premenstrual Syndrome Scale. The statistical data analysis was performed using matched-pairs t-test, ANOVA, Tukey, Mann Whitney U, Kruskal Wallis.

RESULTS: The leukocyte values were found higher in follicular phase than luteal phase ($p < 0.001$). PMS has been found over 50% sample groups. The adolescent girls with first-degree obesity were determined to experience premenstrual syndrome more severely than the thin or normal-weight girls ($p < 0.05$). In early follicular phase; adolescent girls with higher fasting blood glucose values were found to experience less fatigue while they suffer more sleep disorders with lower iron levels compared with higher levels. Adolescent girls with low or normal MEHC values struggled with more anxiety than ones with higher MEHC values ($p < 0.01$).

CONCLUSIONS: This study has demonstrated that adolescent girls with iron insufficiency, low fasting blood glucose level and high body mass index experience the PMS symptoms more frequently. The presence of increased leukocyte values before menstrual cycle indicates that menstrual cycle is an associated condition with inflammation. This study reveals the importance of body mass index, anemia and controlling the blood glucose level in reducing PMS symptoms.

Keywords: blood parameters, follicular phase, luteal phase, premenstrual syndrome

Effects of Growth Hormone in the prevention of age related sleep disturbances

Ruben Rial Planas¹, Jesus A. F. Tresguerres², Roman Kireev², Lluís Gené¹, Sara Aparicio¹, Pere Barceló¹

¹Iunics, Universitat de les Illes Balears, Spain

²Depto de Fisiologia, Universidad de Madrid, Spain

OBJECTIVE: Aged individuals suffer deep sleep disturbances. Likewise, GH secretion stops in ageing individuals. This report aims at rejuvenating sleep in old rats (24 months) through the chronic administration of exogenous GH.

METHODS: Three groups of Wistar rats have been used: 1) Young controls, (12 animals, 90 days old) receiving two daily saline injections, 2) Old controls (12 animals, 24 months old) also received two daily saline injections 3: Old experimental group (12 animals, 24 months old) injected with two daily subcutaneous injections of GH (1mg/kg). After 30 days of treatment, all animals received chronic electrodes for conventional polysomnography and their sleep was recorded during 2h during light time. Digitized sleep EEG samples were submitted to the Fast Fourier Transform to analyze the power in delta, theta, alpha and beta power and the statistical differences between groups were analyzed.

RESULTS: Old controls showed the typical age-related sleep impairments, but GH administration increased the NREM delta power in old experimental group up to levels with no difference from young controls. No changes were observed in alpha, theta and Beta EEG power. During REM, the EEG power increase was extended to the whole EEG range, from 0.5 to 30 Hz reaching levels without significant difference with those of young controls.

CONCLUSIONS: GH administered to old rats (24 months) rejuvenated the main EEG traits of sleep until reaching levels similar to those recorded in young (90 days) animals.

Keywords: GH, Sleep, ageing, EEG, rats

Changes of progesterone and the multifarious role of IL-10 during pregnancy and early postpartum

Sibel Akyol¹, Kilic Aydinli²

¹I.U.Cerrahpasa School of Medicine, Physiology, Istanbul, Turkey

²I.U.Cerrahpasa School of Medicine, Gyneacology, Istanbul, Turkey

OBJECTIVE: In our study, our aim was to show the relationship between IL-10, IL-4, IL-12 cytokines and progesterone (PRG) changes in each trimester of pregnancy including early postpartum of healthy pregnant women.

METHODS: A checkup, including ultrasound, to verify healthiness was performed to each subject at the University of Istanbul, Cerrahpasa Gynecology. 1) Control group (midluteolitic phase, 21st day) (n=20); 2) Pregnant group (1st trimester, 10-12 weeks) (n=30). Normal pregnant women were followed in each trimester of pregnancy until the end of the first month of postpartum. Blood samples were obtained at the 21st day of midluteolitic phase from the control group and in 12, 24, 30 weeks and 4th weeks of postpartum from pregnant women. The flow cytometric method was used for determining the relative levels of cytokine molecule IL-2. ELISA was used for obtaining levels of IL-4, IL-10, IL-12 cytokines and PRG in plasma.

RESULTS: Comparing with the control group, we obtained significant increases in IL-2, IL-4, IL-12 and IL-10 in the first trimester. IL-2 decreased significantly, IL-4, IL-10, IL-12 increased significantly in the 2nd trimester; and in the mid-3rd trimester the most significant increase was seen in IL-4, IL-2, IL-10, IL-12. Starting from midluteal phase E2, PRG levels increased in the 3rd trimester of the pregnancy, and also it was seen at the similar level in the third trimester of postpartum. This suppression was connected by PRG.

CONCLUSIONS: These data suggest that IL-10 may act as a key contributor to the balance of pro-inflammatory versus anti-inflammatory signals that orchestrate proper pregnancy outcomes.

Keywords: cytokines, pregnancy, postpartum, progesterone

Changes in thyroid hormones metabolism in mice associated with high-fat diet-induced obesity

Stanislav Pavelka¹, Zuzana Macek Jílková², Jan Kopecky²

¹Central-European Technology Institute, Masaryk University, Brno and Department of Radiometry, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague

²Department of Adipose Tissue Biology, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

OBJECTIVE: Previous studies have established that thyroid hormones (TH) play important roles in the development and function of brown and white adipose tissue (WAT). However, data about local transformations of TH in adipose tissue are scarce. We decided, therefore, to measure changes in the enzyme activity of the type 3 iodothyronine deiodinase (D3) in murine WAT during development of dietary obesity.

METHODS: High-fat-feeding induction of obesity was followed in male C57BL/6J mice maintained on a special high-fat (HF) diet for 2 weeks before analysis, in comparison with the same animals maintained on a standard low-fat (LF) diet. Serum total thyroxine (T4) and triiodothyronine (T3) concentrations were determined using RIA kits. D3 activity in WAT was measured with the aid of our newly developed radiometric enzyme assay (see the accompanying posters from the same Institutes).

RESULTS: HF-diet feeding resulted in an increased size of adipocytes and in a significantly higher weight of both epididymal-visceral and dorsolumbar-subcutaneous fat depots. Total T4 and total T3 plasma levels were significantly elevated in mice fed HF-diet, in comparison with mice maintained on LF-diet (n=17, p<0.05). Development of HF-diet-induced obesity in the mice was associated with an enhancement of D3 activity in WAT, especially in subcutaneous fat depots (n=14, p<0.05).

CONCLUSIONS: HF-diet-induced obesity in mice was associated with proliferation and differentiation of WAT and stimulation of thyroid hormones metabolism in WAT. Support from the Ministry of Education of the Czech Republic (Project No. MSM0021622413), Academy of Sciences of CR (Project No. AV0Z50110509), and from the Czech Science Foundation GA CR (Grant No. 304/08/0256) is acknowledged.

Keywords: Metabolism, Obesity, Thyroid hormone, White adipose tissue

Effects of high doses of dexamethasone on hemodynamic and immunohistochemical parameters in acute paraquat intoxication

Nuran Ekerbicer¹, Tugba Gurpinar², Figen Tarakci¹, Elgin Turkoz³, Sevinc Inan³

¹Department of Physiology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey

²Department of Pharmacology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey

³Department of Histology & Embriology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey

OBJECTIVE: Paraquat (1,1'-dimethyl-4,4'-bipyridinium; PQ), a non-selective contact herbicide, is highly toxic to humans. Kidney is one of major target organs affected during PQ poisoning. Dexamethasone (Dexa) has anti-inflammatory effects used in PQ poisoning. The aim of this study was to investigate hemodynamic and immunohistochemical effects of Dexa treatment in acute PQ poisoning on rat kidney.

METHODS: Adult male Sprague Dawley rats (240–270 g) were divided as: 1) Control, 2) Dexa: 100 mg/kg/ip, 3) PQ: 25 mg/kg/ip 4) PQ+Dexa groups. Total blood volume (TBV), mean arterial pressure (MAP) and heart rate (HR) were recorded during the experimental period. Kidney samples were obtained and were fixed in 10% formalin solution. Routine paraffin tissue procedure was executed and paraffin blocks were stained with Hematoxylin-Eosin. Anti-cyclooxygenase-1 (COX1), anti-cyclooxygenase-2 (COX2), anti-angiotensin converting enzyme (ACE), anti-aquaporin-1 (AQU-1), anti-vascular cell adhesion molecule (VCAM) primary antibodies were used for immunohistochemical examination. Immunoreactivities were scored as mild, moderate or severe, and results were evaluated comparatively using ANOVA statistical test.

RESULTS: TBV was increased when 10 and 20 min values were compared in PQ group. MAP value at 10 min was significantly increased especially in Dexa group. HR was also increased in all groups compared with the control group at the 120 minute. Immunoreactivities were observed as moderate/severe in the control group, mild in Dexa group whereas it was severe in the PQ group. Immunoreactivities were observed as moderate in group PQ + Dexa, as closer or the same in the control group.

CONCLUSIONS: The decreasing effect of Dexa and increasing effect of paraquat on TBV were found to be related to acute changes in the tissue level.

Keywords: Dexamethasone, Paraquat, Kidney, Total Blood Volume, Cyclooxygenase, Angiotensin Converting Enzyme, Aquaporin, Vascular Cell Adhesion Molecule, Immunohistochemistry.

Separate and combined effects of long-term black tea and nicotine on cardiovascular parameters and heart resistance to myocardial injury in rat

Siyavash Joukar¹, Hamideh Bashiri²

¹Department of Physiology and Pharmacology, Afzalipour Medical Faculty, Kerman University of Medical Sciences, Kerman, Iran

²Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

OBJECTIVE: The present study was designed to elucidate the outcome of chronic co-administration of black tea and nicotine on cardiovascular performance in the presence of isoproterenol-induced injury.

METHODS: All experimental procedures were carried out according to the national guidelines for conducting animal studies (Ethical committee permission No 86/123KA—Kerman University of Medical Sciences). Main animal groups were control (CTL), black tea (T), nicotine (N) and black tea plus nicotine (N+T) groups. Test groups received nicotine (2mg/kg sc) and black tea brewed (2.5% w/v p.o) each alone and in combination for 4 weeks. On the twenty-eighth day, myocardial damage was induced by isoproterenol (50 mg/kg i.p.) and blood samples were taken for cardiac troponin I measurement. On day of 29, after hemodynamic parameters recording, hearts were removed for histopathological evaluation.

RESULTS: Tea or nicotine consumption had no significant effects on hemodynamic indices before induction of heart' damage. When cardiac injury was induced, tea consumption maintained the Maximum dp/dt and nicotine significantly decreased the pressure-rate product (PRP). Moreover, severity of heart lesions was lower in the presence of chronic nicotine or black tea. Concomitant use of these two materials did not show extra effects on mentioned parameters more than the effect of each of them alone.

CONCLUSIONS: The results suggest that long-term administration of black tea or nicotine may have mild to moderate cardioprotective effects, while concomitant use of these materials can not intensify this beneficial effect

Keywords: Black tea, Nicotine, Isoproterenol, Myocardial injury, Mean arterial pressure

Histo-physiological changes observed in the small intestine with hypovolemia

Mehmet Caglar¹, Sevinc Inan², Emine Esra Nal², Nuran Ekerbicer¹

¹Department of Physiology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey

²Department of Histology & Embriology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey

OBJECTIVE: Administration of fluids to maintain or restore intravascular volume is a common intervention after hypovolemia. Therefore, this study was designed to investigate the histo-physiological changes of resuscitation with Ringer lactate (RL) solutions on small intestine tissue, after acute hemorrhage in rats.

METHODS: Wistar-Albino rats (n=12) were divided into three groups, each including 4 rats. First group was control; second group was assigned as hypovolemia. Then the third group was isovolemic resuscitated with RL. Haematocrit (Hct), mean artery pressure (MAP) and heart rate (HR) were recorded for 120 minutes. For microscopic examination, tissue samples were fixed in 10% formalin and prepared using routine paraffin procedure. Sections were stained with Hematoxylin-Eosin and anti-vascular endothelial growth factor (VEGF), anti-endothelial nitric oxide synthase (eNOS) and anti-inducible nitric oxide synthase (iNOS) primary antibodies were used for immunohistochemical examination. Immunoreactivities were scored as mild, moderate or severe, and results were evaluated comparatively using ANOVA statistical test.

RESULTS: MAP and HR values were significantly reduced in hypovolemia. Hct results at 5 min were significantly decreased especially in RL compared to the hypovolemia group. While strong immunoreactivities of VEGF, eNOS and moderate immunoreactivities iNOS were observed in the hypovolemia group; mild/moderate and moderate immunoreactivities were seen in the control and the resuscitated groups, respectively.

CONCLUSIONS: Even in the acute period, histo-physiological changes were observed in the small intestine with hypovolemia. Ischemia and angiogenesis caused by hypovolemia were increased especially eNOS, and VEGF immunoreactivity in small intestine.

Keywords: Plasma volume expanders, hypovolemia, immunohistochemistry, eNOS, iNOS, VEGF, hemodynamic parameters, haematocrit.

Biomechanical Parameters of the Fibrin Clot in Patients with Diabetes Mellitus

Veronica Sfredel¹, Maria Mota², Mirela Diana Sfredel³, Suzana Danoiu¹, Anca Lelia Riza⁴, Tudor Adrian Balseanu¹, Smaranda Mitran¹, Catalina Pisoschi⁵

¹Department of Physiology and Pathophysiology, University of Medicine and Pharmacy of Craiova, Romania

²Department of Nutrition and Metabolic Diseases, University of Medicine and Pharmacy of Craiova, Romania

³PhD student, University of Medicine and Pharmacy of Craiova, Romania

⁴Student, University of Medicine and Pharmacy of Craiova, Romania

⁵Department of Biochemistry, University of Medicine and Pharmacy of Craiova, Romania

OBJECTIVE: In our study, we aimed at investigating the haemostatic status of diabetic patients through an innovative method called fibrinresistometry (FBR). FBR measures the breakage resistance of the fibrin clot (BRFC), a biophysical parameter that describes the adhesion and extension resistance of the fibrin network until its breaking point.

METHODS: The study lot consisted of 65 diabetic patients (31 women, 34 men, ages 31 to 80) grouped according to the time since diagnosed with Diabetes Mellitus, and 60 controls (31 women, 29 men, ages 35 to 79). We determined and statistically analyzed BRFC, fibrinogen, and standard coagulation time tests: PT, aPTT, platelet count, mean platelet volume. Normal range for FBR is 200-300 fibrinresistometric units (FU).

RESULTS: Mean BRFC values for the diabetic patients (323.34 ± 49.88) was significantly greater ($p < 0.001$) than controls (248.64 ± 25.71). Also, mean BRFC increased steadily with the time since DM diagnosis. The history of DM correlated with BRFC (chi square=21.55, $p < 0.001$), but not with fibrinogen (chi square=2.98, $p = 0.40$). BRFC correlated with platelet count (Pearson coefficient=0.50) and mean platelet volume (Pearson coefficient=0.52). Standard coagulation tests were within normal range. No significant differences were found between men and women.

CONCLUSIONS: Not necessarily correlated with coagulation time tests, FBR could be recommended as an independent predictor of the prothrombotic status present in DM, thus opening a new line of research in this field. The method we propose, a novelty in itself, is showing more and more convincing arguments of its clinical use.

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Keywords: haemostasis, Diabetes Mellitus, fibrinresistometry, thrombosis

Prolonged QT Dispersion in Inflammatory Bowel Disease

Elif Yorulmaz¹, Aslıhan Sezgin², Hatice Yorulmaz³, Gupse Adalı¹, Hilmi Çiftçi², Ümit Akköse², Ilyas Tuncer¹

¹Department of Gastroenterology, Göztepe Training and Research Hospital, Istanbul, Turkey

²Department of III. Internal Medicine, Göztepe Training and Research Hospital, Istanbul, Turkey

³Department of Physiotherapy and Rehabilitation, Halic University, Istanbul, Turkey

OBJECTIVE: Inflammatory Bowel Diseases (IBD) is concerned with two different chronic idiopathic inflammatory diseases: Crohn's disease (CD) and ulcerative colitis (UC). This study aimed to investigate the frequency and factors of prolonged QT dispersion that may lead to severe ventricular arrhythmias in the IBD diseases.

METHODS: This study included 63 ulcerative colitis and 41 Crohn's disease patients with 47 healthy patients as control group with no previous chronic disease, electrolyte imbalance and medication except IBD treatment. By electrocardiography, corrected QT dispersion (QTcd) for heart rate using the Bazett's formula was calculated. Homeostasis Model Assessment (HOMA) was used to determine insulin resistance (IR). HOMA values <1 were considered normal and values >2.5 indicated a high probability of IR.

RESULTS: This study included 63 ulcerative colitis patients (mean age 40.89±12.25), 41 Crohn's disease patients (mean age 38±7.79) and 47 healthy patients in control group (mean age 41.21±10.55). Of the patients; 12.2% ulcerative colitis and 14.5% Crohn's disease patients had prolonged QTcd than the control group (p<0.05). A significant difference was found between the values of insulin (p<0.05) and HOMA (p<0.01) of the UC and Crohn's disease patients with and without prolonged QTcd. The structurizing and penetrating types of involvements were determined in Crohn's disease patients with prolonged QTcd. The increased systolic arterial pressure (p<0.01) and age (p<0.05) in ulcerative colitis patients were significantly associated with prolonged QTcd.

CONCLUSIONS: Further studies are necessary to determine the association between etiopathogenesis of IBD and IR. The routine follow-up of the IBD patients should include determination of HOMA values and ECG examination.

Keywords: Inflammatory bowel disease, insulin resistance, QT dispersion

Changes in Tongue Papillae and Taste Perception in Experimentally Induced by Zinc Deficiency Animal Models

Kader Aydın¹, Ilknur Özcan¹, Hüsniye Birman², Tamer Lütü Erdem¹, Hülya Koçak Berberođlu³

¹Department of Oral Diagnosis and Radiology, Faculty of Dentistry, Istanbul University, Çapa Istanbul

²Department of Physiology, Istanbul Faculty of Medicine, Istanbul University, Çapa, Istanbul

³Department of Oral Surgery, Faculty of Dentistry, Istanbul University, Çapa, Istanbul

OBJECTIVE: Zinc (Zn) is a precious rare element on behalf of its metabolic activities of the organism and contributes to the development of taste sensation. This study is designed to investigate the effects of Zn in the papillae on the tongue which are the most important anatomic structures responsible for taste perception.

METHODS: For this purpose, in rats consuming pelleted food with different concentrations of Zn, growth and development, daily food and water consumption, taste preferences and consumption rates were determined. Additionally, in histological analysis, alternations of the fungiform papillae and taste buds along with mucosal properties were evaluated. Animals were assigned into four groups each containing 6 rats. Animals were fed diet containing 20, 40, 60 mg/kg Zn for 32 days.

RESULTS: No significant differences in water, salt solution and food consumption were found between the groups. The average muscle thickness showed a significant decrease in the group fed with insufficient Zn.

CONCLUSIONS: The test results and the histological findings seem consistent with each other. No difference in the diameters of the papillae was found related with Zn concentration. The average muscle thickness decreased as a result of Zn deficiency.

Keywords: zinc deficiency, histological, taste perception, experimentally

The constrictor effect of pro-oxidant agents on rat tracheal smooth muscle

Claudia Borza, Germaine Savoiu, Smaranda Gotia, Laura Gotia, Cristian Oancea
University of Medicine and Pharmacy, Timisoara, Romania

OBJECTIVE: In vitro, the pro-oxidant agents have been reported to induce constriction of the airway smooth muscle. The aim of this paper was to study the effect of cumene hydroperoxide and hypochlorite on the tracheal smooth muscle in vitro.

METHODS: There were 12 spiral trachea obtained from male Wistar rats (200 g). A dose-response curve was performed using acetylcholine concentrations from 10^{-5} to 10^{-6} M. After 5 doses response curves and a washing period, samples were incubated for 30 minutes with hypochlorite (10^{-3} M), respectively cumene hydroperoxide (10^{-4} M) and a new acetylcholine dose-response curve was performed. For each study we have performed 4 to 6 experiments. Statistical analysis included calculation of mean values, standard deviation and Student's t-test.

RESULTS: The hypochlorite enhanced the acetylcholine constrictor effect ($29.2 \pm 3.7\%$; $p < 0.001$) for all doses of acetylcholine. Incubation with cumene hydroperoxide enhanced the acetylcholine constrictor effect ($36.5 \pm 1.3\%$; $p < 0.001$), but only for intermediate doses of acetylcholine. The maximal effect of acetylcholine dose response curve did not change significantly.

CONCLUSIONS: The possible mechanism involved in hyperconstriction induced by cumene hydroperoxide is mediated by destruction of epithelial layer.

Keywords: hypochlorite, acetylcholine, cumene hydroperoxide, tracheal smooth muscle.

The metabolic syndrome components in hypertensive patients

Claudia Borza, Rodica Mateescu, Germaine Savoiu, Smaranda Gotia, Laura Gotia, Corina Serban, Gabriela Negrisanu

University of Medicine and Pharmacy, Timisoara, Romania

OBJECTIVE: The correlations between arterial hypertension, hyperglycemia, abnormal lipid profile and obesity are the characteristics of metabolic syndrome (MS) established by the criteria from WHO, NCEAP/ATP III (revised) and IDF. The aim of this study was to evaluate the metabolic syndrome components in hypertensive patients.

METHODS: We investigated 137 patients (38.6% female) with a mean age of 53.8 +/- 2.7 years with a high level of arterial pressure and an abnormal lipid/glucose profile. All patients were evaluated for personal history, anthropometric and biochemical parameters (arterial pressure, lipid profile, blood glucose and body mass index)

RESULTS: Hypertension (II stage) was present in 52,8% of patients and a high triglycerides level (329.72+/- 38.75 mg/dl) was detected in 58% of patients. 45.9% of patients were obese (BMI higher than 30 kg/m²) with a high percentage of abdominal obesity. BMI was correlated with total, LDL-cholesterol values and also with the parameters of glucose metabolism (p<0.001). In study group, 52.2% of patients with MS presented type II mellitus diabetes.

CONCLUSIONS: The results show a strong interrelationship between metabolic syndrome, obesity, abnormal lipidic profile and arterial hypertension. These correlations pointed out the fact that is necessary in hypertensive patients with obesity to control the risk factors and life style which can develop the metabolic syndrome.

Keywords: hypertension, metabolic syndrome, obesity, hyperglycemia

Medical ozone vs. hyperbaric oxygen treatment in excisional wound healing

Mehmet Ozler¹, Bulent Uysal¹, Esra Erdogan², Serdar Sadir¹, Emin Oztas², Ahmet Korkmaz¹, Sukru Oter¹

¹Department of Physiology, Gulhane Military Medical Academy, Ankara, Turkey

²Department of Histology and Embryology, Gulhane Military Medical Academy, Ankara, Turkey

OBJECTIVE: Hyperbaric oxygen (HBO) therapy has been widely used for the treatment of inflammation-based pathophysiological processes. A relative new therapeutic modality, medical ozone therapy (OT), has also been reported to represent beneficial effects in inflammatory pathologies. In this study, the efficacy of HBO and OT were compared in an excisional wound model of rats.

METHODS: First, under ketamine/xylazine anesthesia, two full-thickness excisional skin wounds were created on the back of SD-rats by a 10-mm punch biopsy. Then, the animals were divided into three groups: control, HBO, OT (n=12 for each). Daily HBO and OT treatments were started immediately after wound creation. Ozone was injected at a dose of 1 mg/kg and HBO administrations were set at 2.5 atm for 90 min. One half of the animals were sacrificed on day 5, and the other half on day 8. Evaluation was made by measuring the wound surface areas and excising the wound tissues for histopathologic analysis.

RESULTS: On day 5, both HBO and OT resulted in significantly smaller wound areas than controls, and the wound area of ozone-treated animals was also significantly less than the HBO group. On day 8, no difference was recorded among HBO and OT animals, but both of the treatment groups represented significantly better wound healing compared with control animals. Histopathological evaluations revealed that inflammation decreased whereas the amount of collagen, angiogenesis and epithelialization increased significantly with HBO and OT.

CONCLUSIONS: In conclusion, both HBO and OT efficaciously accelerated wound healing in the model used in this study whereas OT was more powerful during the acute phase of the healing process.

Keywords: Excisional wound; Hyperbaric Oxygen; Ozone therapy; Wound healing

The Effects of Radiotherapy and Chemotherapy on Root Development: Report of 2 Cases

Taha Emre Köse, Oya Barut, Ilknur Özcan, Tamer Lütfi Erdem
Department of Oral and Maxillofacial Radiology, Istanbul University, Istanbul, Turkey

OBJECTIVE: Rhabdomyosarcoma is a malignant neoplasm of primitive mesenchyme exhibiting skeletal muscle differentiation. It is the most common soft tissue sarcoma in children. Head and neck region is the most frequently affected area by rhabdomyosarcoma. Radiotherapy and chemotherapy, the treatment method of rhabdomyosarcoma, affects the development of teeth and root formation. In this study, tooth development and root formation anomalies in 2 rhabdomyosarcoma cases are presented.

METHODS: The 2 cases were a 20-year-old-boy and a 16-year-old-boy who both underwent radiotherapy and chemotherapy because of rhabdomyosarcoma in their head and neck area appealed to our clinic for dental treatments. The patients were 6 and 10 years old respectively when they underwent radiotherapy and chemotherapy. Root genesis anomaly and rhizomicry due to the radiotherapy and chemotherapy were determined with intraoral and extraoral radiological examinations.

RESULTS: Dental treatments of the patients were planned with conservative aspects. Strict oral hygiene was ordered to the patients because of the poor conditions of their teeth roots. They were also invited for 6 month routine control and oral hygiene follow-ups.

CONCLUSIONS: In conclusion, tooth development and root formation abnormalities generated by radiotherapy and chemotherapy; their radiologic appearances seen in rhabdomyosarcoma are emphasized in this study.

Keywords: Root Development, Rhabdomyosarcoma, Radiotherapy, Chemotherapy

Ischemic preconditioning attenuates intestinal ischemia-reperfusion injury

Hakkı Gökbel¹, Mehmet Öz¹, Aysel Kıyıcı², Nilsel Okudan¹, Muaz Belviranlı¹, İdris Mehmetoğlu³

¹Department of Physiology, Meram Faculty of Medicine, Selçuk University, Konya, Turkey

²Department of Biochemistry, Selçuklu Faculty of Medicine, Selçuk University, Konya, Turkey

³Department of Biochemistry, Meram Faculty of Medicine, Selçuk University, Konya, Turkey

OBJECTIVE: Ischemic preconditioning due to exposure of a tissue to ischemia for a brief period followed by reperfusion provides a defensive ability of the tissue to survive the sustained ischemia. In this study, we aimed to evaluate impact of ischemic preconditioning on intestinal ischemia reperfusion injury in an experimental mesenteric ischemia model.

METHODS: The study protocol approved by the Ethics Committee of the Selçuk University Experimental Medicine Research and Application Center. Thirty-two Wistar male rats were divided into four groups, eight rats in each: Group I: sham operated, group II: ischemia/reperfusion (IR) injury, group III: ischemic preconditioning, group IV: IR injury after ischemic preconditioning. Mesenteric artery was clamped for 45 minutes and reperfusion was provided for 120 minutes following ischemia in group III and group IV. Rats in group III and IV were exposed to ischemic preconditioning by mesenteric artery clamp for 10 minutes, and a following reperfusion phase for 10 minutes. Serum ischemia modified albumin (IMA) and intestinal tissue hydroxyproline levels were determined with spectrophotometric methods.

RESULTS: Although serum IMA levels were significantly increased in IR injury, ischemic preconditioning attenuated IR induced overproduction of IMA probably due to suppressive effect on reactive oxygen species. However, tissue hydroxyproline levels were not affected from IR injury, as well as preconditioning.

CONCLUSIONS: Ischemia/reperfusion injury causes a disturbance on serum IMA levels probably due to overproduction of reactive oxygen species. We concluded that ischemic preconditioning has beneficial effects on ischemia/reperfusion injury.

Keywords: Hydroxyproline; ischemia/reperfusion injury; ischemic preconditioning; ischemia modified albumin

Histopathological effects of personal communication devices and microwave ovens frequencies (2450 MHz) radiation in rat kidney tissue: Possible ameliorating effects of melatonin

Nurhan Gumral¹, Nermin Karahan², Mustafa Saygin¹, Salih Dogan³, Mustafa Naziroglu³, Selcuk Comlekci⁴, Sadettin Caliskan¹

¹Department of Physiology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

²Department of Pathology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

³Department of Biophysics, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

⁴Department of Electronics and Communication, Faculty of Engineering, Suleyman Demirel University, Isparta, Turkey

OBJECTIVE: There are very few studies on the biological effects of personal communication devices (Wi-Fi, bluetooth) and microwave ovens frequencies (2450 MHz) in the literature. This study was designed to investigate the histopathological effects of 2450 MHz electromagnetic radiation (EMR) in rat kidney tissue and possible ameliorating effects of melatonin.

METHODS: Thirty two male Wistar Albino rats were randomly grouped as follows (n=8/group): Cage-control group [dimethyl sulfoxide (DMSO) 10 mg/kg/day, i.p., without stress and EMR; Group I], shame-control rats stayed in restrainer without exposure to EMR and DMSO (10 mg/kg/day, i.p.); Group II, rats exposed to 2450 MHz EMR; Group III, 2450 MHz EMR exposed+melatonin (10 mg/kg/day, i.p.) treated group; Group IV. Group III and Group IV were exposed to 2450 MHz EMR 60 minutes/day for 30 days. At the end of 30 days, tissue samples were taken for histopathological examination. Data was estimated by using the chi square test of SPSS v15.0.

RESULTS: There was no significant difference between the groups by means of glomerular sclerosis, tubular necrosis, interstitial fibrosis, thickening of the wall of vessel, hydropic degeneration of tubular epithelial cells. However, in the Group III, vascular congestion ($p<0.05$), interstitial mononuclear cell infiltration ($p<0.01$) were observed significantly increased when compared with Group I, II. In the Group IV, vascular congestion, interstitial mononuclear cell infiltration reduced, but no significant difference when compared with Group III.

CONCLUSIONS: Exposure to 2450 MHz EMR may cause histopathological changes, but treatment by melatonin can diminish these changes. We suggest that melatonin may have a beneficial effect in preventing renal impairments in rats.

Keywords: histopathological changes, melatonin, 2450 MHz electromagnetic radiation, rat kidney tissue,

Age-Related Changes in Respiratory Function of Liver and Heart Rat Mitochondria

Elena Dorina Gheorgheosu¹, Oana Maria Duicu¹, Simona Lavinia Trancota¹, Silvia Nicoleta Mirica¹, Andreea Ionela Anechitei¹, Ovidiu Fira Mladinescu¹, Cristina Adriana Dehelean², Mirela Danina Muntean¹
¹Department of Pathophysiology, University of Medicine and Pharmacy Timisoara, Romania
²Department of Toxicology, University of Medicine and Pharmacy Timisoara, Romania

OBJECTIVE: The present study was purported to compare mitochondrial respiratory rates (RR) in liver and heart mitochondria isolated from aged (18-20 months) vs. adult (4-6 months) Sprague-Dawley rats (n=8/group).

METHODS: Mitochondria were isolated by differential centrifugation and oxygen consumption was measured at 37°C by classic and high-resolution respirometry, respectively. The Substrate-Uncoupler-Inhibitor Titration protocol was used as follows: complex I and complex II-dependent respiration was stimulated by glutamate + malate and succinate+rotenone, respectively (LEAK state) and subsequent ADP addition (OXPHOS state); cytochrome c addition evaluated mitochondria integrity; ATP synthase was inhibited by oligomycin; uncoupled respiration was obtained by FCCP titration in steps of 0.1 µM optimal concentration (ETS capacity); respiration was inhibited with antimycin A.

RESULTS: RR (expressed in $\text{nmol O}_2/\text{min}/\text{mg}$) in aged vs. adult liver mitochondria for CI dependent respiration were: Leak 21.6 ± 0.9 vs. 24.6 ± 0.8 ($p < 0.05$); Oxphos 100.7 ± 2.1 vs. 124.8 ± 5.6 ($p < 0.01$); ETS 88.5 ± 3.6 vs. 131.9 ± 6.6 ($p < 0.001$). A more important decrease of Leak state was obtained for CII supported respiration (33.5 ± 0.8 vs. 42.6 ± 0.8 , $p < 0.001$) with comparable results for the other parameters. For heart mitochondria RR (expressed in $\text{pmol}/\text{s} \cdot \text{ml}$) were: (i) for CI dependent respiration: Leak, 41.6 ± 5 vs. 48.3 ± 2 ; Oxphos 480.2 ± 85.2 vs. 673.4 ± 32.5 , ETS 470.4 ± 63.8 vs. 548 ± 24 and RCR (the ratio between Oxphos and Leak states) 11.5 ± 1.9 vs. 13.62 ± 1.10 ($p < 0.01$); (ii) for CII dependent respiration: Leak 138.1 ± 16.7 vs. 159.03 ± 9.6 Oxphos 536.7 ± 101.8 vs. 630.6 ± 25.53 ; ETS 588.08 ± 100.7 vs. 667.7 ± 28.6 ($p < 0.01$).

CONCLUSIONS: Our results showed an important decline for all respiratory parameters in the senescent group vs. adult group, in both liver and heart mitochondria energized with complex I and II substrates. Research supported by PhD fellowship POSDRU/88/1.5/S/63117.

Keywords: ageing, mitochondria, respirometry

The Importance of Timing for Magnesium Orotate-Induced Cardioprotection During Post-ischaemic Reperfusion in Isolated Rat Hearts

Silvia Nicoleta Mirica¹, Elena Dorina Gheorgheosu¹, Oana Maria Duicu¹, Andreea Ionela Anechitei¹, Maria Danila¹, Ovidiu Fira Mladinescu¹, Denis Angoulvant², Mirela Danina Muntean¹

¹Department of Pathophysiology, University of Medicine and Pharmacy Timisoara, Romania

²Inserm U1060 CarMeN, University Lyon 1, Hôpitaux de Lyon, Lyon, France

OBJECTIVE: Acute administration of magnesium orotate (Mg-Or) at reperfusion has been previously shown to elicit significant protection in isolated rat hearts. We hypothesized that Mg-Or (1mM) induced cardioprotection against post-ischemic reperfusion injuries depends on the timing of intervention.

METHODS: Isolated male rat hearts (n = 6-8/group) were subjected to 30 min global ischaemia followed by 120 min reperfusion. They were randomized to receive: (i) no additional intervention (Ctrl); (ii) early administration of Mg-Or, 2 minutes before the onset of reperfusion (Mg-Or-E) and (iii) delayed administration of Mg-Or 3 minutes after the onset of reperfusion (Mg-Or-D). Functional recovery was assessed after 30 min of reperfusion by measuring the functional parameters: LVDP, contractility (dP/dtmax) and relaxation (dP/dtmin) indices. Infarct size (IS) was measured by TTC staining.

RESULTS: Significant protection of ventricular function was observed in Mg-Or-E vs. Ctrl group: LVDP (67.8±2.8% vs. 39.2±3.2, p < 0.001), dP/dtmax (61.5±2.9% vs. 38.8±4.6%, p < 0.01) and dP/dtmin (72±4.45% vs. 47.6±4.6%, p < 0.01). In Mg-Or-D group important albeit less significant protection of systolic function was present whereas no protection was observed for the relaxation index. Mg-Or induced significant IS reduction in both treated groups as compared to Ctrl (Mg-Or-E, 32.07±1.8%, Mg-Or-D, 35±3% vs. Ctrl 70.4±3.7%, p < 0.001).

CONCLUSIONS: Early administration of Mg-Or before the onset of reperfusion reduces infarct size in the same magnitude as delayed administration, but induces significantly better recovery of myocardial function. Early administration of Mg-Or during the postischaemic reperfusion may also have beneficial effects on non-infarcted dysfunctional cardiomyocytes (stunned myocardium). Research supported by National Authority for Scientific Research grant 42-122/2008, Hungary-Romania Cross-Border Cooperation HURO/0802/011

Keywords: magnesium orotate, ischaemia/reperfusion injury, infarct size, cardioprotection

Light Microscopic Investigation of NAC's Effects on Liver Injury Induced By Methotrexate in Rats

Murat Yagmurca¹, Figen Bala², Orhan Bař³

¹Department of Histology and Embryology, Fatih University, Faculty of Medicine, Ankara, Turkey

²Department of Histology and Embryology, Afyon Kocatepe University, Faculty of Medicine, Afyonkarahisar, Turkey

³Department of Anatomy, Rize University, Faculty of Medicine, Rize, Turkey

OBJECTIVE: Methotrexate (MTX), a folic acid antagonist, is a cytotoxic chemotherapeutic agent used for the treatment of several malignancies and various inflammatory disease, and is known to have hepatotoxic effects on the liver. Acetylcysteine is an antioxidant material. Acetylcysteine has a role in glutathione synthesis in the liver and lung for upregulation glutathione synthesis. The aim of this study is to investigate the effects of NAC (N- Acetylcysteine) on liver damage, which has occurred by MTX using light microscopy.

METHODS: In this study 40 Wistar albino male rats ranging between 250-350 g were used. Rats were evenly divided into four groups (control, methotrexate, methotrexate+NAC and NAC groups). 0.9% sodium chloride (0.5 ml, intraperitoneal [ip]) was administered to Group 1 (control) rats. Group 2 (methotrexate) rats received 0.9% sodium chloride (0.5 ml, ip) in the first two days and a single dose of methotrexate (100 mg / kg, ip) in the third day. For Group 3 (methotrexate+NAC) rats, NAC (180mg/kg, i.p) was started two days before the single dose administration of methotrexate (100 mg / kg, i.p) and it was continued for 9 days. Group 4 (NAC) rats received only NAC (180mg/kg, i.p) for 9 days. Rats were sacrificed under anesthesia and the liver tissues were removed on the 7th day after the administration of methotrexate.

RESULTS: It was found that on the liver of rats, which was given MTX, sinusoidal dilatation, hepatocyte degeneration, vascular congestion-thrombosis, inflammatory infiltration on light microscope.

CONCLUSIONS: As a result of this study, it was found that NAC may decrease MTX-induced liver damage.

Keywords: Methotrexate, NAC, light microscope, hepatotoxicity, rat

Comparative Investigation of Conconi and V-Slope Methods on Accurate Anaerobic Threshold Estimation During Incremental Exercise Test: Modifying the Conconi Test

Oguz Ozcelik¹, Ihsan Serhatlioglu¹, Bayram Yilmaz², Haluk Kelestimur³

¹Firat University, Faculty of Medicine, Department of Biophysics, Elazığ

²Yeditepe University, Faculty of Medicine Department of Physiology, Istanbul

³Firat University, Faculty of Medicine, Department of Physiology, Elazığ

OBJECTIVE: The aims of this study were to compare the accuracy of Conconi and V-Slope methods used in Anaerobic threshold (AT) estimation and also determine the validity of a new method based on work rate divided to heart rate ratio (i.e. modifying Conconi method) on AT estimation.

METHODS: Twenty trained male subjects (19 ± 0.7 yr, 61.3 ± 1.7 kg) have participated to the study after giving signed written informed consents, which were approved by the local ethical committee. Each subject performed an incremental exercise test (15 W/min) using an electromagnetically braked cycle ergometer. Ventilatory and gas exchange parameters were evaluated breath-by-breath. The heart rate was recorded as beat-by-beat. AT estimated using V-Slope, heart rate plotted versus work load and also heart rate divided to work rate plotted versus VO₂. A paired t- test was used to evaluate the values.

RESULTS: Heart rate-work rate relationships were observed as linear increases in 15 subjects, left side deflection in 3 subjects (associated with the V-slope break point) and right side deflection in 2 subjects. The work rate to heart rate ratio showed a clear break point in all subjects and closely associated with the V-slope break point: 1.89 ± 0.03 L/min vs 1.92 ± 0.04 L/min ($p=0.2$).

CONCLUSIONS: In conclusion, absent of break point in heart rate work rate relationship (75%) and extremely low percent of association in AT estimation between Conconi and V-slope method (15 %) may reduce reliability of Conconi test. Thus, caution should be taken by investigators especially when making important decision for patients and sports training. However, work rate to heart rate ratio could provide easy and reliable AT estimation.

Keywords: Anaerobic threshold, V-slope, Conconi, Exercise test, heart rate

The effects of coenzyme Q10 supplementation on exercise performance and oxidative stress in patients with end stage renal failure

Hakkı Gökbel¹, Süleyman Türk², Nilisel Okudan¹, Hüseyin Atalay², Muaz Belviranlı¹, Yalçın Solak²

¹Department of Physiology Meram Faculty of Medicine Selçuk University, Konya, Turkey

²Department of Nephrology Meram Faculty of Medicine Selçuk University, Konya, Turkey

OBJECTIVE: The aim of the study was to investigate the effects of coenzyme Q10 (CoQ10) supplementation on exercise performance and oxidative stress and antioxidant defense in patients with end stage renal failure.

METHODS: Twenty-eight patients with end stage renal failure were participated in the study. At the beginning of the study, the participants performed a 6-min walk test (6MWT) and submaximal exercise test with 24 hours interval. Before, immediately after and 30 min after the submaximal exercise test blood samples were taken. After completing the tests, CoQ10 (200 mg/day) were given orally to 14 patients and placebo to 14 patients for 12 weeks and tests and measurements were repeated. After 4 weeks of wash-out period, placebo was given to the subjects who used CoQ10 first time and vice versa. At the end of the study, all measurements were repeated.

RESULTS: While CoQ10 supplementation caused to increase blood CoQ10 concentrations, it did not affect 6MWT and maximal oxygen consumption. Before and after exercise, there were no difference between the groups in MDA, oxidized LDL and uric acid levels and SOD and GPx activities. While MDA levels increased immediately after the exercise, it was decreased 30 min after the exercise. Although, SOD activities and uric acid levels increased after exercise, there were no changes in oxidized LDL levels and GPx activities.

CONCLUSIONS: CoQ10 supplementation does not affect 6MWT and submaximal exercise performance and exercise-induced oxidative stress and antioxidant defense in patients with end stage renal failure.

Keywords: Coenzyme Q10, exercise performance, haemodialysis, oxidative stress.

Effects of Work Load Intensity on Body Metabolism and Substrate Utilisation during Muscular Exercise Performance

Seda Ugras¹, Bayram Yilmaz², Ihsan Serhatlioglu¹, Nida Aslan¹, Oguz Ozcelik¹

¹Firat University, Faculty of Medicine, Department of Biophysics (Physiology)

²Yeditepe University, Faculty of Medicine, Department of Physiology

OBJECTIVE: Body substrate utilisations at anaerobic threshold (AT), which describes the changes in body metabolic system from aerobic to anaerobic, and at critical power (CP) outputs, which describes the highest sustainable work rate, was examined comparatively in sedentary male subjects.

METHODS: Six subjects performed 6 different exercise tests after giving signed written informed contents which were approved by the local ethical committee. They initially performed an incremental exercise test (15 W/min) to estimation of AT and maximal exercise capacity (Wmax) using cycle ergometer. Each subject performed 5 different constant load exercise tests (30 min): work load corresponded to 25% below AT (46% of Wmax), at the AT (62% of Wmax), at the CP (72% of Wmax), at the 25% above AT (77% of Wmax), and at 100% above AT (122% of Wmax). Ventilatory and pulmonary gas exchange parameters were evaluated breath-by-breath. Metabolic changes were determined using respiratory quotient (RQ). AT was estimated using V-Slope method and CP was by other conventional methods. A paired t- test was used to evaluate values.

RESULTS: Fat oxidation ratio was higher in CP (0.92 ± 0.01) than AT (0.95 ± 0.01) and 25% below AT (0.95 ± 0.005) ($p < 0.05$). RQ was systematically increased work load at the 25% above AT (1.04 ± 0.02) and at 100% above AT (1.55 ± 0.05).

CONCLUSIONS: Interestingly, increasing exercise intensity (from 25% below to AT to CP) caused increases in fat oxidation ratio. However, work load intensity above the CP results in increases carbohydrate oxidation rate due to the anaerobic glycolysis. Increases in fat oxidation at the work load at corresponded to CP could be an important training protocol for clinical medicine.

Keywords: Anaerobic Threshold, RQ, Critical Power Output, Exercise Test

The effect of moderate or high intensity exercise on stressed rats with hypothyroidism

Özgür Çakır Kasımay¹, Dilek Özbeyli³, Ebru Şenel¹, Gazi Contuk², Ayliz Velioglu⁴, Şule Çetinel², Berrak Ç. Yeğen¹

¹Marmara University School of Medicine, Department of Physiology

²Marmara University School of Medicine, Department of Histology and Embryology

³Marmara University Animal Center

⁴Vocational School of Health Related Professions, Medical Laboratory Technics Program

OBJECTIVE: The responses to stress show discrepancy in hypothyroidism. The aim was to study the impact of regular exercise on the peripheral tissues of hypothyroid rats exposed to acute stress.

METHODS: Male Sprague Dawley rats were administered intraperitoneally with 6-n-propyl-2-thiouracil (PTU, 10 mg/kg) for 15 days to induce hypothyroidism. Exercise was performed on a treadmill for 6 weeks at a moderate intensity (MIE; 30min/5days/week) or high intensity (HIE; 60min/5days/week), but the control group did not exercise. Six weeks later, rats were exposed to a 30-min water avoidance stress and then the rats were decapitated to obtain samples of heart, stomach, liver and small intestine for the measurement of lipid peroxidation (LP), glutathione (GSH) levels, myeloperoxidase (MPO) activity and for histological analysis. Data were analyzed by Student's t test or ANOVA.

RESULTS: MPO activities, LP and GSH levels in the tissues of control rats and stressed rats with hypothyroidism were not different. In hypothyroid rats with MIE, MPO levels were increased in the stomach and small intestine as compared to control group ($p<0.05$), while in HIE rats with hypothyroidism cardiac and hepatic MPO activities and LP levels were decreased ($p<0.01-0.001$). IL-1alpha and IL-6 levels were decreased in exercised rats with hypothyroidism. The histological results were parallel to the biochemical data.

CONCLUSIONS: Results revealed that stress did not further increase oxidant damage in tissues affected by hypothyroidism. Moderate exercise enhanced oxidant damage parameters in the gastrointestinal tissues, while high intensity exercise in hypothyroidism depressed oxidant damage in the cardiac and hepatic tissues.

Keywords: exercise, hypothyroidism, stress

Effects of various types aerobic exercise methods on myokines and some other cytokines that secreted from muscle and adipose tissues

Halil DUZOVA¹, Esin Güllü², Güner Çicek³, Burcu Kırımhan Köksal¹, Başak Kayhan⁴, Abdullah Güllü², Ibrahim Şahin⁵

¹Inönü Univ. Medical Faculty, Dept of Physiology, Malatya, Turkey

²Physical Education and Sports High School, Inönü University, Malatya, Turkey

³Physical Education and Sports High School, Hitit University, Çorum, Turkey

⁴Inönü Univ. Medical Faculty, Dept of MEDical Biology and Genetics, Malatya, Turkey

⁵Inönü Univ. Medical Faculty, Dept of Endocriology, Malatya, Turkey

OBJECTIVE: Running-walking and aerobic-steps are the most commonly used exercise methods. It has been demonstrated that exercise increases muscle derived cytokines (myokines) by increasing muscle size and decrease adipose tissue derived cytokines/hormones by reducing adipose tissue size. On the other hand, leptin, tumor necrosis factor- α , resistin and adiponectin secreted from fat tissue may have an effect on muscle tissue.

It was aimed to investigate whether IL-17 and myokines or adipokines were interrelated and to find out which type of aerobic exercise is more efficient on myokine and adipokine homeostasis in females. Any relationship between myokines, insulin resistance and low grade inflammations were investigated during exercise program.

METHODS: Twenty five women were included in this study. They were assigned into two groups. One group (n=10) exercised by performing aerobic steps five days a week for ten weeks. The second group (n=15) exercised by performing running-walking for ten weeks. Serum IL-6, 15, 17,18, TNF α , leptin, adiponectin, and resistin levels were detected by ELISA on the blood samples taken before and 10 min and 2 hours after the study. Results were statistically analyzed and reported.

RESULTS: TNF α , leptin, IL-15 and -18 levels of exercising aerobic-steps group differed significantly from that of walking-running groups. Differences between the levels of leptin, IL-15, and resistin before and after the exercise in running-walking group were found to be statistically significant, whereas in aerobic steps group only TNF α level differed ($p < 0.05$).

CONCLUSIONS: We suggest that running and walking exercises may influence myokine and lipokine systems more efficiently in women.

Keywords: exercise, myokine, interleukin-17, aerobic steps, running-walking, female, ELISA

Association Analyses of Depression, Anxiety, and Physical Fitness Parameters in Turkish Adults

Hakan Mollaoğlu¹, Kagan Uçok¹, Aslı Kaplan¹, Abdurrahman Genç¹, Hasan Mayda², Halil İbrahim Güzel², Ümit Şener¹, Emine Uygur³, Ömer Özbulut²

¹Department of Physiology, Afyon Kocatepe University Faculty of Medicine, Afyonkarahisar, Turkey

²Department of Psychiatry, Afyon Kocatepe University Faculty of Medicine, Afyonkarahisar, Turkey

³Vocational School of Health Services, Namik Kemal University, Tekirdag, Turkey

OBJECTIVE: This study aims to investigate the associations among depression, anxiety, aerobic exercise capacity, body fat percentage, sum of skinfolds, abdomen circumference, and waist to hip ratio on the basis of body mass index (BMI) in adults.

METHODS: The subjects of the study were 60 obese participants (30 women, 30 men) with BMIs over 30 kg/m² and 60 healthy controls (30 women, 30 men) with BMIs of 18-25 kg/m². Body fat percentage was calculated from the skinfold thickness using the formula. Body circumference measurements were performed using a tape measure. Maximal aerobic capacity (VO₂max) was determined by Astrand submaximal exercise protocol. Two self-reported questionnaires, Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI), were administered to all participants.

RESULTS: BMI, body fat percentage, sum of skinfolds, abdomen circumference, and waist to hip ratio were found to be higher in obese groups as compared to the controls, while VO₂max (ml/kg/min) values were lower in both genders. In males, BAI scores and mild-level anxiety percentage values were higher in the obese group than in the control group. There was no significant difference for BDI scores and levels between the obese and control groups in both genders. There was also no significant difference in BAI scores and levels between the obese and control groups in women.

CONCLUSIONS: The fact that physical fitness is found to be poor in obese individuals shows the existence of a condition that might constitute an increased tendency for obesity-related disorders. In addition, it was suggested that, in Turkey, attitudes toward obesity change depending on gender.

Keywords: Obesity, Physical fitness, Anxiety, Depression, Anthropometry, Body fat

Psychophysiological states in athletes

Georgiy Korobeynikov, Lesia Korobeinikova

Sport Biology Department, National University of Physical Education and Sport, Kiev, Ukraine

OBJECTIVE: The functional states of athletes reflect the integral complex of functional system elements which are responsible for the effectiveness of activity. Many works deal with the psychological diagnostics, influence of physical performance on cognitive functions in athletes. But the modern methods diagnostics are ignored the integral criteria of psychophysiological states which are used for athletes.

METHODS: A total of 27 athletes (men) were examined. An automated method of testing of a number of cognitive functions was studied.

RESULTS: The results of the investigation showed that 8 subjects had a high estimated, 14 have the intermediate and 5 have a low level of psychophysiological states. This explains the fact that, during sporting activities in athletes with higher level of psychophysiological states deteriorate earlier than in low level. The elaboration method of psychophysiological diagnostics gives possibilities to control for functional states of higher qualification athletes with individual-typological peculiarities. According to our studies the adaptation to activity performed resulted in athletes with increasing of psychophysiological states level are characterised of reduction meanings of the variability of the psychophysiological parameters. Thus, the specific features of psychophysiological states in athletes are manifested with optimized the adaptive compensatory brain mechanisms of decline of visual perception and information processing capability in athletes.

CONCLUSIONS: The analysis of the studied athletes show that the biggest level of psychophysiological state in higher qualification sportsmen is related with increasing of tension and compensation of psychophysiological organization of information processing.

Keywords: psychophysiological states, athletes, of information processing, cognitive functions

The effects of docosahexaenoic acid supplementation and exercise on growth hormone and insulin-like growth factor I serum levels during chronic hypoxia in rats

Sebahat Turgut¹, Haydar Ali Erken², Gülten Erken³, Ceylan Ayada¹, Osman Genç¹, Günfer Turgut¹

¹Department of Physiology, Faculty of Medicine, University of Pamukkale, Denizli, Turkey

²State Hospital of Balıkesir, Balıkesir, Turkey

³Department of Physiology, Faculty of Medicine, University of Balıkesir, Balıkesir, Turkey

OBJECTIVE: In this study it was examined the effects of docosahexaenoic acid (DHA) on growth hormone (GH), insulin-like growth factor I (IGF-I) and insulin-like growth factor binding protein-3 (IGFBP-3) in response to chronic hypoxia and exercise training in hypoxic conditions.

METHODS: Thirty-five rats were divided into five groups; control group (C), hypoxia group (H), hypoxia-exercise group (HE), hypoxia-docosahexaenoic acid group (HD), hypoxia-exercise- docosahexaenoic acid group (HED). A treadmill exercise was performed as 30 m/min for 20 min/day, five days per week for 28 days at level grade in exercise groups. DHA was given HD and HED groups every day orally (36 mg/kg). The animals except controls were exposed to hypoxia during twenty-eight days.

RESULTS: Serum levels of GH and IGF-I in H group decreased after chronic hypoxia ($p<0.001$). GH and IGF-I in HD group also decreased compared with C group ($p<0.05$, $p<0.01$, respectively). GH in controls did not show significant difference compared with HE and HED groups. Decreased serum level of IGF-I was observed for HED group ($p<0.05$).

CONCLUSIONS: According to findings, we can say that chronic hypoxia exposure decreases serum levels of GH and IGF-I and exercise training have slightly positive effect on GH/IGF-I axis during hypoxia. Also DHA supplementation slightly increases GH and IGF-I serum levels in hypoxic conditions. But this effect on GH/IGF-I axis during hypoxia is not strong compared with exercise. That's why we concluded that exercise and/or DHA supplementation doesn't have additional positive effect on these hormones in hypoxic conditions.

This study was supported by Pamukkale University Research Fund.

Keywords: Hypoxia, GH, IGF-I, exercise, DHA

Physical Effort, Mouth Ecosystem and Body Homeostasis

Smaranda Rodica Gotia¹, Smaranda Laura Gotia¹, Camelia Gurban², Nicoleta Filimon³

¹Department of Physiology, University of Medicine and Pharmacy, Timisoara, Romania

²Department of Biochemistry, University of Medicine and Pharmacy, Timisoara, Romania

³Biology Department, West University, Timisoara, Romania

OBJECTIVE: Mouth as interface of the body with the environment, is responsive to environmental factors that affect body homeostasis. Food qualities, content of nutrients, vitamins, minerals, influence the mouth ecosystem, saliva composition, number and variety of bacterial species that inhabit the oral environment and forming dental plaque and tartar. This is the first step toward oral pathology. The purpose was to discuss the mouth ecosystem correlated with physical effort.

METHODS: Ten trained teenagers (13.35 ± 0.47 years old) that performed a submaximal effort to a medicinal bicycle were investigated. Before and after physical effort, salivary flow, pH, epithelial cells were determined and salivary leukocyte phagocytosis was estimated by NBT test.

RESULTS: After the physical effort salivary flow was decreased by 22%, pH by 10%, and cell viability by 3.09%. Salivary epithelial cells increased by 20%, and leukocytes by 37.08%, associated with high values of phagocytosis. Smaller amount of saliva produces dry mouth, can affect nutrition, teeth health, and activates thirst sensation and ADH secretion in order to establish the water homeostasis. Dental plaque triggers oral cavity defense mechanisms, inducing leukocyte recruitment into the gum, gingival fluid. Leukocyte enzymes trigger and maintain inflammation, endothelial injury, activate the complement system, and amplify gingival lesions. Salivary leukocytes were active in local defense.

CONCLUSIONS: Physical exercise decreased salivary flow, increased gingival epithelium desquamation, lowered salivary pH, and salivary leukocyte defense capacity. Reducing the environmental factors that impact negatively on oral and rigorous oral hygiene may be a condition in the prevention of oral cavity lesions.

Keywords: physical effort, saliva, body homeostasis

Investigation of Oxidative Stress, Aerobic Capacity, Daily Physical Activity and Pulmonary Functions in Patients with Mild to Moderate COPD

Abdurrahman Genç¹, Kagan Uçok¹, Ümit Şener¹, Tülay Koyuncu², Olcay Albaş², Sefa Çelik³, Mehmet Ünlü²

¹Department of Physiology, Afyon Kocatepe University Faculty of Medicine, Afyonkarahisar, Turkey

²Department of Pulmonary Medicine, Afyon Kocatepe University Faculty of Medicine, Afyonkarahisar, Turkey

³Department of Medical Biochemistry, Afyon Kocatepe University Faculty of Medicine, Afyonkarahisar, Turkey

OBJECTIVE: In this study, investigation of total oxidant and antioxidant status, aerobic exercise capacity, daily physical activity, pulmonary functions, body composition, and health related quality of life changes as well as the associations among these parameters was aimed in patients with chronic obstructive pulmonary disease (COPD) versus healthy controls.

METHODS: A total of thirty male patients with COPD and thirty healthy male controls were included in this study. Total oxidant and total antioxidant status were measured in venous blood sera. Aerobic exercise capacity, daily physical activity, pulmonary function tests, body composition, skinfold thickness and circumference measurements were determined.

RESULTS: Aerobic exercise capacity was found to be lower in patients with COPD in comparison to the ones in healthy controls. In terms of daily physical activity, no significant differences were found between two groups. The total oxidant value was higher in patients with COPD than the ones in healthy controls, whereas the total antioxidant value was lower in COPD group compared to the control group. Body fat percentage and waist to hip ratio were found higher in patients with COPD than the ones in healthy controls.

CONCLUSIONS: Unaffected daily physical activity in patients with mild to moderate COPD might be attributed to the mild levels of pulmonary dysfunction. It seems that oxidative stress, pulmonary functions and aerobic capacity are interrelated parameters in patients with COPD and we think they might have a role in the pathogenesis of COPD. This study also suggests that abdominal obesity might be considered in the COPD phenotype.

Keywords: Aerobic exercise capacity, daily physical activity, chronic obstructive pulmonary disease, oxidative stress, body composition

Investigation of Physical Activity and Quality of Life Differences Between Male and Female Young Adults

Abdurrahman Genç, Ümit Şener, Hatice Karabacak, Kagan Uçok
Afyon Kocatepe University Faculty of Medicine Department of Physiology, Afyonkarahisar, Turkey

OBJECTIVE: The aims of this study were to inquire the physical activity and quality of life differences between male and female young adults and to investigate associations among these parameters.

METHODS: International physical activity questionnaire short form (IPAQ) and life quality questionnaire (SF36) were applied to a total of 710 (387 female, 323 male) volunteer young adults. Weekly vigorous, moderate and mild (walking) physical activity times and daily sitting times were determined with using IPAQ. Physical and mental health scores of SF36 were calculated. Total physical activity times of participants were calculated and then classified as low, moderate and high for both genders.

RESULTS: Body Mass Index, vigorous physical activity, moderate physical activity, total physical activity, SF36- physical health score and SF36- mental health score were found higher in males than in females. In terms of physical activity level, there were statistically significant differences among the percentages of males and females shown as low, moderate, and high. Only, vigorous physical activity time showed mild positive correlations with SF36- physical health score.

CONCLUSIONS: Total physical activity being found higher in males compared to females, may contribute to the higher quality of life in males. Because of vigorous and moderate physical activity differences between males and females excluding mild physical activity, total physical activity was found different in males and females. Usually, vigorous and moderate physical activities are enjoyable team activity. These enjoyable vigorous and moderate physical activities may be proposed to increase quality of life in young females.

Keywords: Physical activity, life quality, young adults

Investigation of Aerobic Exercise Capacity, Resting Metabolic Rate, and Respiratory Functions and Body Composition in people having different BMI

Emine Uygur¹, Kagan Uçok², Abdurrahman Genç², Ümit Şener², Ramazan Uygur³, Ahmet Songur⁴

¹Vocational School of Health Services, Namik Kemal University, Tekirdag, Turkey

²Department of Physiology, Afyon Kocatepe University Faculty of Medicine, Afyonkarahisar, Turkey

³Department of Anatomy, Namik Kemal University Faculty of Medicine, Tekirdag, Turkey

⁴Department of Anatomy, Afyon Kocatepe University Faculty of Medicine, Afyonkarahisar, Turkey

OBJECTIVE: The aim of this study is to make a comparative analysis of aerobic exercise capacity, resting metabolic rate (RMR), respiratory functions, body mass index (BMI), lean body mass as well as body fat and water percentage, thereby investigating the associations among these parameters in obese and normal weighted adult people.

METHODS: We enrolled a total of 60 obese participants (30 women, 30 men) whose the BMI is 30 kg/m and over and 60 control subjects (30 women, 30 men) with BMI of 18.5-25 kg/m for this study. Body composition was determined using the bioelectrical impedance analyzing system. RMR was measured with indirect calorimeter equipment. Aerobic exercise capacity was determined by following Astrand submaximal exercise protocol. Pulmonary function tests were carried out by portable spirometer. Statistical analyses were done by SPSS 16.0 computer program using t-test and Pearson correlation tests.

RESULTS: In both genders, we found higher body fat %, the total body fat, total water amount, net body weight and RMR in obese subjects than the control ones; while body water %, VO₂max (ml/kg/min) and VO₂max (ml/lean kg/min) was found lower. Only forced expiratory volume in 1 second (FEV₁) among the pulmonary function tests was higher in male obese group than in controls. Forced vital capacity (FVC) and FEV₁ were higher in female obese group than in controls.

CONCLUSIONS: These results suggest that RMR might be affected by body fat besides the lean body weight. Also, physical fitness has been found lower and some respiratory functions were diminished in obese subjects in comparison to the normal control ones.

Keywords: Obesity, body composition, resting metabolic rate, respiratory functions, aerobic exercise capacity

Anterior cruciate ligament reconstruction improves the metabolic energy cost of level walking at customary speeds

Uğur Dal¹, Mehmet Çolak², İrfan Ayan², Turan Yaroğlu², Figen Dağ¹, Cengiz Yılmaz², Hüseyin Beydağı¹
¹Department of Physiology, School of Medicine, University of Mersin, Mersin, Turkey

²Department of Orthopaedics and Traumatology, School of Medicine, University of Mersin, Mersin, Turkey

OBJECTIVE: The metabolic energy cost of walking is altered by pathological changes in gait. It is thought that anterior cruciate ligament (ACL) deficiency alters the energy requirement for level walking through its effect on gait pattern. In this study, it is hypothesised that the metabolic energy cost of walking would improve after ACL reconstruction

METHODS: Eight patients who were undergoing ACL reconstruction for an isolated rupture were included in this prospective study. Clinical examinations, Lysholm scores and metabolic tests were performed preoperatively and at 3, 6 and 12 months after ACL reconstruction using autologous quadruple hamstring tendons. For the metabolic evaluation, net oxygen cost was calculated while walking on a treadmill at 50-70-90-m/min velocities. A two-way factorial ANOVA was performed in order to evaluate the primary effects and interactions of the time point and velocity variables on net oxygen cost.

RESULTS: All patients had positive Lachman and anterior drawer tests preoperatively that became negative postoperatively and remained negative until the last follow-up point. The mean preoperative Lysholm score was 66, whereas the mean postoperative follow-up scores were 85, 91 and 94, respectively. The interaction between follow-up time point and velocity was not significant. Regardless of the selected velocity, the net oxygen cost was lower than that at preoperative levels at each postoperative time point ($p < 0.05$).

CONCLUSIONS: The results of the present study indicate that the energy cost of level walking in chronic ACL-deficient patients improves after ACL reconstruction. Cause-effect-based studies with correlation evaluations that compare kinetic, kinematic and electromyographic data and metabolic cost calculations should facilitate more accurate analyses.

Keywords: Anterior cruciate ligament Anterior cruciate ligament reconstruction Energy expenditure of walking, Gait, Oxygen cost

Effects of smoking, fruit consumption and physical activity on blood oxidant and antioxidant levels and pulmonary functions

Songül Doğanay¹, Sedat Akar¹, Serap Yildirim¹, Nezahat Kurt², Abdulkadir Yıldırım²

¹Department of Physiology, Medical Faculty, Ataturk University, Erzurum, Turkey

²Department of Biochemistry, Medical Faculty, Ataturk University, Erzurum, Turkey

OBJECTIVE: It has been reported that fruit consumption can prevent deleterious effects of smoking. The aim of this study was to investigate the effects of smoking, fruit consumption and physical activity on lung functions and serum oxidant and antioxidant parameters in young persons.

METHODS: This study was carried out on 164 healthy volunteers aged 18-28. Smoking, physical activity and fruit consumption status were determined with an inquiry. Pulmonary functions tests (PFT) were measured using pony spirometer. Serum malondialdehyde (MDA), total oxidant and total antioxidant levels were determined with spectrophotometry.

RESULTS: Working groups non-smoker, leaving, among active-smokers, passive smokers, or, in terms of PFT values did not differ significantly. Likewise, serum MDA and TOS and TAS levels were not significantly different between study groups.

CONCLUSIONS: In this study, there was no relationship between PFT and smoking, fruit consumption or exercise. This may be due to lack of oxidant/antioxidant imbalance in young smokers having low pack-year at present. Therefore, smokers, especially having low pack-years, should be advised to quit smoking before a pulmonary disturbance occurs.

Keywords: Pulmonary function tests, physical activity, malondialdehyde, total antioxidant capacity, fruit consumption

The effect of submaximal acute exercise on platelet activation: The role of NO

Kutluhan Ertekin, Gülriç Ersöz

Ankara University, Faculty of Medicine, Department of Physiology, Ankara, Turkey

OBJECTIVE: The key role of platelets in the pathogenesis of atherosclerosis prompted considerable interest on the effect of physical exercise on platelets. The studies have shown that various intensities of acute exercise affect platelets differently. The exact mechanisms and the regulatory pathways concerned in the effect of acute exercise on platelet function are not completely understood. Shear induced nitric oxide (NO) release has been suggested a probable mechanism that involves in exercise-induced platelet responses. In the present study we aimed to examine the effect of submaximal exercise on platelet activation and the role of NO.

METHODS: Twenty-one healthy, sedentary male volunteers performed 15 minutes of cycling exercise at a workload that increased their heart rate to 60% of maximal. Platelet glycoprotein IIb/IIIa (GPIIb/IIIa) as a marker of platelet activation and plasma nitric oxide (NO) were evaluated by enzyme-linked immunassay before and immediately after the exercise

RESULTS: Platelet GPIIb/IIIa decreased after the exercise protocol ($p=0.036$). NO significant difference was found between plasma NO levels measured before and after the exercise.

CONCLUSIONS: The exercise protocol performed in the present study has inhibited the platelet activation since NO levels did not increase. We found no clear-cut relationship between platelet GPIIb/IIIa and plasma NO. Since various mediators can activate or inhibit platelets, the balance between platelet activating and inhibiting systems during exercise are needed to be examined.

Keywords: Exercise, Platelet activation, Nitric Oxide, Glycoprotein IIb/IIIa, Endothelium

Effects of Exhausting Exercise On Muscle Cytokine (IL-6) In Long-Term Exercise Trained and Untrained Rats

Elcin Taylan Deveden¹, Lamia Pınar¹, Güleser Goktas², Cigdem Ozer¹, Deniz Erdoğan²

¹Department of Physiology, Gazi University, Ankara, Turkey

²Department of Histology, Gazi University, Ankara, Turkey

OBJECTIVE: In this research, long-term exercise trained (12 weeks/5days/30min) and untrained rats were forced to make exhausting exercise. Immune responses of muscle (IL-6) against the destructive activity of the fast-twitch muscles were compared in two groups.

METHODS: Both groups were sacrificed just after exhaustion; one day after exhaustion and three days after exhaustion. The immunoreactivity of the muscles was examined by IL-6 immunohistochemical evaluation in Histology Laboratories of Gazi University Medical Faculty.

RESULTS: In both acutely running and exercise-trained rats, IL-6 levels were found to increase significantly compared to controls. However, the strongest IL-6 immunoreactivity emerged in both experimental groups 'one day after exhaustion group', being greater in the acutely run group. Three days after exhaustion, IL-6 levels were found weaker especially in the exercised-trained group.

CONCLUSIONS: We found that IL-6 immunoreactivity in the rat plantar muscles that exposed to vigorous contractions and destructions mostly exacerbated one day following exhaustion, and lasted about 3 days. This experiment and the other results related with muscle damage after exhausting exercise (that were not shown here), revealed that muscles of the exercise-trained rats were more resistive to this type of destructive muscle contractions than untrained rats. The IL-6 levels that not prominently increased just after the exhaustion but one day after exhaustion make us think that those pro-inflammatory factors might be suppressed by another agent -most probably by cortisol- in the beginning of the muscle damage and increased after the diminishing effects of these agents.

Keywords: Exhausting exercise, Myokines, Muscle damage

Long-Term Exercise Training Lowered the Destructive Effects of the Oxidants In Muscles Exposed to Exhausting Exercise

Elcin Taylan Deveden¹, Güleser Goktas², Cigdem Ozer¹, Arzu Aral³, Deniz Erdoğan², Lamia Pınar¹

¹Department of Physiology, Gazi University, Ankara, Turkey

²Department of Histology, Gazi University, Ankara, Turkey

³Department of Immunology, Gazi University, Ankara, Turkey

OBJECTIVE: In this study we evaluated the muscle damage depend on exhausting exercise and the effects of exhausting exercise on oxidative stress in rats that underwent acute and chronic exercise.

METHODS: 48 male Wistar albino rats were used and long-term (12week/5day/30min) exercise trained (chronic) and untrained (acute) rats were forced to make exhausting exercise, and they were sacrificed; just after exhaustion, 1 day and 3 days following exhaustion. Free oxygen radicals (MDA), that are reported being responsible for muscle damage after exercise, and antioxidants (GSH) as a scavenger of the oxidants were assessed in rats spectrophotometrically and extend of muscle damage were assessed histologically respectively.

RESULTS: After exhaustion, there were significantly increase in oxidants and also the signs of destruction in the muscle fibers in both groups. However the findings of muscle damage and oxidant levels were found higher and antioxidant levels lower in acutely running rats in all groups comparing to the chronically exercise-trained rats. Maximum muscle damage and oxidant levels emerged 1 day after exhaustion in both trained and untrained groups that were analyzed. The drop of oxidants and increase in antioxidants and the visualized regeneration process in histological samples appeared more significantly in the third day after exhaustion in the trained animals but not in the acutely run animals.

CONCLUSIONS: These data showed that degeneration process in the muscles intensified one day after exhaustion in both trained and untrained groups, however regeneration process developed significantly 3 days after exhaustion in chronic groups, but the regeneration signs was not significant in acutely run rats 3 days after exhaustion.

Keywords: Antioxidants, Exhausting Exercise, Muscle damage, Oxidants

Effect of Combined Aerobic and Progressive Resistive Exercise on Aerobic Capacities of Obese People

Gülin Fındıkoğlu¹, Aysun Özlü¹, Emine Kılıç Toprak², Füsun Ardıç¹

¹Department of Physical Medicine and Rehabilitation, Pamukkale University, Faculty of Medicine, Denizli, Turkey

²Department of Physiology, Pamukkale University, Faculty of Medicine, Denizli, Turkey

OBJECTIVE: The aim of this study was to investigate the effect of combined aerobic and strengthening exercises on the aerobic capacities of obese people.

METHODS: Fifty patients who applied Physical Medicine and Rehabilitation Department of Pamukkale University, with a body mass index (BMI)>30 and completed the exercise program participated to this study. Patients completed a total of 30 sessions of exercise program for 10 weeks, 3 times a week. Aerobic capacities were evaluated by stress exercise testing with Modified Bruce Protocol made before and after the exercise program. Intensity of aerobic exercise program was determined by 50-70 % of the maximum heart rate calculated with Karvenon formule. Strengthening exercise was applied by weights corresponding to 50-60 % of 1 repetition maximum of pectoral, abdominal, lower and upper extremity muscles over 2 sets of 10 repetitions. Paired T test was used for statistics.

RESULTS: Mean age, weight, height and BMI of the 45 women and 5 men registered in our study were 48.48±9.9; 87.62±11.16 kg; 161.2±6.4cm; 33.81±3.4kg/m², respectively. A statistically significant difference was observed in relative VO₂max (33.26±7.15 vs 43.57±7.12 ml/kg/min), absolute VO₂max (2.81±0.66 vs 3.90±1.52 L/min) and MET (9.49±2.05 vs 12.06±2.6). No statistically significant alteration was obtained in maximal heart rate reached at peak exercise level.

CONCLUSIONS: The results of this study indicate that the increase observed in aerobic capacity is independent of the weight change.

Keywords: obesity, combined exercise, aerobic capacity, aerobic exercise, resistive exercise

Effect of Combined Aerobic and Progressive Resistive Exercise on Body Composition of Obese People

Gülin Fındıkoğlu¹, Aysun Özlü¹, Emine Kılıç Toprak², Füsun Ardıç¹

¹Department of Physical Medicine and Rehabilitation, Pamukkale University, Faculty of Medicine, Denizli, Turkey

²Department of Physiology, Pamukkale University, Faculty of Medicine, Denizli, Turkey

OBJECTIVE: The aim of this study was to investigate the effect of combined aerobic and resistive exercise on body composition.

METHODS: Among patients who applied to PMR Department, 50 patients with body mass index (BMI)>30 who completed exercise programme were evaluated. Patients completed a total of 30 sessions of exercise programme for 10 weeks, 3 times a week. Body composition was measured by TANITA in the fasting state. Intensity of aerobic exercise programme was determined by 50-70 % of the maximum heart rate calculated with Karvenon formule. Strengthening exercise was made by weights corresponding to 50-60 % of 1 repetition maximum (RM) of pectoral, abdominal, lower and upper extremity muscles over 2 sets of 10 repetitions.

RESULTS: The mean age, weight, height and BMI of the 45 women and 5 men registered in our study were 48.48±9.9; 87.62±11.16 kg; 161.2±6.4 cm; 33.81±3.4 kg/ m² respectively. A statistically significant difference was detected before and after exercise measurements in weight, BMI percent of fat, mass of fat, percent of lean body weight, waist circumference and hip circumference. There was no significant alteration in lean body mass, waist to hip ratio and basal metabolic rate.

CONCLUSIONS: Optimal exercise programme for obese people aims at decreasing fat mass while preserving muscle mass. Aerobic exercises play a role in decreasing mass and percent of fat, whereas strengthening exercise contributes to preservation of muscle mass. Combination of these exercises was found to be effective.

Keywords: obesity, combined exercise, body composition, aerobic exercise, resistive exercise

Alterations in red blood cell rheological properties in healthy persons living at low and mid-altitudes

Sami Aydogan, Aise Seda Artis, Erdem Başaran, Nazan Dolu

University of Erciyes, Medical Faculty, Department of Physiology, 38039, Kayseri, Turkey

OBJECTIVE: After living at high altitude for a long time, the body begins to adapt to the oxygen shortage. The most important adaptation may be an increase in the number of red blood cells (RBC). On the other hand, in microcirculation, blood flow and adequate oxygen delivery to tissue are mainly dependent on RBC rheologic properties. In this study, the changes of hemorheological properties of two groups of healthy people who lived at low (1300 m) and mid (2300 m) altitudes were investigated.

METHODS: Twenty-six healthy volunteers in each group were studied and hematologic and hemorheologic parameters were measured from blood samples. Hemoglobin (HGB), packed cell volume (PCV), RBCs, MCV, MCH and MCHC were measured by means of an electronic particle counter. Deformability of RBC was measured by Rheodyne SSD Laser Diffractionmeter. Aggregation measurements were done by Myrenne Aggregometer. RBC osmotic fragility was determined in a series tubes with increasing concentrations of NaCl solution by spectrophotometer and calculated as 50 % hemolysis.

RESULTS: RBC, HGB and PCV values were higher at mid altitudes than those at low altitudes. However, there were no significant differences in MCV, MCH and MCHC as red cell indices between two groups. In the other hands, as rheological parameters, erythrocyte deformability and osmotic fragility values were lower who lived at mid altitude compared to low altitude, but an increased were observed in RBC aggregation index (M1).

CONCLUSIONS: We conclude that especially hemorheological changes of red blood cells at different altitudes can be an important signal for high altitude diseases.

Keywords: Altitude, RBC, Hematologic and Rheologic properties

Exercise-induced alterations in hemorheological parameters in sedentary and exercise-trained individuals living at mid-altitude

Sami Aydogan, Erdem Başaran, Aise Seda Artis, Nazan Dolu

University of Erciyes, Medical Faculty, Department of Physiology, 38039, Kayseri, Turkey

OBJECTIVE: The effects of high altitude on humans are considerable, starting from as low as 1300 m above sea level. Usually by 2000 m, they will experience more fatigue, and a greater potential for dehydration. Altitude also affects individuals differently. The aim of this study was to investigate the effects of moderate regular exercise on hematologic and blood rheologic properties on subjects who had been living at mid-altitude.

METHODS: Venous blood samples were obtained from 21 sedentary and 23 regularly trained male, who stayed for a long time at 2300 m. Hemoglobin (Hb), hematocrite, red blood cells (RBCs), MCV, MCH and MCHC were measured by using an electronic particle counter. RBC deformability was measured by Rheodyne SSD. Aggregation measurements were done by an aggregometer. Osmotic RBC fragility was determined in a series of NaCl solution by spectrophotometer.

RESULTS: Hematological parameters (RBC, Hb, MCV, MCH and MCHC) and erythrocyte deformability indices of trained subjects were almost identical to those of sedentary ones, except the hematocrite values which were higher in the trained group than the sedentary group. But erythrocyte aggregation index (M1) and osmotic fragility values were lower in the trained subjects.

CONCLUSIONS: As a result, moderate exercise when living at mid-altitude causes increased hematocrite values probably due to dehydration, as well as lower erythrocyte aggregation and osmotic fragility. But other hematologic parameters and erythrocyte deformability indices do not change significantly. If you live at higher altitudes, your body has long-term adaptation to altitude that allows easily compensate for the lack of oxygen by regular exercising.

Keywords: Altitude, Exercise, Hemorheological parameters

Influence of acute daily mountain climbing on some hematological parameters and red blood cell rheology in winter

Nazan Dolu¹, Erdem Başaran², Aise Seda Artis¹, Sami Aydoğan¹

¹Erciyes University, Faculty of Medicine, Department of Physiology, Kayseri, Turkey

²Erciyes University, Institute of Health Sciences, Department of Physiology, Kayseri, Turkey

OBJECTIVE: Accurate reports of some hematological parameters and red blood cell rheology during acute daily mountain climbing activity are sparse. The purpose of this study was to investigate some hematological parameters (erythrocyte, leucocyte number, hematocrit value, hemoglobin concentration together with erythrocyte deformability, aggregation and osmotic fragility, etc) during a daily winter mountain climbing to Erciyes Mountain (3500 m) in Kayseri, Turkey.

METHODS: Seventeen university students, who are regular mountain climbers, participated in this study. They completed their ascent and descent to Erciyes in 24 hours. Hematological parameters, erythrocyte deformability, aggregation and osmotic fragility were determined from blood samples immediately before and after mountain climbing activity.

RESULTS: When we compared hematological parameters, white blood cells (neutrophil, lymphocyte, basophilic) were statistically increased; eosinophil and hematocrit values were decreased after climbing. We observed a significant decrease in the second measurements of erythrocyte deformability and osmotic fragility compared to the initial measurements. But there were an increase in aggregation indexes in the second measurements.

CONCLUSIONS: We suggest that differences in white blood cells and red blood cell rheology may result from low temperature in mountains. Physical activity may also change hematological parameters by itself. There was no sign of infectious diseases in the people studied. We need further studies for identify these results.

Keywords: mountain climbing, hematological parameters, red blood cell rheology

Muscle Damage, Total Oxidant Status, and The Liver Enzyme levels Related to Coenzyme Q10 Application in Endurance Skiers During The Training Program

Nevzat Demirci¹, Ebru Beytut², Nadide Nabil Kamiloğlu², Pervin Toptaşdemirci¹

¹Kafkas Üniversitesi Eğitim Fakültesi Beden Eğitimi Anabilim Dalı, Kars, Turkey

²Kafkas Üniversitesi Veteriner Fakültesi Fizyoloji Anabilim Dalı, Kars, Turkey

OBJECTIVE: In our study, it was aimed to investigate the relations between the muscle damage, the changes in the TOS, the liver enzyme levels and the use of CoQ10.

METHODS: The treatment in the control groups was planned to be 100 mg and 200 mg of CoQ10. During the research, three groups followed a training programme with 70-80% maximum repeated 2 hours every day, every day for one week. Before and after the training the blood was taken two times from the sportsmen to identify enzyme activities CoQ10, with TOS, AST, ALT, ALP, GGT, LDH and CK. The blood samples were analyzed in the laboratory of Kafkas University Medicine Faculty. The levels of CoQ10 and the TOS were measured using HPLC and the kit of TOS according to the manufacturer's instructions.

RESULTS: When the CoQ10 and TOS levels of Endurance Skiers were compared before and after the training; although it was understood that these was an increasing having level of $p < 0.01$ there was a decrease in the TOS levels experimental group about using of CoQ10; However, it was understood that here were important increases ($P < 0.001$) in the levels of AST, ALT, ALP, GGT, LDH and CK. But, about the experiment group it was found these were decreases in value of the activity of enzyme ($p < 0.05$).

CONCLUSIONS: As a result, it was understood that the levels of TOS, liver and muscle activities of experimental group were lower than the control group and the reason of this result may depend on the use of CoQ10.

Keywords: CoQ10, enzyme activities, muscle damage, total oxidant, Training

Effects of exercise preconditioning on remote organ injury in intestinal ischemia-reperfusion induced rats

Hakkı Gökbel, Mehmet Öz, Nilsel Okudan, Muaz Belviranlı

Department of Physiology, Meram Faculty of Medicine, Selcuk University, Konya, Turkey

OBJECTIVE: Intestinal ischemia-reperfusion (IR) injury affects not only the intestines but also remote organs due to activated neutrophils and formation of free radicals. The present study aimed to investigate the effects of exercise preconditioning on heart and lung injury in intestinal IR induced rats.

METHODS: Sixty male Wistar rats were divided into six groups: Sham-operated (SHAM), IR, Exercise (E), E+IR, Ischemic preconditioning (IPC), IPC+IR. Intestinal IR injury was induced in rats by clamping the superior mesenteric artery (SMA) for 45 minutes followed by 120 minutes of reperfusion. The IPC groups underwent a short-term IR prior to long-term IR. Animals in exercise groups were run on a treadmill for four weeks. Interleukin-6 (IL-6) and tumor necrosis factor (TNF)-alpha levels were measured in serum samples and malondialdehyde levels and superoxide dismutase activities were measured in heart and lung tissues.

RESULTS: Exercise training decreased serum concentrations of TNF-alpha compared with that of control rats ($p<0.05$), whereas IL-6 levels did not differ among groups. In the IR group MDA levels increased in lung tissue. MDA levels of the lung and heart tissues were lower in the E+IR and IPC+IR groups compared to the IR group ($p<0.05$). SOD activities of the lung and heart tissues were higher in IPC+IR group compared to the IR group ($p<0.001$).

CONCLUSIONS: Exercise preconditioning alleviates the severity of remote organ injury induced by intestinal IR in rats.

Keywords: Exercise preconditioning, intestinal ischemia-reperfusion, oxidative stress, antioxidant defenses, lung injury.

Effects of Aerobic and Anaerobic Regions of Incremental Exercise Test on Cardiorespiratory and Metabolic Changes In Trained Subjects

Nida Aslan, Ihsan Serhatlioglu, Seda Ugras, Fethi Ahmet Ugur, Oguz Ozcelik
Firat University, Faculty of Medicine, Department of Biophysics (Physiology)

OBJECTIVE: Clinical exercise test can provide an objective evaluation of the body's response to exercise stress. The cardiorespiratory and metabolic changes in response to aerobic and anaerobic regions of the incremental exercise test were comparatively examined in trained subjects.

METHODS: After giving a signed informed consent which was approved by the local ethical committee, total of 24 male trained subjects (19.75 ± 0.3 yr, 70.1 ± 1.8 kg) performed an incremental exercise test. Following a 4 min of warm-up period at 20 W, the work rate increased by a work rate controller as 15 W/min until the subjects could not tolerate. During exercise, ventilatory and pulmonary gas exchange parameters were measured using metabolic system and turbine volume transducer and evaluated breath-by-breath. Anaerobic threshold (AT) was estimated using V-slope method. Paired t test was used to analyze data ($p < 0.05$).

RESULTS: Maximal exercise capacity, work rate at the AT, maximal O₂ uptake (VO_{2max}) and VO_{2max} for body weight was found to be 232.7 ± 6.2 W, 156.2 ± 5.04 W, 2.93 ± 0.06 L/min and 42.3 ± 0.001 ml/kg/min, respectively. Minute ventilation to work rate ratio was 3.78 ± 0.1 L/min for aerobic region and 1.63 ± 0.1 L/min for anaerobic region ($p < 0.05$). Work rate to heart rate ratio was found to be 2.752 ± 0.19 W/beat/min for aerobic and 2.205 ± 0.11 W/beat/min for anaerobic regions ($p < 0.05$).

CONCLUSIONS: The patterns of cardiopulmonary variables throughout the range of exercise intensities were shown to be affected with the metabolic activity. Consequently, determining the metabolic effect on cardiopulmonary variables could be important alternative endpoints that might be better used to indicate sedentary subjects or patients capacity to cope with the metabolic demands encountered during physical activity.

Keywords: Incremental Exercise Test, Anaerobic Threshold, Heart rate, Ventilation, Trained subjects

Determination of the Relationships Between Heart Rate-Work Rate Ratio and Anaerobic Threshold During Exercise in Sedentary Females

Gulsen Cakmak¹, Ihsan Serhatlioglu², Seda Ugras³, Fethi Ahmet Ugur³, Oguz Ozcelik²

¹Firat University Faculty of Engineering, Department of Bioengineering, Elazig

²Firat University, Faculty of Medicine Department of Biophysics, Elazig

³Firat University, Faculty of Medicine Department of Physiology, Elazig

OBJECTIVE: The heart rate in response to exercise test is a widely used criteria for determination of subject's aerobic fitness and establishing optimal training work protocol. We aimed to determine the effects of anaerobic threshold (AT) on work rate to heart rate (WR-HR) ratio during incremental exercise. Thus, WR-HR ratio was comparatively evaluated in the aerobic and anaerobic region of incremental exercise.

METHODS: Twenty one sedentary females (age: 20 ± 0.2 yr, weight: 54.9 ± 1.4 kg) were participated to the study after giving a signed written informed contents which were approved by the local ethical committee. Each subjects performed an incremental exercise test (15 W/min) using an electromagnetically braked cycle ergometer. Ventilatory parameters were estimated using spirometry. The heart rate was recorded as beat-by-beat. WR-HR ratio was averaged in aerobic and anaerobic region of the AT. During exercise, AT estimated using ventilation to work rate relationships. A paired t- test was used to evaluate values ($P < 0.05$).

RESULTS: The work rate at maximal exercise capacity was 128.3 ± 3 W. The WR-HR ratio in response to the incremental exercise was not linear and the WR-Hr break point associated with AT: 78.1 ± 2 W vs 77.8 ± 2 W ($R = 0.92191$, $P < 0.0001$). The WR-HR ratio was similar below and above the AT: 1.895 ± 0.07 W/beat and 1.743 ± 0.11 W/beat, respectively. However, averaged WR-HR ratio was 0.349 ± 0.01 W/beat in aerobic region and 0.591 ± 0.01 W/beat in anaerobic region ($p < 0.05$).

CONCLUSIONS: The work production capacity for each heart beat was significantly affected with metabolic activity. Thus, determination of averaged HRWR ratio for aerobic and anaerobic exercise test could be a useful index when evaluating subject's with different aerobic fitness level.

Keywords: Anaerobic threshold, Heart Rate, Exercise test, aerobic metabolism, anaerobic metabolism.

Protective Effects of Hypoxic Adaptation in Liver After Exhaustive Exercise

Sibel Dinçer¹, Şevin Güney¹, Ayşe Şebnem İlhan²

¹Gazi University, Faculty of Medicine, Department of Physiology, Ankara, Turkey

²Başkent University, Faculty of Medicine, Department of Physiology, Ankara, Turkey

OBJECTIVE: It is known that hypoxic adaptation increases exercise performance. Alterations in antioxidant/oxidant systems by hypoxia and exercise have been reported with several studies. Liver plays a prominent role in antioxidant defense, and is more vulnerable to hypoxia than other organs in the body. Relationship between hypoxia and antioxidative defense in liver is mostly associated with ischemia/reperfusion conditions. However hypoxia can occur in physiologic conditions or can be temporary event in pathologic events. By present study, it was aimed to search the effects of hypoxic adaptation on exercise performance in sedentary rats in terms of oxidant/antioxidant parameters.

METHODS: Eight weeks-old Wistar Albino male rats were divided in normoxia (n=6) and hypoxia (n=7) groups. Rats in the hypoxia group were exposed to 10% hypoxia in a hypoxic chamber for 2 days. After the hypoxic exposure, all animals were subjected to exhaustive exercise on a treadmill. Upon exhausted, they were sacrificed by cardiac puncture, and liver samples were collected for analyses of nitrate, malon dialdehyde, and glutathione levels. Analysis of variance was performed by One Way ANOVA. The significancy levels of the differences were analyzed by Mann Whitney U Test. Values are expressed as mean \pm S.D. $p < 0.05$ was considered significant.

RESULTS: By the hypoxic adaptation, exhaustion time, liver nitrate, and glutathione levels were increased after the exhaustive exercise. Moreover in malon dialdehyde levels in the liver tissue have a tendency to decrease, but this decrement was not statistically significant.

CONCLUSIONS: Hypoxic adaptation might increase exercise performance in sedentary animals by enhancing antioxidant GSH levels.

Keywords: Hypoxic adaptation, liver, exercise, oxidant/antioxidant systems

Nociception before and after exercise in trained rats under light-induced functional pinealectomy

Yaşar Gül Özkaya, Aliye Gündoğdu, Özgür Özdemir, Mehmet Seyran
School of Physical Education and Sports, Akdeniz University, Antalya, Turkey

OBJECTIVE: Exercise is known to influence pain perception. This phenomenon, termed exercise-induced hypoalgesia (EIH) typically reported during and/or following exercise, but less is known the mechanisms responsible for EIH. Melatonin is a hormone synthesized principally in the pineal gland that has been classically associated with endocrine actions. However, several lines of evidence suggest that melatonin plays a role in pain modulation. In this study, we investigated the nociceptive response before and after exercise in both sedentary and exercise trained rats under light-induced functional pinealectomy.

METHODS: Male wistar rats were assigned to one of four groups: sedentary control (C), sedentary pinealectomized (Px), exercise trained (T), and trained under pinealectomy (T-Px) groups. Exercise trained groups were subjected to 6 weeks of motor-driven treadmill exercise. C and T groups exposed to normal light/dark cycle (12:12-hour light/ dark cycle) and Px and T-Px rats exposed to continuous light for 6 weeks. Hot plate test was repeated before and after exhaustive exercise.

RESULTS: Animals in T group had significantly higher pain thresholds compared to other groups both before and after exercise. Although Px was found to be ineffective on pain threshold before and after exercise, pain threshold in T-Px group was found to be returned to baseline levels observed in control animals.

CONCLUSIONS: Our findings support the hypothesis that endogenous melatonin plays a role in the exercise-induced hypoalgesia.

Keywords: exercise, hypoalgesia, pinealectomy, melatonin, pain perception

The Effect of Grape Seed Extract (GPSE) on Epileptiform Activity in Rats Performing Various Duration Swimming Exercise

Recep Soslu¹, Erkut Tutkun¹, Mustafa Ayyıldız², Erdal Agar²

¹Department of Yasar Dogu Physical Education and Sports, University of Ondokuz Mayıs, Samsun, Turkey

²Department of Physiology, Faculty of Medicine, University of Ondokuz Mayıs, Samsun, Turkey

OBJECTIVE: The aim of this study was to investigate the effect of GPSE on epileptiform activity in rats performing various duration exercise.

METHODS: Totally 40 adult male Wistar rats weighing 180–220 g composed the material of this study after at least 1 week of acclimatization. Each animal group included eight rats. All animals were adapted to water before the beginning of the experiment. Swimming exercise was performed in two training glass tanks filled with tap water. Animals were swim-exercised for 90 days with 15, 30 and 60 minutes/day. In addition, 200 mg/kg GPSE was given by gavage once in two days for 90 days. Thereafter, the epileptiform activity was induced by a single microinjection of penicillin (500 units) into the left somatomotor cortex. GPSE (200 mg/kg) was administered intraperitoneally (i.p) 30 minutes after penicillin injection. The electrocorticography activity was continuously monitored on a four-channel recorder. Statistical comparisons were made using Sigmastat software.

RESULTS: GPSE given short, moderate and long duration swimming exercising rats (for 90 days) decreased the mean frequency of penicillin-induced epileptiform activity in the 60,40 and 40 minutes after penicillin injection compared to control group, respectively.

CONCLUSIONS: The results of this study presented that short, moderate and long duration swimming exercise reduced penicillin-induced epileptiform activity in rats given 200 mg/kg GPSE for three months, indicating the GPSE application, which is a potent antioxidant, may provide a safer swimming exercise in epileptic patients.

Keywords: ECoG, epilepsy, epileptiform activity, GPSE, swimming exercise

An Important Biomechanical Parameter In Elite Wrestlers: Pre and Post Training Hand Grip Strength

Omer Kaynar¹, Suleyman Dasdag²

¹Department of Physical Training and Education, Institute of Health Sciences of Ataturk University, Erzurum, Turkey

²Department of Biophysics, Medical School of Dicle University, Diyarbakir 21280, Turkey

OBJECTIVE: Hand grip strength (a biomechanical parameter) plays an important role in daily life and especially during the sportive activities to get the maximum efficiency. Hand grip strength is an important indicator in wrestling in terms of performance.

METHODS: This study was carried out on 22 elite wrestlers who wrestle for professional Turkish wrestling clubs in Ankara. Hand grip strength of wrestlers in 66 kilogram category was only measured in this study for standardization. Maximum and minimum right and left hand grip strengths of the wrestlers were measured when the arms positioned in 90 ° and 180 ° degree angles. Each measurement was repeated three times.

RESULTS: The results of the study were compared by using paired samples t test. MedCalc statistical program were used for the statistics. Although pre training hand grip strengths were found higher than post training hand grip strength the results were not found to be statistically significant ($p>0.05$).

CONCLUSIONS: In conclusion, no significant difference was observed in hand grip strength of the elite wrestlers before and after training.

Keywords: Wrestling, muscles, force, hand grip strength, biomechanical parameters

Time-domain heart rate variability in professional handball players

Yıldırım Kayacan¹, Sedat Yıldız²

¹Physical Training and Sport Section, Artvin Coruh University, Artvin, Turkey

²Department of Physiology, Faculty of Medicine, Inonu University, Malatya, Turkey

OBJECTIVE: Aim of the current study was to determine time-domain heart rate variabilities in professional handball players competing in Turkish premier league and to compare these data with those of sedentary controls.

METHODS: Electrocardiography (ECG) of handball players (n=12) and sedentary subjects (n=12) were recorded for 5 min before and after an exercise programme (5 min running with a stable speed). These recordings were used to find QT intervals and time-domain heart rate variability parameters including AVNN, SDNN, RMSSD, PNN50 and PNN20.

RESULTS: Heart rate variability was higher for handball players than sedentary subjects and for exercising period than resting period ($P<0.05$). There was significant negative correlation between heart rate and RMSSD ($r=-0.599$; $P<0.001$). Although corrected QT interval was longer for exercising period than resting period and for handball players than sedentary subjects ($P<0.001$), these values were not considered as short- or long-QT intervals.

CONCLUSIONS: (1) As increase in heart rate variability is positively associated with health parameters, sedentary people are recommended to exercise regularly, and (2) heart rate variability appears to be a useful index for planning exercise protocols.

Keywords: Heart rate variability, handball, QT interval, sedentary, sport

Zinc Supplementation Prevents Cellular Damage to the Brain Tissue of Diabetic Rats Subjected to Acute Swimming Exercise

Mursel Bicer¹, Abdulkerim Kasim Baltaci², Rasim Mogulkoc², Mustafa Akil³, Nihal Savuran²

¹School of Physical Education and Sports Selcuk University, Konya-Turkey

²Selcuk University, Selcuklu Medical School, Department of Physiology, Konya-Turkey

³Karabuk University, Hasan Dogan High School of Physical Education and Sports, Karabuk-Turkey

OBJECTIVE: The present study aims to demonstrate the effects of zinc supplementation on brain tissue damage in diabetic rats subjected to acute swimming exercise.

METHODS: The study included 80 adult male Sprague-Dawley rats which were equally divided into 8 groups: Group 1, general control; Group 2, zinc-supplemented control; Group 3, zinc-supplemented diabetic control; Group 4, swimming control; Group 5, zinc-supplemented swimming; Group 6, zinc-supplemented diabetic swimming; Group 7, diabetic swimming; Group 8, diabetic control group. In order to induce diabetes, the experimental animals were injected with 40 mg/kg intraperitoneal (ip) streptozotocin (STZ) "Sigma, S-0130". The injections were repeated at the same dose after 24 hours. The animals with blood glucose level 300 mg/dl and above 6 days after the last injection were considered as diabetic. These animals were supplemented with 6 mg/kg/day ip zinc sulfate for 4 weeks. Levels of MDA (nmol/gram/protein) and GSH (mg/dl/gram protein) were determined in the brain tissue samples of rats decapitated at the end of the 4-week study.

RESULTS: The highest MDA values in brain tissue were obtained in groups 7 and 8. MDA levels in Group 4 were lower than those in groups 7 and 8, but higher than the levels in all other groups. Groups 3, 5 and 6 had MDA levels lower than groups 4, 7 and 8. Groups 4, 5 and 6 had the highest brain GSH values. The lowest GSH levels in brain tissue were found in groups 7 and 8.

CONCLUSIONS: Results of this study demonstrate that lipid peroxidation in the brain tissue caused by forced swimming exercise in diabetic rats can be prevented by zinc sulfate supplementation.

Keywords: Zinc, diabetes, brain tissue, lipid peroxidation, exercise.

Oxidative stress in health and disease

Beti Dejanova¹, Sunchica Marko Petrovska¹, Petar Dejanov², Sanja Mancevska¹, Jasmina Pluncevic¹, Elizabeta Sivevska¹, Slobodan Nikolic¹, Vaska Antevska¹

¹Institute of Physiology, Medical Faculty, Skopje, Macedonia

²Clinic of Nephrology, Clinical Center, Skopje, Macedonia

OBJECTIVE: The aim of this study was to evaluate oxidative stress (OS) level in healthy subjects and in chronic disease patients.

METHODS: In 70 healthy subjects (38 ♂ and 32 ♀) and in 115 chronic disease patients (75 ♂ and 40 ♀), 85 related with chronic renal failure and 30 with diabetes mellitus, OS was examined by using the fluorimetric method for lipid peroxidation (within its end product malonyldialdehyde). The values were expressed in $\mu\text{mol/L}$. Healthy subjects were divided concerning different age, sex and hygienic-dietetic issues (lipid profile, smoking habits, living conditions: city-village). Patients were divided concerning age, sex, chronic disease duration, using different substitution therapies as hemodialysis, iron, erythropoietin, L-carnitine.

RESULTS: Regarding the age of both healthy subjects and patients, older ones showed higher OS: for healthy subjects 3.94 ± 0.8 ($p < 0.05$); and for patients 4.99 ± 0.7 ($p < 0.01$). OS in different sex groups did not show statistical difference neither in healthy subjects nor in patients. Impaired lipid profile in healthy subjects showed higher OS 4.24 ± 0.4 ($p < 0.05$) as well as the ones with smoking habits, 4.3 ± 0.3 ($p < 0.01$). No statistical differences were found concerning living conditions. The longer chronic disease, hemodialysis duration and iron supplementation increased OS level ($p < 0.05$). Erythropoietin supplementation showed decreased OS level to 4.2 ± 0.9 and so did L-carnitine supplementation, 4.1 ± 0.4 ($p < 0.05$).

CONCLUSIONS: Higher OS was found in healthy older people with impaired lipids who smoke and in a patients with longer chronic duration who were more exposed to hemodialysis and iron therapy. On the contrary, erythropoietin and L-carnitine therapies showed beneficial effects towards OS.

Keywords: oxidative stress, healthy subjects, chronic disease patients.

Melatonin prevents aluminum-induced oxidative damage in rat intestine

Güner Sarıkaya¹, Ömür Karabulut Bulan¹, Pelin Arda Pirincci²

¹Division of General Biology, Department of Biology, Faculty of Science, Istanbul University, Turkey

²Division of Zoology, Department of Biology, Faculty of Science, Istanbul University, Turkey

OBJECTIVE: Aluminum enters into the body from the environment, diet and medications, is extensively used in modern daily life. Aluminum shows toxic effect in various tissues by expose of reactive oxygen species (ROS). Melatonin, produced by the pineal gland, has been shown to be an effective antioxidant and free radical scavenger. In this study, we aimed to investigate the effects of melatonin on intestinal injury induced by aluminum in rats.

METHODS: Wistar albino rats used in this study were divided into five groups. Group I: control animals, group II: melatonin control animals which were injected with physiological saline and ethanol, group III injected with 10mg/kg melatonin, group IV 5 mg/kg aluminum sulfate injected and, group V 5 mg/kg aluminum sulfate and 10 mg/kg melatonin injected. All groups were injected three times a week for one month. Jejunum samples were prepared for light microscopic examination and were stained with Masson's trichrome. Myeloperoxidase (MPO) as an oxidative stress marker and glutathione (GSH), which is a parameter of antioxidant system, were examined in the intestinal homogenates by ELISA.

RESULTS: Administration of aluminum resulted in a common injury in the small intestine by causing degenerative changes. In addition, aluminum exposure promoted oxidative stress with a significant increase myeloperoxidase (MPO) levels and decrease in glutathione (GSH) levels in small intestine. Melatonin administration decreased degenerative changes. Melatonin administration decreased myeloperoxidase (MPO) levels and increased GSH levels biochemically.

CONCLUSIONS: Our results showed that melatonin has protective effects on aluminum-induced small intestinal injury in rats. Also we suggested that melatonin may be useful as a therapeutic agent in aluminum-induced intestinal disorders.

Keywords: aluminum, melatonin, oxidative stress, small intestine

Distribution Of Spleen Connective Tissue Fibers In Diabetic And Vitamin C Treated Diabetic Rats

Dilşad Okan¹, Nesrin Özsoy², Suna Cebesoy², Cigdem Ozer³

¹Kastamonu University, Faculty of Science and Art, Department of Biology, Kastamonu

²Ankara University, Faculty of Science, Department of Biology, Ankara

³Gazi University, Faculty of Medicine, Ankara

OBJECTIVE: In this study, the distribution and the localization the of connective tissue fibers in the rat spleen were investigated.

METHODS: Albino Wistar rats were divided into diabetic, Vitamin C-treated diabetic and control groups. Diabetes was induced by a single dose of STZ (45 mg/kg) administered intraperitoneally. Vitamin C (20 mg/kg) was administered intragastrically for 21 days. Collagen, reticular and elastic fibers were stained by Massons's trichrome, Gomori's silver impregnation and Elastic Van Gieson (EVG) techniques, respectively. The sections were investigated by light microscopy.

RESULTS: In the diabetic spleen the reticular fibers were disorganized and they more or less accumulated in the white and the red pulps. Focal reticular fiber thickening was observed in the fiber-dense areas while it was rare in the Vitamin C-treated diabetic group. The collagen fibers were localized into trabeculae, capsules, splenic sinusoids and around central artery, in the diabetic group. There were increased collagen fibers in these regions. In the central artery, elastic fibers could not be detected in the EVG stained diabetic and Vitamin C treated diabetics. However, some of the Vitamin C treated diabetic groups had zigzag shaped elastic fibers in the internal elastic lamina of the splenic artery at the hilum region.

CONCLUSIONS: Further studies are needed to assess the positive effects of Vitamin C on the connective tissue fibers of the diabetic spleen.

Keywords: spleen, connective tissue fibers, diabetes, Vitamin C

Effects of different calorie restriction types on circulating blood cells and oxidant and antioxidant status in male Wistar rats

Muharrem Balkaya¹, Ferda Belge¹, Cengiz Ünsal¹, Aykut Göktürk Üner¹, Selim Sekkin², Ayşegül Bildik³, Hümevra Ünsal¹, Mümin Alper Erdoğan¹, Ece Koç¹

¹Department of Physiology, Faculty of Veterinary Medicine of Adnan Menderes University, Aydın, Türkiye

²Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine of Adnan Menderes University, Aydın, Türkiye

³Department of Biochemistry, Faculty of Veterinary Medicine of Adnan Menderes University, Aydın, Türkiye

OBJECTIVE: To investigate possible effects of calorie restriction on hematologic variables and oxidant/anti-oxidant status in blood and tissues of rats.

METHODS: Eighteen male Wistar rats were randomized into three groups. First group were fed normal rat chow ad libitum (Control), second group fasted or fed ad libitum on alternate days (FAD-group), while third group received a 30% restricted diet of ad libitum (CRD-group) for 35 days. Body weights and daily food/water intakes were recorded. Anticoagulated blood samples were obtained to determine hematologic variables weekly and total oxidant status (TOS) and anti-oxidant capacity (TAC) at the end of the study. Brains, livers and kidneys were collected after euthanasia for determination of SOD, GSH, MDA and catalase values.

RESULTS: Significant changes in mean body weights and food and water intakes were noted. Mean body weight increased in controls and decreased in both food-restricted groups significantly, partially with time. A significant treatment effect and group-time interactions were found for some hematologic variables ($P < 0.05$). Mean WBC and lymphocyte counts, PLT, Pct, MPV and PDW decreased in both restricted groups compared to controls. In addition, the mean Hb and PCV values were higher in FAD-group compared with others. Mean MCV decreased in CRD-group at day 21. The mean PDW in CRD-group was lower than control group. Compared with controls, TOS decreased whereas TAC increased in both restricted groups. But, this was not statistically confirmed. Dietary interventions affected also the anti-oxidative capacity only in liver and kidneys of rats.

CONCLUSIONS: Calorie restriction affects blood variables and anti-oxidative capacity in plasma and certain organs in a restriction and organ-dependent manner.

Keywords: Male Wistar rats, calorie restriction, blood cells, organs, oxidants, antioxidants

Effects Of Extremely Low Frequency (ELF) Magnetic Field On The Oxidative Stress Parameters Of Some Internal Organs

Mehmet Zulkuf Akdag¹, Suleyman Dasdag¹, Ozcan Erel²

¹Department of Biophysics, Medical School of Dicle University, Diyarbakir, Turkey

²Department of Biochemistry, Medical School of Yildirim Beyazit University, Ankara, Turkey

OBJECTIVE: The aim of this study was to investigate the effect of 100 μ T and 500 μ T ELF magnetic field, on the oxidative stress parameters of some internal organs of rats.

METHODS: The study was carried out on 30 male Sprague–Dawley adult male rats. Rats in the first (n: 10) and the second (n: 10) experimental groups were respectively exposed to 100 μ T and 500 μ T ELF magnetic fields for 2 h/day for 10 months. At the end of the ten months of exposure some of the oxidative stress parameters such as catalase, myeloperoxidase, malondialdehyde (MDA), total oxidant status (TOS), total antioxidant capacity (TAC) and oxidative stress index (OSI) were measured in liver, lung and kidney of rats in the sham and two exposed groups.

RESULTS: Myeloperoxidase and OSI decreased in the kidney of the rat exposed to 100 μ T and 500 μ T ELF magnetic fields ($p < 0.05$). However, increase of TAC in the kidney of rats exposed to 100 μ T was found to be significant ($p < 0.05$). By the way, decrease of TAC in the lung of the rats exposed to 500 μ T ELF magnetic field was found significant ($p < 0.05$). On the other hand, OSI decreased in the liver of rats exposed to 100 μ T and 500 μ T ELF magnetic fields while TAC increased in the liver of rats exposed to 100 μ T magnetic field ($p < 0.05$).

CONCLUSIONS: Long-term exposure of 100 μ T and 500 μ T ELF magnetic fields can alter some of the oxidative stress parameters in the liver, lung and kidney of the rats.

Keywords: ELF Magnetic Field, oxidative stress parameters, liver, lung and kidney

Selenium glutathione peroxidase in the antarctic teleost *Gymnodraco acuticeps*: molecular characterisation and regulation of gene expression

Francesco Volpe, Francesco Boldrin, Diana Ferro, Ester Piccinni, Gianfranco Santovito
Department of Biology, University of Padua, Padova, Italy

OBJECTIVE: Glutathione peroxidase (GPX, EC 1.11.1.9) is a family of metalloenzymes that catalyze the detoxification of hydrogen peroxide, using glutathione as reducing factor, and is one of the most important antioxidant enzymes involved in antioxidant homeostatic control. For their ubiquitous distribution, those proteins are well suited for molecular evolution studies, especially in animals adapted to peculiar environments.

Despite numerous previous studies on GPX from aquatic animals, the gene structure and expression of teleost GPXs have not been comprehensively studied. In particular, very little is known about the GPX of Antarctic teleost. The Antarctic species have an interesting evolutionary history because they have developed some adaptations that allow them to survive and to breed in waters where the temperature, oxygen and salt concentration deviate significantly from the average recorded in the temperate waters. They represent paradigmatic cases for adaptation to different temperatures and salinities in their environment.

METHODS: Specimens of *Gymnodraco acuticeps*, a teleost fish widely distributed in Antarctic Ocean, were sampled in the Ross Sea (Terra Nova Bay, 74°42'S, 164°7'E) during the XVII Italian Antarctic Expedition. cDNA sequence of Se-GPX has been obtained from hepatic tissue by a combination of RT-PCR, 3' and 5'RACE techniques.

RESULTS: The obtained nucleotide sequence and the deduced amino acid sequence have been compared with those of homologous genes already available in Genbank and have been used for phylogenetic analyses. Preliminary results were also obtained regarding the regulation of gene expression.

CONCLUSIONS: The obtained results represent the start-up for further studies on physiological antioxidant responses to the Antarctic environment.

Keywords: Antarctica, gene expression, molecular evolution, glutathione peroxidase, teleosts, *Gymnodraco acuticeps*

Interaction melatonin and vagus nerve on the oxidative system during gastric ischemia/ reperfusion in male rats

Shahla Nourizad, Mohamad Khaksari Hadad, Nader Shahrokhi Sardoo
Department of Physiology, Medical University, Kerman, Iran

OBJECTIVE: There are few studies about interaction of melatonin and peripheral parasympathetic nerves in ischemia / reperfusion (I / R) injury of the stomach that often occurs in surgical pathology. The objective of this study was to assess the interaction of melatonin and vagus nerve in stomach I/R.

METHODS: In this study, rats were randomly divided into 7 groups. Celiac artery ischemia for 30 minutes was followed by 3 h perfusion. Cervical vagus nerve was cut on both sides of the neck and in the other group the right cervical vagus nerve was stimulated by stimulator and in therapeutic group, melatonin, or vehicle was injected ip.

RESULTS: Vagus nerve stimulation in ischemia - reperfusion significantly ($p < 0.001$) increases gastritis and melatonin administration in ischemia-reperfusion + vagus nerve stimulation significantly ($p < 0.001$) reduces gastritis. Results show that malondialdehyde in gastric tissue in I/R + vagus nerve stimulation significantly ($p < 0.001$) increased and melatonin administration significantly reduced ($p < 0.001$) malondialdehyde activity levels. Superoxide dismutase in vagus nerve stimulation significantly ($P < 0.01$) compared to I/R decreased and melatonin administration in vagus nerve stimulation + I/R significantly ($p < 0.001$) was increased. Catalase levels in vagus nerve stimulation + I/R group, significantly compared to I/R increased ($p < 0.01$) and also melatonin administration caused increase ($p < 0.001$). Glutathione peroxidase, vagus nerve stimulation group + I/R significantly compared to I/R increased ($p < 0.01$) and with melatonin administration compared with ischemia / reperfusion significantly increased ($p < 0.001$).

CONCLUSIONS: This study showed that gastric ulcer healing and antioxidant enzyme activity by melatonin has a cholinergic action and is mediated by acetylcholine.

Keywords: Stomach, melatonin, vagus nerve, ischemia / reperfusion

Vitamin E reduces oxidative stress and improves hemorheological parameters in aged rats

Vural Küçükataş¹, Melek Bor Küçükataş¹, Gulsah Gundogdu¹, Gülten Erken², Tonguç Olgun Özcan¹, Fatma Demirkaya Miloglu³, Yucel Kadioğlu³

¹Pamukkale University, Faculty of Medicine, Department of Physiology Kınıklı, 20020, Denizli/TURKEY

²Balıkesir University, Faculty of Medicine, Department of Physiology, 10100 Balıkesir/TURKEY

³Ataturk University, Faculty of Pharmacy, Department of Analytical Chemistry, 25240, Erzurum/TURKEY

OBJECTIVE: The harmful effects of aging on blood rheology have been well known. These effects in the aging have been found to be associated with an increase in oxidative stress. The aim of this study was to seek whether treatment of Vitamin E which is a potent antioxidant could improve the age-related hemorheological abnormalities.

METHODS: For this purpose, male Wistar rats at the age of 3 and 24 months were used and the following parameters were evaluated: Red blood Cell (RBC) deformability and aggregation, plasma viscosity, plasma Vitamin E level, Total Oxidant Status (TOS) and Total Antioxidant Status (TAS).

RESULTS: The results show that aging is associated with a decrease in RBC deformability and increase in RBC aggregation and plasma viscosity. Compared with young group, aged rats had significantly lower TAS and higher level of TOS in plasma. We also found significantly lower plasma Vit E level in aged rats than young rats. Vitamin E administration produced significant increase in RBC deformability and decrease in RBC aggregation in aged group with respect to young and aged control groups. While TOS levels were decreased, an increment in TAS levels was seen in plasma of old rats treated by Vitamin E.

CONCLUSIONS: In conclusion, these findings suggest that blood rheology impairs with age and Vitamin E has ameliorating effects on aged-induced hemorheological abnormalities probably by reducing increased oxidative stress in old age. This study was supported by Pamukkale University Research Fund.

Keywords: Aging, Blood rheology, Erythrocyte aggregation, Erythrocyte deformability, Oxidative stress, Vitamin E

The effect of phosphodiesterase-5 inhibition by sildenafil citrate on inflammation and apoptosis in rat experimental colitis

Berna Karakoyun¹, Ünal Uslu², Feriha Ercan³, Mehmet Şerif Aydın³, Meral Yüksel⁴, Ayliz Velioglu Ögünç⁴, Inci Alican⁵

¹Department of Nursing, Marmara University Health Sciences Faculty, Istanbul, Turkey

²Department of Histology and Embryology, Yeditepe University School of Medicine, Istanbul, Turkey

³Department of Histology and Embryology, Marmara University School of Medicine, Istanbul, Turkey

⁴Department of Medical Laboratory, Marmara University Vocational School of Health Related Professions, Istanbul, Turkey

⁵Department of Physiology, Marmara University School of Medicine, Istanbul, Turkey

OBJECTIVE: The aims of this study were to investigate the effects of sildenafil citrate (SIL) on tissue integrity, oxidant-antioxidant status and apoptosis in rats with colitis.

METHODS: Colitis was induced by trinitrobenzenesulphonic acid (TNBS) in 40% ethanol (30 mg/ml; 0.8 ml) given intrarectally to Sprague-Dawley rats. Sildenafil (25 mg/kg/day) was administered after the induction of colitis and the treatment was continued for 7 days. After decapitation, the distal colon was scored and stored for the measurement of malondialdehyde (MDA) and glutathione (GSH) levels, myeloperoxidase (MPO) activity and apoptosis. Oxidant generation was monitored by using chemiluminescence (CL). Blood was collected for tumor necrosis factor (TNF)- α and interleukin (IL)-10 assays.

RESULTS: The macroscopic lesion score of the colitis group was reduced by SIL ($p < 0.01$). The increase in colonic MDA along with a concomitant decrease in GSH of the colitis group compared to control group was reversed by SIL ($p < 0.01$ and $p < 0.001$, respectively). Sildenafil also reduced the elevated tissue MPO activity ($p < 0.001$), colonic lucigenin CL level and serum TNF- α levels in the colitis group ($p < 0.001$ and $p < 0.01$, respectively). The serum anti-inflammatory cytokine IL-10 level of the colitis group showed a marked reduction compared to control group ($p < 0.001$). However, SIL treatment did not show a significant effect on this parameter. The colonic apoptotic index of rats with colitis was significantly higher than that of the control animals ($p < 0.001$) and reduced by SIL ($p < 0.01$).

CONCLUSIONS: Sildenafil is beneficial in rat experimental colitis via the maintenance of oxidant-antioxidant status, prevention of apoptosis, superoxide production and cytokine release.

Keywords: Apoptosis; colitis; inflammation; phosphodiesterase; rat; sildenafil citrate

Chlorpyrifos-Induced Oxidative Stress in Rat Brain Tissues And the Effects of Catechin and Quercetin

Fatma Gökçe Uzun¹, Filiz Demir¹, Hatice Baş², Suna Kalender³

¹Department of Biology, Gazi University, Ankara, Turkey

²Department of Biology, Bozok University, Yozgat, Turkey

³Department of Science Education, Gazi University, Ankara, Turkey

OBJECTIVE: Chlorpyrifos (CPF) is an organophosphate that is widely used as an insecticide. It has been reported that CPF enhance the production of reactive oxygen species (ROS), and it alters the enzyme activities associated with antioxidant defense mechanisms in different tissues of rats. Quercetin and catechin are the most common flavonoids in the human diet and, like other flavonoids, have been shown to potent free radical scavengers and antioxidant.

METHODS: The mature male Wistar rats (n=36) were divided into six groups having six animals, i.e., Group I (control), Group II (catechin, 20 mg/kg bw), Group III (quercetin, 20 mg/kg bw), Group IV (chlorpyrifos 5.4 mg/kg), Group V (catechin+chlorpyrifos) and Group VI (quercetin+chlorpyrifos). Rats were given chlorpyrifos, catechin and quercetin daily via gavage for 4 weeks.

RESULTS: In the chlorpyrifos, catechin plus and quercetin plus chlorpyrifos treated groups, there were increased the levels of malondialdehyde (MDA) content, and decreased superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities in brain tissues compared to the control group. Whereas, when catechin plus and quercetin plus chlorpyrifos- treated groups compared to chlorpyrifos treated group, there were significantly decreased in MDA content and increased SOD, CAT, GPx and GST activities.

CONCLUSIONS: In the present study, even though chlorpyrifos was given at 1/25 of the oral LD50, we observed changes in the antioxidant enzyme activities and MDA content in rat brain tissues. The antioxidant properties of flavonoids may scavenge some radical species. Thus, it appears that catechin and quercetin ameliorate chlorpyrifos-induced neurotoxicity but are not completely protective.

Keywords: Chlorpyrifos, Catechin, Quercetin, Antioxidant Enzymes, Lipid peroxidation, Brain

Investigation of 3-nitrotyrosine levels and antioxidant effect of taurine in spleen tissues of endotoxin-treated guinea pigs

Filiz Sezen Bircan¹, Barbaros Balabanlı¹, Nurten Türközkan², Gonca Ozan³

¹Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey

²Department of Biochemistry, Faculty of Medicine, Gazi University, Ankara, Turkey

³TUBITAK, Ankara, Turkey

OBJECTIVE: Nitric oxide and superoxide radicals react each other to form peroxynitrite (ONOO-) that is far more toxic and reactive than its precursors, and is a relatively long-lived oxidant. ONOO- is a potent oxidant and nitrating agent which causes modifications, and reacts with numerous biomolecules including proteins, lipids, thiols, sulfhydryl groups and DNA bases. A well known reaction of ONOO- is forming 3-Nitrotyrosine (3-NT) by nitrating free or protein-bound tyrosine residues from ortho position. 3-NT, a stable and specific end product of ONOO-, is a biomarker that is used commonly for determining ONOO--mediated tissue damage in human disease and animal models. On the other hand, taurine is a free sulfur-containing beta-amino acid which has antioxidant, antiinflammatory and detoxificant properties. In this study, the role of endotoxemia on ONOO- formation via 3-NT detection, and the antioxidant effect of taurine in lipopolysaccharide (LPS)-treated guinea pigs were investigated.

METHODS: Forty adult male guinea pigs were injected LPS (4 mg/kg), taurine (300 mg/kg) or taurine plus LPS intraperitoneally, and levels of 3-NT and taurine were measured by HPLC in spleen tissues. The One Way ANOVA test was used to analyze the significance of the differences between control and experimental groups.

RESULTS: LPS administration significantly decreased the concentration of taurine whilst increased levels of 3-NT. It was determined that taurine decreased the levels of 3-NT in taurine plus LPS-treated group. The group in which taurine was administered alone, contradictory to its well-known antioxidant effect, taurine caused elevated concentration of 3-NT.

CONCLUSIONS: Taurine may act as an antioxidant during endotoxemia, while it may also act as a prooxidant in healthy conditions.

Keywords: Endotoxemia, Taurine, 3-Nitrotyrosine, Peroxynitrite, Spleen.

Smoking Induced Oxidative Stress in Oral Cavity

Smaranda Laura Gotia¹, Smaranda Rodica Gotia¹, Claudia Borza², Corina Serban²

¹Department of Physiology, University of Medicine and Pharmacy, Timisoara, Romania

²Department of Pathophysiology, University of Medicine and Pharmacy, Timisoara, Romania

OBJECTIVE: Cigarette smoke contains a large amount of oxidative species, and therefore smoking represents a significant source of oxidative stress and is one of the main risk factor for oral tumors. Saliva possesses protection mechanisms from oxidative attack. The purpose was to investigate the smoking effect on salivary leukocytes and salivary antioxidant capacity, appreciated by glutathione level (GSH).

METHODS: Twenty four persons, 10 smokers and 14 non-smokers, aged between 30-60 years, and 12 smoking patients with oral malignant tumors were investigated. Saliva samples were taken in the morning for a period of 5 minutes in order to determine salivary flow. Saliva samples were obtained after two hours of tooth brushing. Unstimulated whole saliva samples in smokers lot, was taken in the morning before and 30 minutes after smoking one cigarette. Salivary GSH was performed by dithio-bisnitro-benzoic acid method (DTNB). Salivary leukocytes were counted and phagocytic capacity was estimated by NBT test.

RESULTS: Salivary flow in non-smoking patients was 32% decreased in comparison with smokers, and increased after smoking and in patients with oral soft tissues tumors. Salivary GSH in non-smokers was 5% increased than in healthy smokers. After 30 minutes smoking salivary GSH increased with 73.48%. The increase of salivary leukocytes observed in smokers, may maintain local inflammation, through mediators and enzymes released from leukocytes. Low values in phagocytic tests complete the negative effects of smoking over oral environment.

CONCLUSIONS: Smoking increased the salivary GSH levels, decreased the salivary leukocytes phagocytosis. Smoking can have noxious effects on oral cavity.

Keywords: oxidative stress, smoking, oral cavity

Effect of Coenzim Q-10 on the level of total lipid, total cholesterol and fatty acids in the rat brain

Hatice Akkaya¹, Sait Celik², Ahmet Ozkaya³, Okkes Yilmaz⁴

¹Experimental Research Center, Faculty of Medicine, University of Yeditepe, Istanbul, Turkey

²Usak University Rectorship, Usak, Turkey

³Department of Chemistry, Faculty of Science, University of Adiyaman, Adiyaman, Turkey

⁴Department of Biology, Faculty of Science, University of Firat, Elazığ, Turkey

OBJECTIVE: Hydrogen Peroxide (H₂O₂) is a powerful reactive oxygen species and causes oxidative damage the proteins and lipids. The aim of the present study was to evaluate the neuroprotective effect of Coenzim Q-10 (Ubiquinone) on H₂O₂-induced oxidative damage in rats.

METHODS: Forty male Wistar albino rats were randomly divided into four groups as control, H₂O₂, Ubiquinone (UB) and H₂O₂+UB groups. H₂O₂ (10 mg/every other day) and UB (8 mg/every other day) were intraperitoneally administered for a period of 38 days. At the end, the rats were decapitated and brains were rapidly removed. After homogenization, total lipid and total cholesterol levels were analyzed by spectrophotometer, and fatty acid levels were determined by gas chromatography.

RESULTS: In brain tissue, total cholesterol levels were not statistically different from control group ($p>0.05$), however total lipid levels were decreased in UB group as compared to average results ($p>0.05$). Also, the ratio of total ω -3-fatty acid and total polyunsaturated fatty acid (PUFA) in the UB group increased ($p>0.05$) compared to control group, but these results were not statistically significant for the brain lipid metabolism.

CONCLUSIONS: We conclude that Coenzim Q-10 does not seem to have a neuroprotective potential in the rat brain.

Keywords: Brain, Coenzim Q-10, oxidative damage, rat.

The hepatoprotective effect of coumarin and coumarin derivatives on carbon tetrachloride-induced hepatic injury by anti-oxidative activities in rats

Mukadder Atmaca¹, Hakkı Murat Bilgin¹, Basra Deniz Obay¹, Hüda Diken¹, Mustafa Kelle¹, Ebru Kale²

¹a Dicle University Faculty of Medicine, Department of Physiology, Diyarbakır, Turkey

²b Dicle University Faculty of Medicine, Department of Biochemistry, Diyarbakır, Turkey

OBJECTIVE: Coumarins are a vast group of natural compounds and some of them possess anti-oxidant activities. The comparison of the antioxidant activity of some coumarins with various chemical molecular structures has not been investigated in previous studies. Therefore this study was aimed to investigate the hepatoprotective effect against CCl₄ (carbon tetrachloride) -induced hepatic injury by coumarin (1,2-benzopyrone) and coumarin derivatives, esculetin (6,7-dihydroxycoumarin), scoparone (6,7-dimethoxycoumarin) and 4-methylumbelliferone (7-hydroxy-4-methyl) in male Sprague-Dawley rats.

METHODS: Product of lipid peroxidation, malondialdehyde (MDA), and activities of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) were evaluated for oxidative stress in hepatic injury. Gamma glutamyl transpeptidase (GGT), and lactate dehydrogenase (LDH) were detected in plasma as biomarkers of hepatic injury.

RESULTS: Significantly elevated levels of MDA and lowered levels of SOD and CAT activities were observed in livers of rats exposed to CCl₄, when compared to control values. Similarly, administration of CCl₄ increased LDH and GGT levels in serum. Pre-treatment of rats with esculetin (35 mg kg⁻¹, orally) and scoparone (35 mg kg⁻¹, orally) significantly prevented CCl₄-induced increases in MDA level and decreases in SOD and CAT activities, whereas 4-methylumbelliferone (35 mg kg⁻¹) and coumarin (30 mg kg⁻¹) had no effect against CCl₄-induced rise in serum enzymes.

CONCLUSIONS: Esculetin and scoparone also showed protective properties as was evidenced in reduced LDH and GGT levels in serum. The results of this study indicate that the chemical structures of coumarins play an important role in the prevention of oxidative stress.

Keywords: CCl₄-induced hepatotoxicity, Antioxidant, Hepatoprotection, Coumarins

Effects of teduglutide on gastric mucosal architecture and oxidative stress in the TNF-alpha/actinomycin D-induced gastric injury

Pelin Arda Pirincci¹, Ismat Burcu Turkyilmaz², Refiye Yanardag², Sehnaz Bolkent¹

¹Istanbul University, Faculty of Science, Department of Biology, 34134-Vezneciler, Istanbul, Turkey

²Istanbul University, Faculty of Engineering, Department of Chemistry, 34320-Avcilar, Istanbul, Turkey

OBJECTIVE: Glucagon-like peptide-2 (GLP-2) is a proglucagon-derived peptide hormone acting as a specific gastrointestinal growth factor. Teduglutide is a dipeptidylpeptidase IV-resistant synthetic analogue of human GLP-2. Blocking the synthesis of protective proteins through a transcriptional inhibitor such as actinomycin D (Act D) sensitizes many cell types to TNF-alpha toxicity. In this study, we aimed to investigate whether teduglutide has effects on gastric mucosal morphology and oxidative stress at gastric injury induced by TNF-alpha/Act D in mice.

METHODS: The gastric injury was induced by intraperitoneal administration of 15 µg/kg TNF-alpha and 800 µg/kg Act D per mouse. Animals were injected subcutaneously 200 µg/kg teduglutide every 12 hr for 10 consecutive days prior to the administration of TNF-alpha and Act D. Samples from the fundic stomach were fixed in Bouin's solution and embedded in paraffin for light microscopic examination. The sections 5 µm in thickness were stained with hematoxylin-eosin. Stomach tissue was homogenized in 0.9 % saline to make up to 10 % homogenate. Glutathione (GSH) and lipid peroxidation (LPO) levels and lactate dehydrogenase (LDH) activity were examined in these homogenates by spectrophotometry.

RESULTS: Administration of TNF-alpha/Act D to mice caused degenerative morphological changes on gastric mucosal architecture. The stomach GSH levels were significantly decreased, while LPO and LDH activities were significantly increased in the TNF-alpha/Act D group as compared with control group. Teduglutide administration to TNF-alpha/Act D group reduced the damage in the gastric mucosa, and reversed these biochemical effects.

CONCLUSIONS: These results suggest that teduglutide pretreatment may prevent TNF-alpha/Act D-induced gastric injury in mice.

Keywords: Teduglutide, TNF-alpha, actinomycin D, gastric injury, oxidative stress

The protective effects of Dexmedetomidine on Hepatic Ischemia Reperfusion Injury

Aysegul Kucuk¹, Faik Yaylak², Aslihan Cavunt Bayraktar³, Murat Tosun⁴, Mustafa Arslan⁵, Faruk Metin Çomu⁶, Mustafa Kavutcu³

¹Dumlupınar University, Medical Faculty, Physiology Department, Kutahya, Turkey

²Dumlupınar University, Medical Faculty, General Surgery Department, Kutahya, Turkey

³Gazi University, Medical Faculty, Biochemistry Department, Ankara, Turkey

⁴Afyon-Kocatepe University, Medical Faculty, Histology-Embryology Department, Afyon, Turkey

⁵Gazi University, Medical Faculty, Anesthesiology and Reanimation Department, Ankara, Turkey

⁶Kırıkkale University, Medical Faculty, Physiology Department, Kırıkkale, Turkey

OBJECTIVE: The aim of this study was to evaluate the effect of dexmedetomidine (100µg/kg-i.p.) on liver ischemia and reperfusion (I/R) in rats.

METHODS: Twenty-four Wistar Albino rats were separated into three groups as control (C), ischemia-reperfusion injury (I/R) and dexmedetomidine group (I/R-D). Ischemia was induced with portal clamping for 45 minutes and reperfusion period was 45 minutes after declamping. Group I/R-D received dexmedetomidine 100µg/kg i.p. 30 min before portal clamping. Malondialdehyde (MDA), glutathione-S-transferase (GST), superoxide dismutase (SOD), Catalase (CAT), and paraoxonase 1 (PON-1) were investigated. Also HSP60 and p53 positive hepatocytes were counted under UTHSCSA Image Tool for Windows 3.0 image analysis program.

RESULTS: All parameters, except GST levels, were significant between the groups ($p < 0.05$). Although HSP60 expression was significantly increased between I/R, I/R-D and C group ($p = 0.015$ and 0.001 , respectively), there were no significant differences between I/R-D and C ($p = 0.443$). On the other hand, p53 expression was also significantly increased between I/R, I/R-D and C group ($p = 0.044$ and 0.003 respectively). At the same time, there were no significant differences between I/R-D and C group ($p = 0.354$).

CONCLUSIONS: All the results suggest that Dexmedetomidine has beneficial effects on liver ischemia/reperfusion stress.

Keywords: Ischemia-reperfusion, liver, oxidative stress, HSP60 expression, p53 expression, Dexmedetomidine, rat

Methyl Parathion and Dichlorvos-Induced Oxidative Stress in Human Erythrocytes and the Protective Effect of Vitamins C and E in vitro

Sema Eroğlu¹, Dilek Pandır¹, Fatma Gökçe Uzun², Hatice Baş²

¹Bozok University, Faculty of Arts and Science, Department of Biology, Yozgat, Turkey

²Gazi University, Faculty of Arts and Science, Department of Biology, Ankara, Turkey

OBJECTIVE: Methyl parathion and dichlorvos are organophosphate (OP) pesticides. They have been shown to induce oxidative stress in erythrocytes through the generation of free radicals and alteration of the cellular antioxidant defense system.

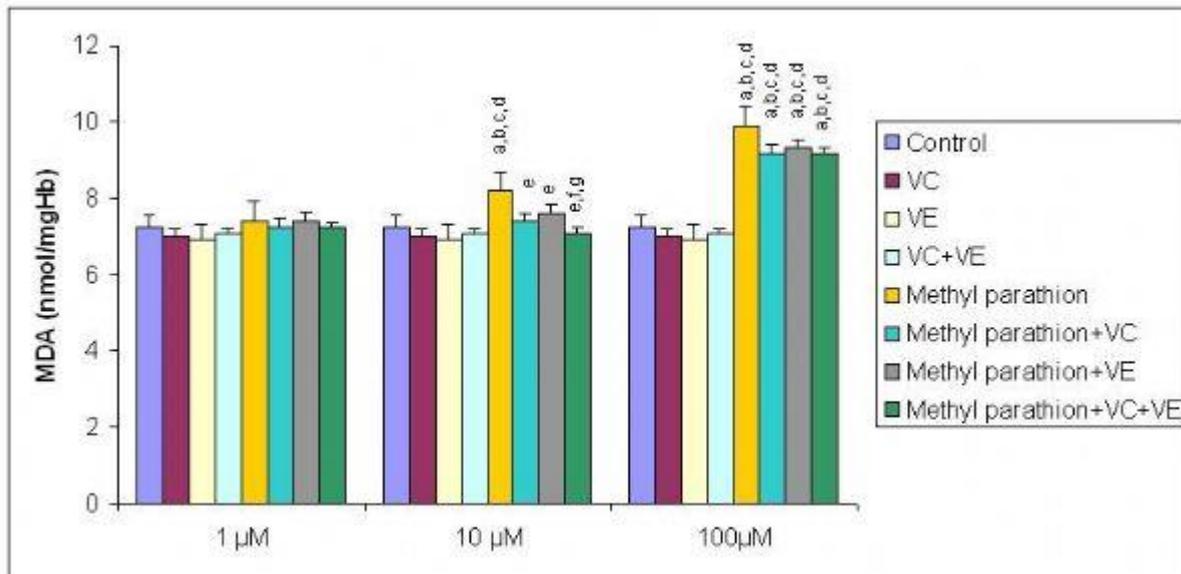
METHODS: In this study, we investigated the effect of several different doses of methyl parathion and dichlorvos (1,10,100 μ M) or methyl parathion or dichlorvos in combination vitamin C (VC; 10 μ M) or vitamin E (VE; 30 μ M), on the levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities in human erythrocytes in vitro. Erythrocytes were incubated under various treatment conditions (methyl parathion/ dichlorvos/vitamins alone, methyl parathion plus vitamins, and Dichlorvos plus vitamins) at 37 °C for 60 min.

RESULTS: Treatment with methyl parathion or dichlorvos alone increased the levels of MDA, and decreased SOD, CAT and GPx activities in erythrocytes ($P < 0,05$). There were no statistical difference among VC-treated, VE-treated, or VC+VE-treated erythrocytes, as compared with nontreated control cells. Treatment of cells with methyl parathion or dichlorvos+VC, methyl parathion or dichlorvos+VE, or a combination of all three agents prevented Methyl parathion or dichlorvos-induced changes in antioxidant enzyme activity and lipid peroxidation. However, this effect was seen only at low concentrations of methyl parathion or dichlorvos (1 and 10 μ M), and combination of VC+VE had a more protective effect than VC or VE alone.

CONCLUSIONS: These results indicated that the presence of vitamins at concentrations that are similar to the levels found in plasma have no effect on methyl parathion or dichlorvos-induced toxicity in erythrocytes at a concentration of methyl parathion or dichlorvos (100 μ M) that are typically used in pesticides.

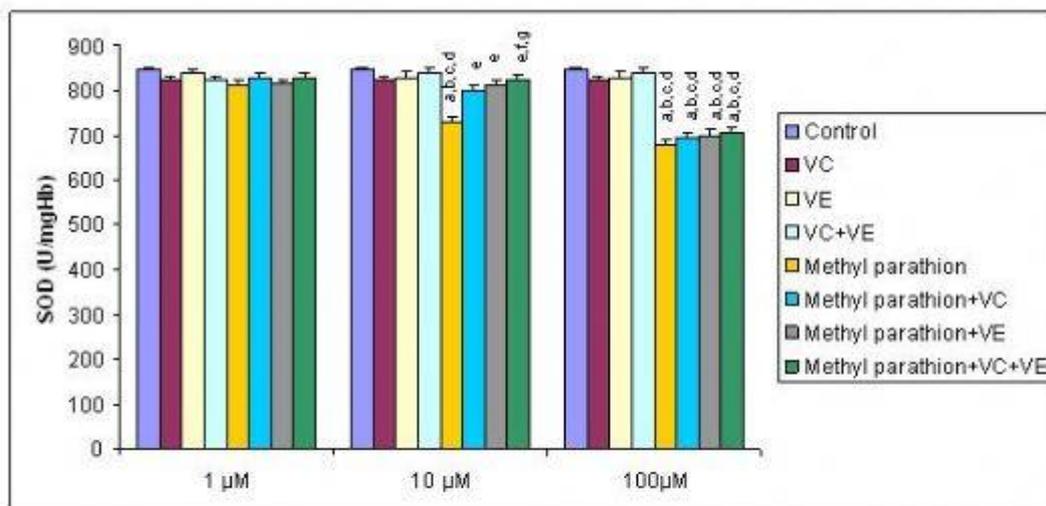
Keywords: Dichlorvos, Methyl parathion, MDA, SOD, CAT, erythrocytes

Figure 1. MDA levels in control and experimental groups of erythrocytes with methyl parathion



a Comparison of nontreated control cells and other groups ($P < 0.05$). *b* Comparison of VC-treated cells with VE-, VE+VC-, methyl parathion-, methyl parathion+VC-, methyl parathion +VE-, and methyl parathion+VC+VE-treated cells ($P < 0.05$). *c* Comparison of VE-treated cells with VE+VC-, methyl parathion-, methyl parathion+VC-, methyl parathion+VE-, and methyl parathion+VC+VE-treated cells ($P < 0.05$). *d* Comparison of VC+VE-treated cells with methyl parathion-, methyl parathion+VC-, methyl parathion+VE-, and methyl parathion+VC+VE-treated cells ($P < 0.05$). *e* Comparison of methyl parathion-treated cells with methyl parathion+VC-, methyl parathion+VE-, and methyl parathion+VC+VE-treated cells ($P < 0.05$). *f* Comparison of methyl parathion+VC-treated cells with methyl parathion+VE- and methyl parathion+VC+VE-treated cells ($P < 0.05$). *g* Comparison of methyl parathion+VE-treated cells with methyl parathion+VC+VE-treated cells ($P < 0.05$). Data represent the means \pm SD of six samples.

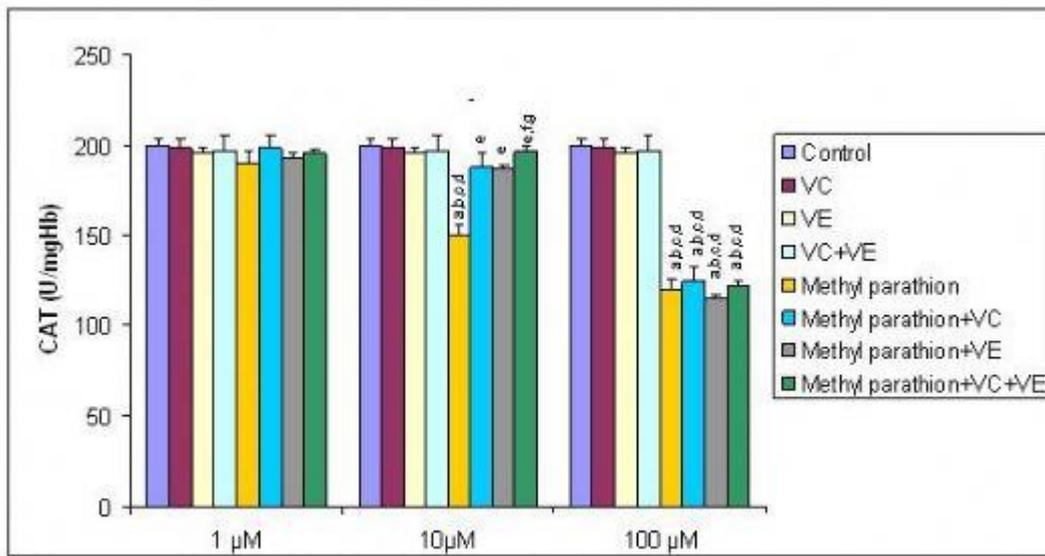
Figure 2. SOD activity in control and experimental groups of erythrocytes with methyl parathion



a Comparison of nontreated control cells and other groups ($P < 0.05$). *b* Comparison of VC-treated cells with VE-, VE+VC-, methyl parathion-, methyl parathion+VC-, methyl parathion +VE-, and methyl parathion+VC+VE-treated cells ($P < 0.05$). *c* Comparison of VE-treated cells with VE+VC-, methyl parathion-, methyl parathion+VC-, methyl parathion+VE-, and methyl parathion+VC+VE-treated cells ($P < 0.05$). *d* Comparison of VC+VE-treated cells with methyl parathion-, methyl parathion+VC-, methyl parathion+VE-, and methyl parathion+VC+VE-treated cells ($P < 0.05$). *e* Comparison of methyl parathion-treated cells with methyl parathion+VC-, methyl parathion+VE-, and methyl parathion+VC+VE-treated cells ($P < 0.05$). *f* Comparison of methyl parathion+VC-treated cells with methyl parathion+VE- and methyl parathion+VC+VE-treated cells ($P < 0.05$).

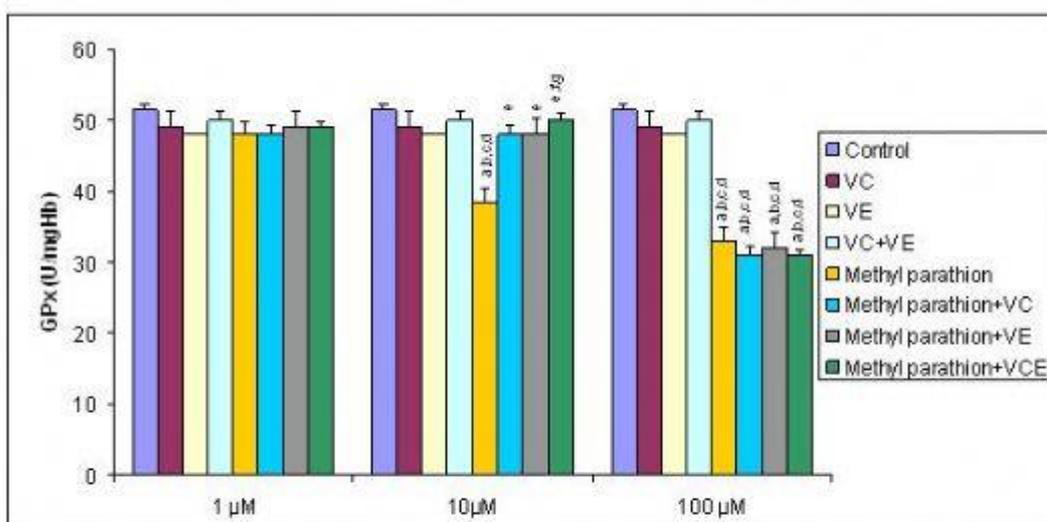
parathion+VC+VE-treated cells ($P<0.05$). gComparison of methyl parathion+VE-treated cells with methyl parathion+VC+VE-treated cells ($P<0.05$). Data represent the means \pm SD of six samples.

Figure 3. CAT activity in control and experimental groups of erythrocytes with methyl parathion



aComparison of nontreated control cells and other groups ($P<0.05$). bComparison of VC-treated cells with VE-, VE+VC-, methyl parathion-, methyl parathion+VC-, methyl parathion +VE-, and methyl parathion+VC+VE-treated cells ($P<0.05$). cComparison of VE-treated cells with VE+VC-, methyl parathion-, methyl parathion+VC-, methyl parathion+VE-, and methyl parathion+VC+VE-treated cells ($P<0.05$). dComparison of VC+VE-treated cells with methyl parathion-, methyl parathion+VC-, methyl parathion+VE-, and methyl parathion+VC+VE-treated cells ($P<0.05$). eComparison of methyl parathion-treated cells with methyl parathion+VC-, methyl parathion+VE-, and methyl parathion+VC+VE-treated cells ($P<0.05$). fComparison of methyl parathion+VC-treated cells with methyl parathion+VE- and methyl parathion+VC+VE-treated cells ($P<0.05$). gComparison of methyl parathion+VE-treated cells with methyl parathion+VC+VE-treated cells ($P<0.05$). Data represent the means \pm SD of six samples.

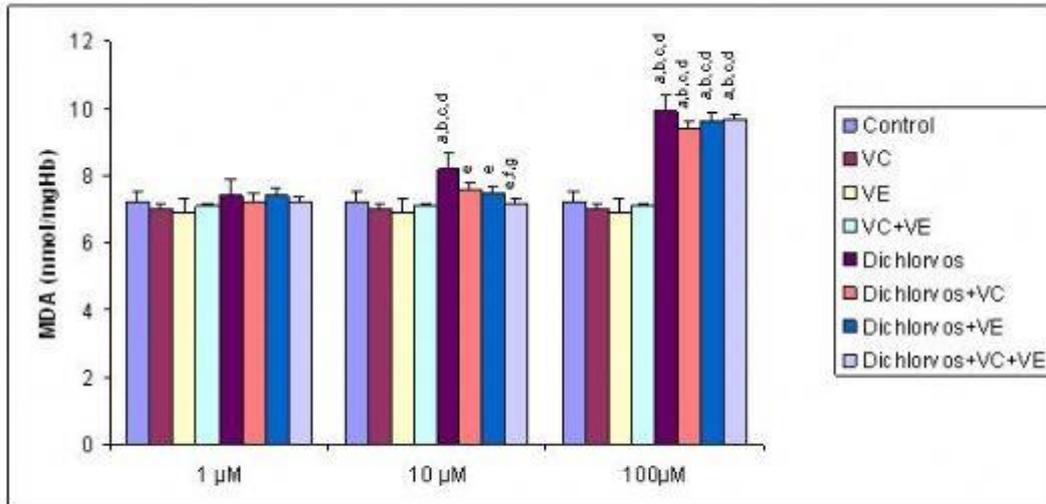
Figure 4. GPx activity in control and experimental groups of erythrocytes with methyl parathion



aComparison of nontreated control cells and other groups ($P<0.05$). bComparison of VC-treated cells with

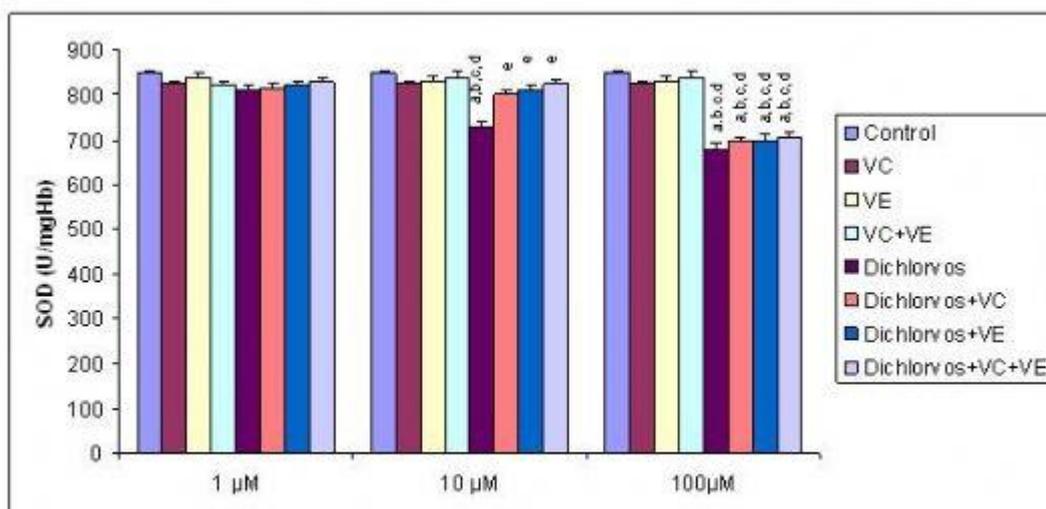
VE-, VE+VC-, methyl parathion-, methyl parathion+VC-, methyl parathion +VE-, and methyl parathion+VC+VE-treated cells ($P<0.05$). cComparison of VE-treated cells with VE+VC-, methyl parathion-, methyl parathion+VC-, methyl parathion+VE-, and methyl parathion+VC+VE-treated cells ($P<0.05$). dComparison of VC+VE-treated cells with methyl parathion-, methyl parathion+VC-, methyl parathion+VE-, and methyl parathion+VC+VE-treated cells ($P<0.05$). eComparison of methyl parathion-treated cells with methyl parathion+VC-, methyl parathion+VE-, and methyl parathion+VC+VE-treated cells ($P<0.05$). fComparison of methyl parathion+VC-treated cells with methyl parathion+VE- and methyl parathion+VC+VE-treated cells ($P<0.05$). gComparison of methyl parathion+VE-treated cells with methyl parathion+VC+VE-treated cells ($P<0.05$). Data represent the means \pm SD of six samples.

Figure 5. MDA levels in control and experimental groups of erythrocytes with dichlorvos



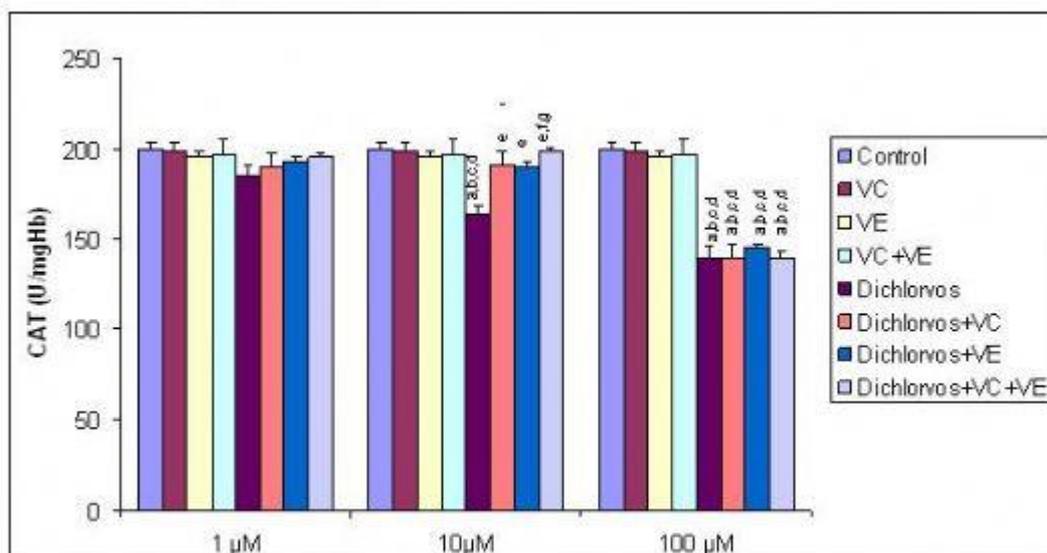
aComparison of nontreated control cells and other groups ($P<0.05$). bComparison of VC-treated cells with VE-, VE+VC-, dichlorvos-, dichlorvos +VC-, dichlorvos +VE-, and dichlorvos+VC+VE-treated cells ($P<0.05$). cComparison of VE-treated cells with VE+VC-, dichlorvos -, dichlorvos +VC-, dichlorvos +VE-, and dichlorvos +VC+VE-treated cells ($P<0.05$). dComparison of VC+VE-treated cells with dichlorvos -, dichlorvos +VC-, dichlorvos +VE-, and dichlorvos +VC+VE-treated cells ($P<0.05$). eComparison of dichlorvos-treated cells with dichlorvos +VC-, dichlorvos +VE-, and dichlorvos +VC+VE-treated cells ($P<0.05$). fComparison of dichlorvos +VC-treated cells with dichlorvos +VE- and dichlorvos +VC+VE-treated cells ($P<0.05$). gComparison of dichlorvos +VE-treated cells with dichlorvos +VC+VE-treated cells ($P<0.05$). Data represent the means \pm SD of six samples.

Figure 6. SOD activity in control and experimental groups of erythrocytes with dichlorvos



a Comparison of nontreated control cells and other groups ($P < 0.05$). *b* Comparison of VC-treated cells with VE-, VE+VC-, dichlorvos-, dichlorvos +VC-, dichlorvos +VE-, and dichlorvos+VC+VE-treated cells ($P < 0.05$). *c* Comparison of VE-treated cells with VE+VC-, dichlorvos -, dichlorvos +VC-, dichlorvos +VE-, and dichlorvos +VC+VE-treated cells ($P < 0.05$). *d* Comparison of VC+VE-treated cells with dichlorvos -, dichlorvos +VC-, dichlorvos +VE-, and dichlorvos +VC+VE-treated cells ($P < 0.05$). *e* Comparison of dichlorvos-treated cells with dichlorvos +VC-, dichlorvos +VE-, and dichlorvos +VC+VE-treated cells ($P < 0.05$). *f* Comparison of dichlorvos +VC-treated cells with dichlorvos +VE- and dichlorvos +VC+VE-treated cells ($P < 0.05$). *g* Comparison of dichlorvos +VE-treated cells with dichlorvos +VC+VE-treated cells ($P < 0.05$). Data represent the means \pm SD of six samples.

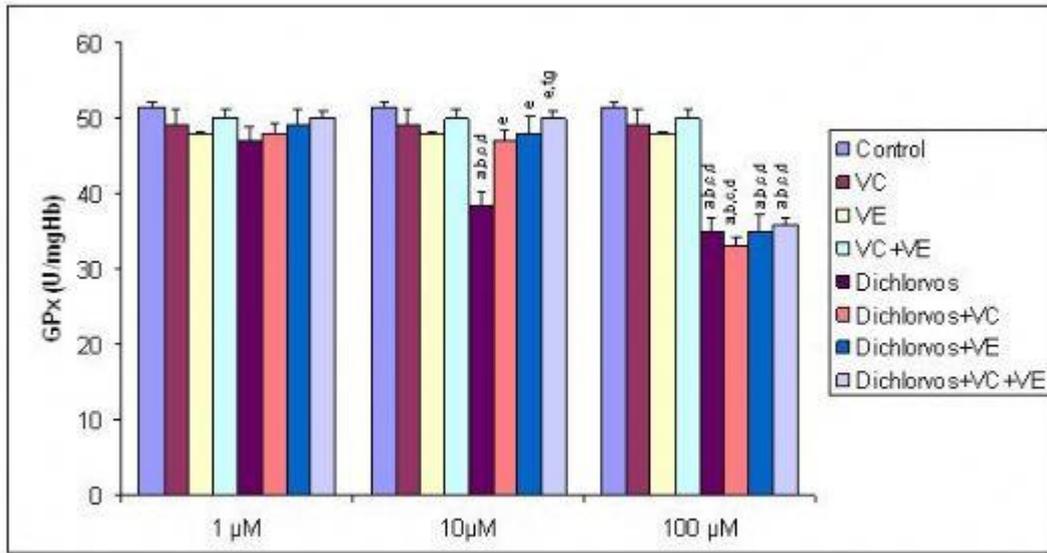
Figure 7. CAT activity in control and experimental groups of erythrocytes with dichlorvos



a Comparison of nontreated control cells and other groups ($P < 0.05$). *b* Comparison of VC-treated cells with VE-, VE+VC-, dichlorvos-, dichlorvos +VC-, dichlorvos +VE-, and dichlorvos+VC+VE-treated cells ($P < 0.05$). *c* Comparison of VE-treated cells with VE+VC-, dichlorvos -, dichlorvos +VC-, dichlorvos +VE-, and dichlorvos +VC+VE-treated cells ($P < 0.05$). *d* Comparison of VC+VE-treated cells with dichlorvos -, dichlorvos +VC-, dichlorvos +VE-, and dichlorvos +VC+VE-treated cells ($P < 0.05$). *e* Comparison of dichlorvos-treated cells with dichlorvos +VC-, dichlorvos +VE-, and dichlorvos +VC+VE-treated cells ($P < 0.05$). *f* Comparison of dichlorvos +VC-treated cells with dichlorvos +VE- and dichlorvos +VC+VE-

treated cells ($P<0.05$). gComparison of dichlorvos +VE-treated cells with dichlorvos +VC+VE-treated cells ($P<0.05$). Data represent the means \pm SD of six samples.

Figure 8. GPx activity in control and experimental groups of erythrocytes with dichlorvos



aComparison of nontreated control cells and other groups ($P<0.05$). bComparison of VC-treated cells with VE-, VE+VC-, dichlorvos-, dichlorvos +VC-, dichlorvos +VE-, and dichlorvos+VC+VE-treated cells ($P<0.05$). cComparison of VE-treated cells with VE+VC-, dichlorvos -, dichlorvos +VC-, dichlorvos +VE-, and dichlorvos +VC+VE-treated cells ($P<0.05$). dComparison of VC+VE-treated cells with dichlorvos -, dichlorvos +VC-, dichlorvos +VE-, and dichlorvos +VC+VE-treated cells ($P<0.05$). eComparison of dichlorvos-treated cells with dichlorvos +VC-, dichlorvos +VE-, and dichlorvos +VC+VE-treated cells ($P<0.05$). fComparison of dichlorvos +VC-treated cells with dichlorvos +VE- and dichlorvos +VC+VE-treated cells ($P<0.05$). gComparison of dichlorvos +VE-treated cells with dichlorvos +VC+VE-treated cells ($P<0.05$). Data represent the means \pm SD of six samples.

The antioxidant effect of chitosan in carbon tetrachloride-induced hepatitis in rats

Cristian Cezar Login¹, Adriana Muresan¹, Cornel Catoi², Adriana Filip¹, Marilena Oarga³, Doina Daicoviciu¹, Nicoleta Decea¹, Andras Nagy², Flaviu Tabaran², Remus Moldovan¹, Simona Clichici¹

¹Department of Physiology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

²Department of Pathology, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

³Department of Professional Diseases, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

OBJECTIVE: Carbon tetrachloride is a solvent known for its hepatotoxicity. Its effects are due mostly to $CCl_3\cdot$ radical production. Chitosan, a natural polymer obtained from the exoskeleton of marine crustaceans, seems to have antioxidant properties. The aim of our study was to assess the antioxidant effect of chitosan in chronic exposure to CCl_4 .

METHODS: The experiments lasted four weeks. Eighty female Wistar rats (215 ± 12 g), divided into four equal groups were used. Group I (control) received twice a week 0.9 ml of sunflower oil. Rats from groups II-IV received twice a week 1.2 ml/kg CCl_4 25% diluted in sunflower oil (by gavage). The animals from group III also received 5 mg/kg vitamin E daily (i.m.), while those of group IV, 3 mg/kg of chitosan (i.p.). After 15 and 30 days, blood and liver tissue samples have been taken. The oxidative stress markers (malondialdehyde, SH groups, GSH) and liver function (AST) were assessed. Liver tissue samples were examined and Knodell Histological Activity Index was calculated.

RESULTS: AST and MDA level showed a significant increase after exposure to CCl_4 : first in the liver tissue (day 15) then in the blood (day 30). Chitosan reduced the oxidative stress markers and increased GSH to values similar to those of the control group. Liver function was improved. The histopathological findings supported the results.

CONCLUSIONS: Chitosan has a good antioxidant effect. It is able to decrease lipid peroxidation and to protect the endogenous antioxidant systems (GSH) against CCl_4 aggression. In the used dose, chitosan has a hepatoprotective and antioxidant effect superior to that of the vitamin E.

Keywords: oxidative stress, chitosan, carbon tetrachloride, toxic hepatitis, vitamin E

Obestatin alleviates indomethacin-induced gastric mucosal injury via the inhibition of neutrophil recruitment

Gülsün Memi¹, Leyla Semiha Şen², Zarife Nigâr Özdemir¹, Demir Kıran³, Feriha Ercan³, Berrak Ç. Yeğen¹,
Cumhur Yeğen⁴

¹Department of Physiology, Marmara University School of Medicine, Istanbul

²Siirt State Hospital, General Surgery Department, Siirt

³Department of Histology and Embryology, Marmara University School of Medicine, Istanbul

⁴Department of General Surgery, Marmara University School of Medicine, Istanbul

OBJECTIVE: Obestatin, a 23-amino acid peptide derived from the ghrelin peptide precursor, is synthesized in the stomach. We have recently demonstrated the protective effect of obestatin in intestinal ischemia-reperfusion injury. We aimed to investigate the putative anti-ulcer and anti-oxidant effects of obestatin in a gastric ulcer model induced by a non-steroidal-anti-inflammatory drug.

METHODS: Sprague Dawley rats of both sexes were injected subcutaneously with indomethacin (25 mg/kg; n=25) or vehicle (5% NaHCO₃; n=5). Indomethacin-treatment was followed by either saline or obestatin (10, 30, and 100 µg/kg, intraperitoneally). One hour after indomethacin injection, gastric mucosal blood flow measurements were made by laser Doppler under urethane (1g/kg, i.p) anesthesia. Three hours later, the rats were decapitated and gastric lesions were scored. Stomach samples were obtained for the measurement of malondialdehyde (MDA) and glutathione (GSH) levels, myeloperoxidase (MPO) activity and for histological examination. Values were compared by ANOVA.

RESULTS: Indomethacin resulted in severe damage of surface mucous cells and gastric pits as observed by light and scanning-electron microscopy. Lesion index, MDA level and MPO activity were increased with the induction of ulcer ($p<0.01-0.001$), while obestatin significantly decreased the gastric ulcer area in a dose-dependent manner and reduced myeloperoxidase activity and lipid peroxidation in the gastric tissue ($p<0.05$), but gastric GSH content was not changed by obestatin treatment. Neither of the obestatin doses significantly changed gastric mucosal blood flow.

CONCLUSIONS: Obestatin alleviates indomethacin-induced gastric mucosal injury by a mechanism that is not associated with mucosal blood flow, but acts via the inhibition of neutrophil recruitment.

Keywords: Obestatin, gastric ulcer, NSAID, oxidant injury

The investigation of serum paraoxonase enzyme activities and malondialdehyde levels in burn cases and healthy controls

Serap Yildirim¹, Akar Karakoç², Songül Doğanay¹, Esra Laloğlu², Enver Aydın³

¹Departments of Physiology, Faculty of Medicine, Ataturk University, Erzurum-TURKEY

²Departments of Biochemistry, Faculty of Medicine, Ataturk University, Erzurum-TURKEY

³Departments of Plastic Reconstructive and Aesthetic Surgery, Faculty of Medicine, Ataturk University, Erzurum-TURKEY

OBJECTIVE: In this study, it was purposed the investigation of possible changes in serum oxidative stress parameters in burn cases and the comparison with healthy controls.

METHODS: Forty-one burn patients and thirty-eight healthy subjects were included in the study. Burn cases were selected from patients hospitalized in burn unit for treatment and having second- and third-degree burns. Malondialdehyde (MDA) levels and PON-1 paraoxonase and arylesterase activities were measured in the fasting serum samples collected from subjects.

RESULTS: Body burn percentage of patients were meanly 13 ± 9 (% 3 – 45). While serum MDA levels and paraoxonase activity of burn cases were increased, the arylesterase activities were shown to be decreased, as compared with healthy subjects. MDA, paraoxonase and arylesterase values in the controls were 8.7 ± 2.7 mM, 106 ± 78 U/mL and 75 ± 20 U/mL, respectively. Those parameters were 9.7 ± 3.6 mM, 114 ± 65 U/mL and 68 ± 21 U/mL in burn cases, respectively. Besides, a significant negative correlation was found between the percentages of body burn and arylesterase activities in patients ($p < 0.05$, $r = -0.367$). In addition, there was a positive correlation between the burn degree and serum MDA levels of patients ($p < 0.05$, $r = 0.317$).

CONCLUSIONS: We concluded that there is an association between the degree of burn and MDA level, and between the percentage of burn and arylesterase activity, which means that oxidative stress may be important in the pathogenesis of burn.

Keywords: Burn; Malondialdehyde; Oxidative Stress; Paraoxonase

The Effects of Heat Stress on Skeletal Muscle Oxidative Protein Damage Which Forms In Hypoxic Conditions

Rıdvan Colak¹, Metin Bastug², Hakan Ficicilar², Eda Agascioglu³, Ali Haydar Demirel⁴

¹Uskup Primary School Bagcilar, Istanbul

²Department of Physiology, Ankara University, School of Medicine, Ankara

³Sports and Physical Education High School, Cankaya University, Ankara

⁴Department of Sports Medicine, School of Medicine, Hacettepe University, Ankara

OBJECTIVE: Hypoxia can lead oxidative damage in tissues. Increase in the heat shock 72 protein level, mainly formed with heat, is involved in the protection of cells from different types of stresses including oxidative stress. The aim of this investigation is to determine that whether heat stress is effective for preventing rat skeletal muscle from hypoxia inducible oxidative protein damage.

METHODS: Thirty-five Wistar Albino male rats randomly assigned to one of the four groups: Hypoxia (HN=9), hypoxia with heat stress (HS=10), normoxia (NN=8) and normoxia with heat stress (NS=8). The hypoxia groups were kept in simulated altitude of 6000m (%9.7 O₂, 90.3 N₂) for up to 15 days. For heat treatment, animals were retained at 41 0 C environment for 60 minutes. Total of three heat sessions, two consecutive days just before the and at the 8th day of experiment were applied. At the end of the 15th day, all rats were anesthetized with sodium pentobarbiturate (50 mg/kg) and then were killed via servical dislocation. Plantaris (PLA) and Extensor digitorum longus (EDL) muscles were used for analyzing protein carbonyl (PCO), advanced oxidation protein products (AOPP) and protein thiol (P-SH). One Way ANOVA and Post Hoc Tukey tests were used for statistical analysis.

RESULTS: Fifteen days of hypoxia resulted in increase in PCO and AOPP and decrease in P-SH levels in both PLA and EDL muscles only in HN group (p<0.05). There were no differences among experimental groups in variables investigated.

CONCLUSIONS: In conclusion, heat stress provided protection against hypoxia-induced oxidative protein damage in rat skeletal muscles.

Keywords: Advanced oxidation protein products, heat stress, hypoxia, protein carbonyl, protein thiol.

The effects of remifentanil preconditioning on intestinal ischemia and reperfusion

Hale Sayan Ozaçmak¹, Inci Turan¹, Veysel Haktan Özaçmak³, Figen Barut², Volkan Hancı³

¹Department of Physiology, Zonguldak Karaelmas University

²Department of Pathology, Zonguldak Karaelmas University

³Anaesthesiology, Zonguldak Karaelmas University, Zonguldak, Turkey

OBJECTIVE: Interruption of blood supply leads to profound functional and structural alterations of the gastrointestinal tract which may lead to deranged gastrointestinal motility. Opioids have been shown to induce preconditioning in myocardial and neuronal tissues. The objective of this study was to determine whether remifentanil could attenuate intestinal ischemia and reperfusion injury.

METHODS: Male Wistar Albino rats were subjected to mesenteric ischemia (30 min) followed by reperfusion (3 hours). Three groups were designed: sham operated control; I/R; I/R and remifentanil preconditioning. Rats in remifentanil preconditioning group were subjected to infusion of remifentanil (3µg/kg/min) for 60 min, half of which started before inducing ischemia. Collecting the ileal tissues, evaluation of damage was based on contractile responses to acetylcholine, levels of lipid peroxidation, and observation of histopathological features in intestinal tissue.

RESULTS: Following reperfusion, a significant decrease in acetylcholine-induced contractile response, a remarkable increase in lipid peroxidation, and a significant injury in mucosa of the tissues were observed ($p<0.05$). The contractile responses of remifentanil-pretreated group were significantly different from those of I/R group ($p<0.05$). Pretreatment also significantly suppressed lipid peroxidation in post-ischemic tissues. I/R group was histopathologically evaluated as grade 2. In preconditioning with remifentanil, mucosal damage was moderate, staging as grade 1.

CONCLUSIONS: Pretreatment with remifentanil can attenuate intestinal I/R injury at a remarkable degree possibly by lowering lipid peroxidation.

Keywords: remifentanil, intestinal ischemia, reperfusion

Protective Effect of Quercetin on Ethanol-induced Testicular Damage in Rats

Ramazan Uygur¹, Murat Yagmurca², Ozan Alper Alkoc³, Abdurrahman Genç⁴, Ahmet Songur⁵, Kagan Ucok⁴, Oguz Aslan Ozen¹

¹Namik Kemal University, School of Medicine, Department of Anatomy, Tekirdag, Turkey

²Fatih University, School of Medicine, Department of Histology&Embryology, Ankara, Turkey

³Duzce University, School of Medicine, Department of Anatomy, Duzce, Turkey

⁴Afyon Kocatepe University, School of Medicine, Department of Physiology, Afyonkarahisar, Turkey

⁵Afyon Kocatepe University, School of Medicine, Department of Anatomy, Afyonkarahisar, Turkey

OBJECTIVE: Ethanol exposure is known to suppress male reproductive activity in laboratory animals and humans. Quercetin is a natural antioxidant. In this study, the effects of quercetin on the testicular changes due to ethanol exposure in rats were investigated.

METHODS: The animals were divided into three groups. Group 1) Control (Saline 3 ml/kg/day, i.g.; n=9), Group 2) Ethanol (40% 3 g/kg/day, i.g.; n=9), Group 3) Ethanol + Quercetin (270 mg/kg/day, i.g.; n=9). After 8-weeks of treatment, all animals were sacrificed and the testes were removed for biochemical and histopathological investigation. Tissue sections from testes were stained with hematoxylin & eosin. Apoptotic cells were analyzed by using TUNEL assay. The activities of SOD, CAT, and GSH-Px as well as MDA and NO levels were measured by spectrophotometry.

RESULTS: It has been observed that the ethanol administered rats have retarded in terms of body weight gain, besides developing degenerative changes in morphologic analyses as well as showing decrease in seminiferous tubule diameters. TUNEL assay also showed an increase in apoptotic cell number. Biochemically, increases in MDA and NO levels were detected, whereas decreases in SOD, CAT, and GSH-Px activities were observed.

Histopathologic changes caused by ethanol have been recovered partly or completely by giving quercetin. It was also found that protection was provided by increasing SOD, CAT and GSH-Px activities and by decreasing the levels of NO in groups administered quercetin.

CONCLUSIONS: In view of the present findings, it is suggested that quercetin treatment may prevent ethanol-induced oxidative damage and apoptosis in rat testes.

Keywords: Ethanol, quercetin, testis, antioxidant enzymes, rat.

The Effects Of Phenytoin And Lamotrigine On Lipid Peroxidation Levels Of Brain Tissue In Offspring Of Epileptic Rats

Handan Soysal¹, Zümrüt Doğan², Yusuf Türköz³, Nihat Ekinci², Özden Kamışlı⁴

¹Medicine Faculty Departments of Anatomy, Adiyaman University, Adiyaman, Turkey

²Medicine Faculty Departments of Anatomy, Inonu University, Malatya, Turkey

³Medicine Faculty Departments of Medical Biochemistry, Inonu University, Malatya, Turkey

⁴Medicine Faculty Departments of Neurology, Inonu University, Malatya, Turkey

OBJECTIVE: Comparison of effects of lamotrigine and phenytoin on lipid peroxidation of brain tissue in offspring of epileptic rats.

METHODS: One hundred and twenty-seven Wistar-Albino offspring rats were used in the study. Acute epileptic seizures were produced by an experimental epilepsy model at the 13th day of pregnancy. Lamotrigine (2 mg/kg) and phenytoin (25 mg/kg) were administered to pregnant rats intraperitoneally at each day of pregnancy period. We measured the MDA levels of brain tissue in offspring rats at 0th, 21th, and 38th days after the birth.

RESULTS: MDA levels of experimental epilepsy group were significantly higher compared with control group ($p<0.05$). While phenytoin significantly reduced brain MDA levels in offsprings of pregnant rats ($p<0.05$), lamotrigine significantly elevated brain MDA levels ($p<0.05$).

CONCLUSIONS: We suggest that lipid peroxidation plays an important role in the pathogenesis of experimental epilepsy and phenytoin showed protective effect on brain tissue by reducing lipid peroxidation.

Keywords: Epileptic pregnant rat, Phenytoin, Lamotrigine, lipid peroxidation

Organic Dried Apricot Prevents the Pre-carcinogenic and Toxic Effects of the Acrylamide in the Large intestine Tissues of the Rats

Erman Erdemli, Yusuf Türköz

Faculty of Medicine, Department of Medical Biochemistry, Inonu Universty, Malatya, Turkey

OBJECTIVE: The main of this study was to analyze the changes in the large intestine of rats, which have been given acrylamide for 3 months, and the possible protective effect of dried organic apricot.

METHODS: Forty female rats were divided into four groups: control group was fed with standard chow, apricot group was fed with standard chow plus 5% apricot, acrylamide group was fed with standard chow and daily acrylamide 500 µg/kg via drinking water, acrylamide+apricot group was fed with standard chow plus 5% apricot and daily acrylamide 500 µg/kg via drinking water for 12 weeks. After 12 weeks all the rats were decapitated and their large intestine samples were used to measure “Glutathione S-Transferase Pi (GST-Pi) gene expression and antioxidant parameters.

RESULTS: GST-Pi gene expression and MDA levels of large intestine in acrylamide group were significantly ($p<0.05$) increased compared to the control group’s levels. On the other hand, those levels were found to be significantly decreased ($p<0.05$) when dried apricot was added to the diet of the rats taking acrylamide.

CONCLUSIONS: The studies on humans and animals show that increase of GST-Pi gene expression in the tissues is related to the pre-carcinogenic changes. In this study, GST-Pi gene expression and MDA levels in acrylamide group large intestine tissues significantly increased. When the organic dried apricot was given at the same time, these levels were decreased to the control group levels. These results show that the acrylamide caused the pre-carcinogenic and toxic changes on large intestine tissues and the organic dried apricot prevented these effects.

Keywords: Rat, large intestine, acrylamide, organic dried apricot, RT-PCR, pre-carcinogenic changes

Preliminary Studies on The in vivo Antioxidant Activity of Cornus mas L. Leaves in Carbontetrachloride - treated rats

Engin Celep¹, Mohammed Charehsaz², Ahmet Aydın², Erdem Yeşilada¹

¹Department of Pharmacognosy, Yeditepe University, Faculty of Pharmacy, Istanbul, Turkey

²Department of Toxicology, Yeditepe University, Faculty of Pharmacy, Istanbul, Turkey

OBJECTIVE: The leaves of *Cornus mas* L. (Cornaceae) have been widely used in the Anatolian folk medicine against diabetes & diarrhea. As a part of our ongoing studies on this plant, this study was designed to investigate the glutathione peroxidase (GSH-Px) activities in the livers of CCl₄-treated Sprague-Dawley rats (270-300 g)

METHODS: Methanolic extract (70%) of plant samples was prepared and administered p.o in 50, 100, 200 mg/kg bw doses to animals for 5 days. On the second and third day of extract administration, CCl₄ were administered 1ml/kg bw s.c to all groups. Silymarin was used as reference and distilled water as negative control. The ALT/AST activities of all groups were measured as an indicator of hepatotoxicity.

RESULTS: According to the result of ALT/AST activities, CCl₄ successfully induced hepatotoxicity. The GSH-Px activities were found as 1125±124, 1130±108, 1273±179 U/L in 50, 100, 200 mg/kg bw dose groups, respectively. Whereas GSH-Px activity of the negative control CCl₄ – treated group was 1089 ± 202 U/L. Although the results showed an increase with respect to negative control group, these differences were not significant.

CONCLUSIONS: It can be concluded that only GSH-Px activity measurement is not enough to evaluate entire antioxidative capacity of a sample. Our studies on other antioxidant enzymes are in progress.

Keywords: *Cornus mas* L., antioxidative capacity, GSH-Px

Effects of MIBI on Antioxidant Defense System and Lipid Peroxidation in the Heart of Sprague Dawley Rats

Gökhan Cesur¹, Duygu Kumbul Doğuç², Mustafa Yıldız³, Serdal Öğüt⁴, Mümin Polat⁴

¹Department of Physiology, Suleyman Demirel University, Isparta, Turkey

²Department of Biochemistry, Suleyman Demirel University, Isparta, Turkey

³Department of Nuclear Medicine, Suleyman Demirel University, Isparta, Turkey

⁴Blood Bank, Suleyman Demirel University Hospital, Isparta, Turkey

OBJECTIVE: Nuclear medicine, which is an important medical discipline, has been using radiopharmaceuticals for diagnostic and therapeutic purposes for many diseases. Technetium-99m methoxyisobutylisonitrile (Tc-99m sestamibi) is a lyphophylic complex that has a positive loaded isonitril group. Aim of the study is to investigate whether Tc-99m sestamibi, which is one of the mostly used radiopharmaceutical in nuclear medicine field, cause oxidative damage or not in rats' heart after an injection.

METHODS: Sixteen male Sprague-Dawley rats were randomly divided into two groups: (I) Tc-99m sestamibi group (n:8), Tc-99m sestamibi administered intravenously with the dose of 0,35 mCi/kg comparable with the dose used in patients. (II) Control group (n:8), one dose of isotonic sodium chloride was administered intravenous with the same volume as Tc-99m sestamibi group. The animals were killed by decapitation 30 min after injection. Malondialdehyde was used as markers of oxidative stress-induced heart impairment. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate the changes of antioxidant status

RESULTS: In the Tc-99m sestamibi group (I), treated Tc-99m sestamibi produced a significant decrease in activities of antioxidant enzymes SOD (P=0,004) and CAT (P=0,001) in myocardial tissue, while MDA (P=0,003) level increased when compared with control group (II). On the other hand, the GSH-Px (P=0,011) activities were significantly increased in the Tc-99m sestamibi treated rats, compared with the untreated rats.

CONCLUSIONS: These findings demonstrate that in vivo acute administration of Tc-99m sestamibi results in the induction of lipid peroxidation and changes the activities of antioxidant enzymes in rat heart tissue, suggesting that free radicals may be involved in the toxic effects of the radiopharmaceutical compounds.

Keywords: Heart, MIBI, Oxidative stress.

Protective effects of melatonin on heart tissue in pinealectomized rats

Alaadin Polat¹, Hakan Parlakpınar², Seda Taşdemir², Ahmet Acet²

¹Inönü Üniversitesi Tıp Fakültesi Fizyoloji Anabilim Dalı

²Inönü Üniversitesi Tıp Fakültesi Farmakoloji Anabilim Dalı

OBJECTIVE: In the free radical theory of aging, it is suggested that accumulated free radical damage may be responsible for the degenerative process during aging. It is known that there is a reduction in serum melatonin concentration during aging. This situation may cause damage in the heart tissue, which is one of the more perfusing organs. The aim of the current study was to expose rats to an aging process via surgical pinealectomy (Px). For this, the effects of lack of chronic physiological melatonin on heart tissue were observed in the current study.

METHODS: The animals were divided into three groups. Group I and group II were designated as sham and Px rats. They were housed for 5 months before the beginning of treatment. Rats in group III were (denoted as) Px and melatonin was injected with 4 mg/kg/day (i.p.) for 28 days. Changes in oxidant substances were evaluated, especially those in lipid peroxidation, nitric oxide (NO), reduced glutathione (GSH) content and SOD activity levels during this period so that the antioxidant effects of long-term exogenous melatonin could be investigated.

RESULTS: In Px group, MDA and NO levels were found as elevated when compared with the sham group. The Px group exhibited reduced SOD activity and GSH content. All of these harmful changes were restored by melatonin supplementation.

CONCLUSIONS: This protective effect may be associated with melatonin's both lipophilic and hydrophilic effects, thus providing on-site protection against free radical-mediated tissue damage.

Keywords: Heart tissue, melatonin, oxidative stress, pinealectomy

Melatonin administration prevents oxidant stress and histological changes in the duodenum and pancreas tissues of pinealectomized rats

Dondu Koykun¹, Abdulkerim Kasim Baltaci¹, Mustafa Cihat Avunduk², Burcu Cevreli³, Nihal Savuran², Rasim Mogulkoc², Bayram Yilmaz³

¹Selcuk University, Selcuklu Medical School, Department of Physiology, Konya-Turkey

²Selcuk University, Meram Medical School, Department of Pathology, Konya-Turkey

³Yeditepe University, Medical School, Department of Physiology, Istanbul, Turkey

OBJECTIVE: The present study aims to examine how pinealectomy and melatonin administration affects oxidant stress and histological parameters in the duodenum and pancreas tissues of rats.

METHODS: The study included 24 adult male Sprague-Dawley type rats, which were allocated to 4 groups: Group 1, control; Group 2, melatonin-administered control; Group 3, pinealectomy (Px); Group 4, pinealectomized, melatonin-administered group. Pineal glands of the animals in groups 3 and 4 were removed under general anesthesia. The animals in groups 2 and 4 were administered 3 mg/kg ip melatonin for 4 weeks. The protocol was approved by the local ethics committee and the study was carried out at the Experimental Research and Center of Yeditepe University. At the end of the experiments, MDA and GSH levels were studied in duodenum and pancreas tissue samples taken from the animals after decapitation. These tissue samples were fixed in 10% buffered formaldehyde. The tissue samples were then subjected to autotechnicon processing. The samples embedded in paraffin were sliced by microtome and the cross-sections were placed on slides. The preparations stained with hematoxylin eosin were examined using light microscopy.

RESULTS: Pinealectomy (group 3) resulted in diffuse edema and an increase in MDA levels in duodenum and pancreas tissues. Melatonin administration (group 4) to pinealectomized animals significantly reversed these negative effects.

CONCLUSIONS: Results of the study demonstrate that pinealectomy leads to diffuse edema and lipid peroxidation in duodenum and pancreas tissues, and melatonin administration prevents these negative changes in the examined parameters.

Keywords: Pinealectomy, melatonin administration, duodenum and pancreas.

Anti-oxidative and anti-genotoxic effects of methanolic extract of *Mentha pulegium* on human lymphocyte culture

Lokman Alpsoy¹, Hilal Sahin¹, Seyda Karaman²

¹Department of Biology, Faculty of Art and Science, Fatih University, Istanbul, Turkey

²Department of Chemistry, Faculty of Art and Science, Fatih University, Istanbul, Turkey

OBJECTIVE: Many plants and vegetables have been found to contain antimutagens so the antimutagenic and antioxidant potential of these plants can be determined by different approaches. *Mentha pulegium* is one of these plants. In the present work, methanolic extract of *Mentha pulegium* from Erzurum, Turkey, was used in order to report the results of anti-oxidant capacity, anti-oxidant activity and anti-genotoxic effects.

METHODS: Total antioxidant capacity and total phenolic content were measured by using CUPRAC, ABTS and Folin–Ciocalteu colorimetric methods. Also, we evaluated the anti-oxidant enzyme activity such as superoxide dismutase (SOD) and glutathione peroxidase, total glutathione (GSH) and malondialdehyde (MDA) in human lymphocyte culture. In addition, anti-genotoxic effect of ME was studied by using sister chromatid exchange (SCE) method.

RESULTS: The total phenolic content was higher than the total antioxidant capacity (for the results of both the CUPRAC and ABTS methods) of methanolic extract of *M. pulegium* (ME). In CCl₄-treated group, the activity of SOD, glutathione peroxidase (GPx) and GSH decreased significantly and the level of MDA increased significantly. A significant increase in the activity of SOD, GPx and the level of GSH were seen when supplemented with ME to CCl₄-treated group. Furthermore, a significant decrease in the level of MDA was observed when compared with CCl₄ alone treated group. As a result, ME has shown anti-genotoxic effect depend on anti-oxidative effect on human lymphocyte culture.

CONCLUSIONS: The results of this study indicate that the extracts of the genus *Mentha* are favorable free radical scavengers as well as primary antioxidants that may react with free radicals and limit ROS attack on biological and food systems.

Keywords: anti-oxidant enzymes, genotoxicity, *Mentha pulegium*, sister chromatid exchange

Antioxidative activity of dried apricot determined in vitro by inducing liver lipid peroxidation

Yavuz Erden¹, Burak Bircan¹, Ersen Eraslan², Suat Tekin³

¹Firat University, Faculty of Sciences, Department of Biology, Elazig

²Firat University, Faculty of Medicine, Department of Physiology, Elazig

³Inonu University, Faculty of Medicine, Department of Physiology, Malatya

OBJECTIVE: In this study, it was detected that the investigation of antioxidant capacity of apricot fruit (*Prunus armeniaca*) dried in the different ways on the liver lipid peroxidation (LPO) are induced with Fe₂Cl (Fenton's reagent; FR) in in vitro condition.

METHODS: Dry apricot samples (SO₂+shine; SG, sun; G) were extracted in methanol. Two adult albino male rats were killed after anaesthetizing with mild chloroform and liver tissues were taken. The tissue samples were homogenized with Tris-EDTA buffer. After the homogenate was centrifuged, supernatant (ST) was used for in the experimental analysis. The groups were prepared as control, FR, FR+SG and FR+G. FR groups were added in ST Fe₂Cl solutions with fruit extracts as simultaneously and incubated for 24h. The levels of LPO was analysed on the HPLC device, according to the methods of Ohkawa et al. The total protein amount was determined by Lowry method.

RESULTS: The LPO amounts were increased at significant levels in all groups compared to control ($p < 0.0001$). LPO level was decreased significantly in the FR group than in the extract ($p < 0.0001$). The protein amount showed a partial reduction in the FR group than the control group ($p < 0.05$), but quite a high level of total protein extract was found ($p < 0.0001$).

CONCLUSIONS: In conclusion, apricot samples prevented LPO. Furthermore, it showed an effect to protect proteins. It is thought that the reason for the occurrence of this effect is related with vitamins and phytochemical compounds that are contained in the apricot fruit.

Keywords: Antioxidants, Apricot, Fenton's reagent, Lipid peroxidation, Total protein

Antioxidants stimulate viability and proliferation rate of primary mixed astroglial cultures

Gonca Dalkurt Mola, Vedat Evren, Dilek Taşkıran, Özlem A. Yılmaz
Department of Physiology, Ege University, Izmir, Turkey

OBJECTIVE: It has been demonstrated that some antioxidants may have trophic effects on cell growth and metabolism besides their protective effects against oxidative damage. Glutathione (GSH), ascorbic acid and α -tocopherol are main antioxidants in many cell types. The purpose of this study is to examine the proliferative effects of glutathione (GSH), ascorbic acid and α -tocopherol in astrocyte cultures.

METHODS: Primary mixed astroglial cultures were prepared from the frontal cortex of 1-2 days-old neonatal rats. Cells were grown in DMEM containing 10 % foetal calf serum and 20 mM glutamine, passaged and re-seeded in 96 well plates (104 cells/well). Astrocytes were treated with GSH (0.1, 0.5 and 1 mM), ascorbic acid (0.1, 0.5 and 1 mM) and α -tocopherol (0.05, 0.25 and 0.5 mM) in DMEM whereas the control group was treated with DMEM alone. After 48 hours, the cell viability and proliferation rate in the cultures were measured by the tetrazolium salt XTT; sodium,3'-[1-[phenylamino-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro) assay. Student-t test was used to evaluate the differences between the groups and p-value smaller than 0.05 was considered statistically significant.

RESULTS: All antioxidants enhanced the proliferation rate in the mixed astroglial cultures compared with control ($p < 0.05$) whereas the most significant effect was observed in 1 mM ascorbic acid-treated group.

CONCLUSIONS: The present findings suggest that antioxidants such as glutathione (GSH), ascorbic acid and α -tocopherol, may have stimulatory effects on viability and proliferation of mixed astroglial cultures.

Keywords: Antioxidant, Astroglia, Cell Viability

Oxidative stress generation after intraperitoneal administration of functionalized multi-walled carbon nanotubes in rats

Simona Clichici¹, Teodora Mocan¹, Alexandru Biris², Stefania Simona², Adriana Filip¹, Flaviu Tabaran³, Andras Nagy³, Adriana Muresan¹

¹Department of Physiology, University of Medicine and Pharmacy, Cluj-Napoca, Romania

²INCDTIM, Cluj-Napoca, Romania

³University of Veterinary Medicine and Agricultural Sciences, Cluj-Napoca, Romania

OBJECTIVE: Carbon nanotubes (CNT) are repetitive structures made of carbon atoms. They can be single-walled (SWCNT) or multi-walled (MWCNT) structures. Various applications have been proposed for CNT. However, their safety for human administration is a controversial subject. In vitro studies have pointed oxidative stress and inflammation as mechanisms involved in their cytotoxic effects, while there are only few in vivo studies, using especially pulmonary models. Based on our previous experience regarding SWCNT, our aim was to evaluate the capacity of functionalized MWCNT to generate oxidative stress after ip administration in rats.

METHODS: MWCNT were characterized by SEM, TEM and Raman spectroscopy. For in vivo administration, the dispersed MWCNT solutions were obtained after single strand DNA (ss-DNA) functionalization through sonication. The concentration of ss-DNA-MWCNTs solution was estimated by UV-Vis-NIR spectroscopy. The presence of MWCNT in blood and liver was detected by Raman spectroscopy at seriate time points after their administration. The oxidative stress was evaluated in dynamics (1h, 3h, 6h, 24 h, 48 h and 6 days) after the administration, both in blood and liver. We also assessed hepatic enzymes, markers of inflammation and angiogenesis.

RESULTS: The most evident alterations were produced in blood of MWCNT treated animals comparatively with controls at 1 hour interval (malondialdehyde 3.01 ± 0.22 vs 1.65 ± 0.15 nmol/ml; reduced glutathione 16.55 ± 1.15 vs 21.5 ± 1.2 nmol/ml) and in liver at 3 hours interval.

CONCLUSIONS: Our results demonstrate that ss-DNA-MWCNT produce oxidative stress both in blood and liver, with a transient pattern, and with a maximum at 1 hour after their ip administration.

Keywords: multi walled carbon nanotubes, oxidative stress

Diazinon-induced Kidney Toxicity and Protection by CAPE

Gökhan Cesur¹, Bora Büyükvanlı², Berit Gökçe Ceylan², Nigar Yılmaz³, Dudu Erduran³

¹Department of Physiology, Suleyman Demirel University, Isparta, Turkey

²Department of Anesthesiology, Suleyman Demirel University, Isparta, Turkey

³Department of Biochemistry, Mustafa Kemal University, Hatay, Turkey

OBJECTIVE: Diazinon, an organophosphate insecticide has been used in agriculture and domestic for several years. The aim of this study was to examine diazinon-induced oxidative stress that promotes production of reactive oxygen species and the role of caffeic acid phenetyl ester (CAPE) on kidney tissue against possible oxidative damage in rats.

METHODS: Thirty rats were used in the study. Animals were randomly grouped as follows: sham-operated control group (n=10) and experimental groups: (a) group II: Diazinon-induced (DI) group (n=10); and (b) group III: Diazinon-induced+CAPE-treated (DI+CAPE) group (n=10). Kidney tissues were removed to study the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and the levels of lipid peroxidation.

RESULTS: In DI group, while tissue malondialdehyde (MDA) levels were increased, SOD, CAT and GSH-Px activities were reduced compared with the control group. CAPE treatment in group III reversed these effects.

CONCLUSIONS: In this study, the increased levels of MDA and the decreased levels of kidney tissue SOD, CAT and GSH-Px activities demonstrate the role of oxidative mechanisms in diazinon-induced kidney tissue damage, and CAPE, via its free radical scavenging and antioxidant properties, ameliorates oxidative kidney injury. These results show that CAPE exhibits a protective effect on diazinon-induced and free radical-mediated oxidative kidney impairment in rats.

Keywords: CAPE, Diazinon, Oxidative stress.

Lipid Peroxidation in Kidney and Testis Tissue in Experimental Hypothyroidism: The Role of Zinc

Abdulkerim Kasim Baltaci¹, Rasim Mogulkoc¹, Mustafa Ayyıldız², Ertugrul Kafali³, Turkan Koyuncuoglu¹

¹Selcuk University, Selcuklu Medical School, Department of Physiology, Konya-Turkey

²Ondokuz Mayıs University, Medical School, Departments of Physiology, Samsun-Turkey

³Selcuk University, Selcuklu Medical School, Department of General Surgery, Konya-Turkey

OBJECTIVE: It has been established in various in vivo and in vitro studies that thyroid hormones affect oxidative stress. Zinc, which is an important element, is not only involved in the structure of numerous enzymes, but also possesses antioxidant characteristics. The objective of the present study was to determine the effect of zinc supplementation and deficiency on lipid peroxidation in kidney and testis tissues of rats with experimentally induced hypothyroidism.

METHODS: This study included 50 adult, male, Sprague-Dawley type rats which were divided into 5 groups: Group 1, general control; Group 2, sham-hypothyroidism; Group 3, hypothyroidism (the group which was injected 10 mg/kg/day ip PTU for 4 weeks); Group 4, hypothyroidism and zinc-deficient group (the group which was injected 10 mg/kg/day ip PTU for 4 weeks and fed on a zinc-deficient diet “0.65 ppm/zinc/gr/diet” in the same period); Group 5, hypothyroidism and zinc-supplemented group (the group which was administered 10 mg/kg/day ip PTU and 3 mg/kg/day zinc sulfate for 4 weeks). At the end of the 4-week procedures, all the animals were decapitated to take kidney and testis tissue samples, which were then analyzed to determine MDA and GSH levels by TBARS and ELLMANN methods, respectively.

RESULTS: An examination of the study results revealed that hypothyroidism in testis and kidney tissues elevated MDA levels, while reducing GSH levels ($p<0.001$). Zinc supplementation together with hypothyroidism was found to reduce the elevated MDA amount and to elevate GSH levels ($p<0.001$). However, zinc deficiency in the presence of hypothyroidism was found to produce opposite results ($p<0.001$).

CONCLUSIONS: The present results demonstrate that experimental hypothyroidism causes lipid peroxidation in kidney and testis tissue. Zinc deficiency together with hypothyroidism increases the degree of lipid peroxidation, whereas zinc supplementation significantly suppresses the increased oxidative damage by activating the antioxidant system.

Keywords: Hypothyroidism, kidney, testis, MDA, GSH, zinc.

Implications of oxidative stress in exercise physiology in experimentally induced postprandial dysmetabolism

Giurgea Natalia¹, Chiş Bogdan Augustin¹, Constantinescu Mihaela Ioana², Chis Irina¹, Adriana Muresan¹
¹Department of Physiology “Iuliu Hatieganu” University of Medicine and Pharmacy Cluj-Napoca, Romania
²Second Surgery Clinic, “Iuliu Hatieganu” University of Medicine and Pharmacy Cluj-Napoca, Romania

OBJECTIVE: Oxidative stress can be defined as a disruption of redox homeostasis or all the oxidative damages as a result of an overproduction of reactive oxygen species or a reduction in the effectiveness of antioxidant systems in the cell or whole organism, or both. Postprandial dysmetabolism is an increasingly common problem in developed countries. The objective of the study was the evaluation of the metabolic changes caused by acute exercise on oxidant/antioxidant levels in postprandial dysmetabolism.

METHODS: The experiment was performed on seven groups (n = 10 animals per group) of male Wistar rats, weighing 200±30 g, in which parameters of oxidative stress were determined: malondialdehyde as a parameter and prooxidant status and free SH groups as markers of antioxidant capacity in relation to exercise. Postprandial dysmetabolism was simulated by intraperitoneal injection of 2 ml 33% glucose, respectively the administration of 2 ml of animal fat by feeding tube. Physical effort has been done using the treadmill.

RESULTS: An increase in malondialdehyde levels was observed after all types of meals, with the decrease of SH groups levels after effort. The correlation between increased malondialdehyde and decreased SH levels was higher with the increased oxidative stress levels.

CONCLUSIONS: Postprandial hyperglycemia and hyperlipaemia leads to increased oxidative stress. Acute postprandial exercise increases oxidative stress and reduces antioxidants levels that appear after a normal lunch, also in the case of hyperglycemic or hyperlipemic lunches.

Keywords: exercise, postprandial dysmetabolism, oxidative stress

Protective effects of a natural grape seed extract over oxidative stress mechanisms

Adriana Muresan, Teodora Mocan, Soimita Suci, Simona Clichici, Doina Daicoviciu, Nicoleta Decea, Remus Moldovan, Adriana Filip
Physiology Department- University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca, Romania

OBJECTIVE: Hypobaric hypoxia has been recently associated with various effects over brain tissue. We aimed to evaluate the effect of acute hypobaric hypoxia (HH) exposure on oxidative stress in the brain. We also tested the protective effects of a recently described natural grape seed extract (Burgund Mare, Recas – Romania-(BMR)) against HH consequences.

METHODS: Forty-nine Wistar rats (180 ± 20 g) were used. 7 equal groups ($n=7$) were constructed. Each group was exposed to one of the following: 1) no intervention; 2) 24 hours of HH (baric chamber, 5500 m, 24 hrs); 3) 5 days of similar HH; 4) 24 hour HH exposure, followed 3 i.p. injections (2, 24 and 72 hrs. post HH exposure) of carboxymethyl cellulose(CMC) (0.5 ml, 0.5% sol.4), as 24-HH hour control; 5). 5 day HH exposure followed by similar CMC injections, as 5-HH day control 6) 24 hour HH exposure, followed 3 i.p. injections (2, 24, 72 hrs. post HH exposure) of BMR (3 x 0.79 AG equivalents/kg body weight), 7) 5 days of HH followed by similar BMR injections. Oxidative stress assays (5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate(DCFDA), malon dialdehyde(MDA), protein carbonyls(PC), thiol groups(SH), Trolox equivalent of antioxidant capacity (TEAC)) were performed on brain tissue.

RESULTS: Significant variations were obtained following exposure to various intensities of HH for DCFDA($p=0.02$), MDA($p=0.03$). BMR groups demonstrate lower levels of oxidative markers as compared to CMC(I, II).(I vs III: MDA($p=0.016$); II vs IV: DCFDA($p=0.023$), PC($p=0.04$). No between-group-significance was obtained for SH and TEAC

CONCLUSIONS: HH can activate oxidative stress in brain tissue. Our results suggest that BMR seed extract might present a good potential for reducing unwanted HH effects over redox equilibrium.

Keywords: oxidative stress, brain, hypobaric hypoxia, grape seed extract

The Effects Of Melatonin on Oxidative Stress Parameters and Zinc of Wound Tissue

Kazime Gonca Akbulut¹, Fehmi Karataş¹, Cigdem Ozer¹, Güler Öztürk³, Suna Ömeroğlu², Füsün Acartürk⁴

¹Departments of Physiology Gazi University School of Medicine, Ankara, Turkey

²Department of Histology and Embriology, Gazi University School of Medicine, Ankara, Turkey

³Department of Physiology, Maltepe University School of Medicine, Istanbul, Turkey

⁴Department of Pharmaceutical Technology, Gazi University Faculty of Pharmacy, Ankara, Turkey

OBJECTIVE: In this experimental study we investigated the effects of subcutaneous (sc) and local administration of MLT on the tissue malondialdehyde (MDA), glutathione (GSH), zinc and plasma thiobarbituric acid reactive substances (TBARS), total sulfhydryl group (RSH) and zinc levels on the rabbit oral cavity wound.

METHODS: A total of twenty-four Five-month old New-Zealand rabbits were used in the study. A standard incision was applied to the oral mucosa of rabbits. Rabbits were divided into four groups as: (Sham): Untreated incisional group; (Sham+PEG) local Polyethylene glycol (PEG) bead, Sham+PEG+MLT (PEG) + MLT (9 mg) bead and Sham+sc MLT administrated group (10 mg/kg 3 day). The substance MLT was given in two different ways as local formulation or subcutaneous (sc) application. On the third day of oral incision, wound tissue strips and plasma were obtained.

RESULTS: MDA levels of the wounded-tissues were significantly lower in the melatonin groups when compared to sham, sham +PEG groups ($p<0.05$). MLT administration significantly decreased the plasma TBARS level compared to Sham, Sham+PEG groups ($p<0.05$). Total plasma RSH levels were significantly increased after sc MLT administration ($p<0.05$), while MLT administration did not have any significant effect on the zinc and GSH levels of the wounded tissues. MLT caused a significant reepithelisation in the wound region which was characterized by a significant increase in fibroblasts and collagen fibers.

CONCLUSIONS: Our results suggest that s.c./local administration of MLT might improve the wound healing by reducing the levels of lipid peroxidation products.

Keywords: wound healing, melatonin, oxidative stress, glutathione

Effects of curcumin supplementation on heart and lung injury induced by intestinal ischemia/reperfusion in rats

Nilsel Okudan, Muaz Belviranlı, Hakkı Gökbel, Mehmet Öz

Department of Physiology Meram Faculty of Medicine Selçuk University, Konya, Turkey

OBJECTIVE: We aimed to investigate the effects of curcumin on the prevention of heart and lung injury induced by intestinal ischemia/reperfusion (IR) in rats.

METHODS: The study protocol was approved by Ethics Committee of the Selcuk University Experimental Medicine Research and Application Center. Male Wistar rats were divided randomly into four experimental groups: Sham, Intestinal IR, Curcumin/Sham, and Curcumin/Intestinal IR groups. Curcumin was given 200 mg.kg⁻¹ via orally for 20 days to curcumin groups. Intestinal IR was produced by 45 min of intestinal ischemia followed by a 120 min of reperfusion. Animals were sacrificed by cardiac puncture. Interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) levels were analyzed in blood samples. Myeloperoxidase (MPO) activity was analyzed in intestinal tissue. Malondialdehyde (MDA) levels and superoxide dismutase (SOD) activities were analyzed in heart and lung tissues.

RESULTS: Serum IL-6 and TNF- α levels were not different among the groups. Intestinal MPO activity was lower in Curcumin/Sham group compared to the IR group. In heart tissue, MDA levels were lower in Curcumin/Sham and Curcumin/IR groups compared to the Sham and IR groups. In lung tissue MDA levels were higher in Curcumin/IR group compared to the IR group. SOD activity was higher in Curcumin/Sham compared to the sham group in heart tissue and was higher in Curcumin/IR group compared to the IR group in lung tissue.

CONCLUSIONS: Curcumin attenuates heart and lung injury induced by intestinal ischemia/reperfusion.

Keywords: Curcumin, intestinal ischemia/reperfusion, heart, lung, oxidative stress

The effect of hepcidin on heart ischemia and reperfusion

Atilla Nizam Mohammed, Tayfun Göktaş, Eser Öz Oyar, Deniz Erbaş
Department of Physiology, Faculty of Medicine, Gazi University, Ankara, Turkey

OBJECTIVE: Hepcidin is a small peptide hormone having a central role for regulating iron metabolism. Hepcidin decreases release of iron from macrophages and enterocytes and blocks the iron transport from enterocyte to plasma by internalizing of ferroportin, a channel protein for iron export. Myocardial ischemia causes increase in the ferritin stores in the cardiac muscle in a dose dependent manner. The iron stores in cardiac myocytes cause oxidant stress in cardiomyocytes. In addition, fenton reaction causes hydrogen peroxide production and it causes DNA damage by changing myocardial function. Hepcidin is also secreted from cardiac myocyte and its release is regulated by hypoxia and inflammation. The scope of this investigation is to test the effect of hepcidin on cardiac ischemia reperfusion for oxidative stress.

METHODS: Wistar-Albino rat hearts (n=12) were loaded on Langendorff system perfusing with Krebs-Henseleit solution. Hepcidin was applied at the onset of no flow ischemia (n=6). In control group (n=6) no substance was applied. MDA, GSH and NOx determinations were made in heart muscle by biochemical methods.

RESULTS: MDA and NOx levels were decreased ($p < 0.05$), but GSH levels did not change significantly in hepcidin applied group ($p > 0.05$).

CONCLUSIONS: Hepcidin protected heart muscle from oxidant factors stress by inhibiting NOx and lipid peroxidation and by preserving antioxidant systems.

Keywords: Hepcidin, cardiac ischemia reperfusion, nitric oxide, oxidant stress

The Protective Effect of Kefir Prophylaxis in the Experimental Necrotizing Enterocolitis Model

Firat Akat¹, Ilke Mjgan Akin², Ali Dođan Dursun¹, Emel Okulu², Begm Atasay², Hakan Ficicilar¹, Metin Bastug¹, Saadet Arsan²

¹Department of Physiology, Ankara University School of Medicine, Ankara, Turkey

²Neonatology, Department of Pediatrics, Ankara University School of Medicine, Ankara, Turkey

OBJECTIVE: Necrotizing enterocolitis (NEC) is a medical condition, primarily seen in premature infants, in that portions of the bowel undergo necrosis. Premature birth and intestinal immaturity are the most important risk factors. The beneficial bacteria like Bifidobacteria, Lactobacilli are called probiotics. Kefir is a fermented dairy product containing various probiotics like Lactobacillus spp. (80% Lactobacillus kefir) and yeast (Candida kefir and Saccaromyces cerevisiae). The effects of probiotics were studied in clinical trials and animal models of NEC. The aim of the study was to test the effects of Kefir in the animal model of NEC.

METHODS: Neonatal Sprague Dawley rats (n=24) were randomly assigned to three groups. Group 1 (n=10); Kefir (0,02ml) + Premature formula (0,18ml) (orogastric feeding 4 times/day), Group 2 (n=7); Premature formula (0,2ml) (orogastric feeding 4 times/day), Group 3(n=7); Breast feeding (Control group). Experimental NEC was constituted by "hypoxia-ischemia-cold damage model", keeping animals in chamber 2 times/day for 10 minutes 100% CO₂, 5 minutes 100% O₂ and 5 minutes +4 oC. Duodenal and jejunal tissues were used for further analysis. After total protein isolation, total antioxidant status (TAS) and total oxidant status (TOS) assays were performed using commercially available kits (Rel Assay). Statistical differences between three groups were evaluated by using one way ANOVA.

RESULTS: There was no statistically significant difference between three groups (p>0.05).

CONCLUSIONS: According to the results of the present study, oxidant or antioxidant status did not change significantly by Kefir administration. NEC is known to damage ileal tissue primarily. However, we had a limitation that we could not use ileal tissue samples because we preserved them for HIF1 α mRNA analysis.

Keywords: probiotics, bifidobacteria, kefir, necrotizing enterocolitis, rat, TAS, TOS,

AOPP, SOD and NO_x Levels in Rat Pancreatic Tissue Exposed to 4-CPA until Prepuberty

Duygu Altın¹, Cigdem Ozer¹, Gamze Turna³, Ediz Yeşilkaya², Aysun Bideci², Peyami Cinaz², Nedret Kılıç³

¹Department of Physiology, Gazi University, Ankara, Turkey

²Department of Pediatric Endocrinology, Gazi University, Ankara, Turkey

³Department of Medical Biochemistry, Gazi University, Ankara, Turkey

OBJECTIVE: The frequent use of 4-chlorophenoxy acetic acid (4-CPA), a PGRH (plant growth regulatory hormone), in agriculture may result in serious damage in endocrine organs due to its deposition on vegetables.

In this study, the effects of 4-CPA administration on advanced oxidation protein products (AOPPs), nitric oxide (NO_x) levels and superoxide dismutase (SOD) activity in pancreatic tissue of rats which are exposed to 4-CPA until prepuberty were investigated.

METHODS: This study was implemented on 20 day-old, male and female Wistar albino rats. Each gender was divided into four groups: 1. Control, 2. SP control (0.5 ml SP/day orally). Group 3 and Group 4 received 25 mg 4-CPA/kg/day and 100 mg 4-CPA/kg/day, respectively. A single daily dose of 4-CPA was given orally for 30 days. The animals were sacrificed by rompun+ketamine anesthesia one day after the last day of the treatment. AOPPs, NO_x levels and SOD activity were then measured in pancreatic tissue. The results were analyzed by one-way ANOVA and Mann-Whitney U tests. $p < 0.05$ was considered to be statistically significant.

RESULTS: There was no significant difference among AOPP, SOD and NO_x levels between the control and the SP groups in both sexes. NO_x and AOPP levels were significantly increased by the administration of 4-CPA whereas SOD levels were significantly decreased in both sexes.

CONCLUSIONS: Our results may suggest that the prepubertal exposure to 4-CPA increases oxidative stress and decreases the antioxidant capacity in pancreatic tissue and these findings are not gender-dependent.

Keywords: 4-CPA, AOPPs, NO_x, Pancreas, SOD

Antioxidant Statues in Senile Macular Degeneration

Serap Yalın¹, Rezan Hatungil², Koray Büyükbozat¹, Mehmet Berköz¹, Ülkü Çömelekoğlu², Pelin Eroğlu¹

¹Mersin University, Faculty of Pharmacy

²Mersin University, Faculty of Medicine

OBJECTIVE: Age related macular degeneration (AMD) is a medical condition, which usually affects elder adults that result in a loss of vision in the macula because of damage to the retina. It occurs in dry and wet forms. It is a major cause of visual impairment in elder adults. AMD can make it difficult or impossible to read or recognize faces, although enough peripheral vision remains to allow other activities of daily life.

METHODS: In this study, we investigated the relationship between blood antioxidant enzymes and AMD. A total of 43 volunteers as 23 AMD patients and 20 control group were included to the study. Blood samples taken from the both groups and the plasma were separated by centrifugation. The paraoxonase, arylesterase and protein carbonyl group levels were measured by using biochemical methods.

RESULTS: The paraoxonase and arylesterase enzyme activities were found to be significantly lower in the AMD patients when compared to control group. Whereas protein carbonyl groups of AMD patients were significantly higher than the control group.

CONCLUSIONS: According to results, there is relationship between AMD and antioxidant enzymes and it may be suggested that antioxidant enzymes may be effective on the pathogenesis of AMD.

Keywords: Age related macular degeneration, antioxidants, paraoxonase, arylesterase, protein carbonyl group.

Does The EGF Supplementation Affect Antioxidants Levels In Wound Base?

Zeynep Kalay, Sule Coskun Cevher

Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey

OBJECTIVE: Cutaneous wound healing is a highly complex process, which includes inflammation, cell proliferation, matrix deposition and remodeling phases. Various growth factors, such as epidermal growth factor (EGF), play an important role during wound healing. However, little is known about relationship between EGF and antioxidant events in cutaneous wound healing models. Thus, we planned to evaluate the relationship between EGF therapy and oxidative stress in the rats.

METHODS: Fifty-four adult male Wistar-Albino rats were randomly divided into 3 groups: Unwounded (n=6), untreated (n=24) and topical EGF administrated (n=24) group. The animals were sacrificed and wound tissues were collected on days 1, 5, 7 and 14. Glutathione (GSH), ascorbic acid (AA) levels and superoxide dismutase (SOD) activity were measured spectrophotometrically. Statistical analysis was performed using ANOVA variance Analysis.

RESULTS: Although there was no significant alteration in SOD activity and AA levels of the EGF therapy group, GSH levels increased when compared to the respective control on the 14th day after operation in EGF administered group.

CONCLUSIONS: EGF may suggest a potentially effective role for antioxidant therapies by contributing in late stages of healing of the wound tissue. However, the exact mechanism of the process whereby EGF enhances antioxidant status in wound tissue remains to be clarified by further experimental studies.

Keywords: AA, Antioxidant, EGF, GSH, Skin, SOD, Wound Healing

A Time Course Study: Effect of TGF beta 1 on Antioxidant Status in Oral Wound Healing

Emine Gülçeri Güleç Peker¹, Sule Coskun Cevher²

¹Department of Nursery, Faculty of Medical Sciences, Giresun University, Giresun, Turkey

²Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey

OBJECTIVE: Transforming growth factor (TGF beta) is a growth factor included in the mechanisms of wound repair and healing that follow inflammatory processes. The objective of this study was to examine the effects of interactions between exogenous TGF beta and antioxidant status in the process of oral wound repair on different days.

METHODS: Five-month-old New Zealand albino male rabbits were used for this study. Animal experimentation was carried out according to Gazi University Animal Ethics Committee Regulations (21.10.2008/ 92-15912). A surgical incision was made in the right mandibula diestema region of the rabbits, which were then divided into controls and TGF beta 1 implanted groups. Glutathione (GSH), ascorbic acid (AA) levels and superoxide dismutase (SOD) activity which are important antioxidants were measured spectrophotometrically.

RESULTS: In the TGF beta 1 implanted groups, SOD activity significantly increased in comparison with those of the control groups on the 1st, 3rd, 5th days after wounding. However, on the 1st day after wounding, the GSH levels significantly decreased in TGF beta 1 implanted group compared to control. Although in the TGF beta 1 implanted groups AA levels decreased in 1st day and 5th days after wounding, on the 3rd day after wounding AA level was found increased comparison with control.

CONCLUSIONS: TGF beta 1 may play a potentially effective role for antioxidant status by promoting inflammatory response and oxidative events in after days of wounding.

Keywords: AA, GSH, SOD, TGF beta, Wound Healing

The Effects Of PDE5 Inhibitory Drugs On Renal Ischemia/Reperfusion Injury in Rats

Aysegul Kucuk¹, Mehmet Yucel², Nilufer Erkasap³, Murat Tosun⁴, Tulay Koken⁵, Mete Ozkurt³, Serdar Erkasap⁶

¹Dumlupinar University, Medical Faculty, Physiology Department, Kutahya, Turkey

²Dumlupinar University, Medical Faculty, Urology Department, Kutahya, Turkey

³Osmangazi University, Medical Faculty, Physiology Department, Eskişehir, Turkey

⁴Afyon -Kocatepe University, Medical Faculty, Histology-Embriology Department, Afyon, Turkey

⁵Afyon-Kocatepe University, Medical Faculty, Biochemistry Department, Afyon, Turkey

⁶Osmangazi University, Medical Faculty, General Surgery Department, Eskişehir, Turkey

OBJECTIVE: The aim of the present study was to evaluate the effects of phosphodiesterase type 5 (PDE5) inhibitory drugs, Tadalafil and Sildenafil, on iNOS gen expression and apoptosis in ischemia/reperfusion (I/R) induced oxidative injury in rat renal tissue.

METHODS: Eighty Sprague-Dawley rats (200-250 g) were divided into four groups. 1) control, 2) ischemia/reperfusion (I/R), 3) Tadalafil+I/R (1 mg/kg oral), and 4) Sildenafil+I/R (1 mg/kg oral) group. Rats were subjected to renal ischemia by clamping the left pedicle for 60 min, and then reperfused for 90 min. The rats were pretreated with Tadalafil in group 3 and Sildenafil in group 4, 1 h before the induction of ischemia. Malondialdehyde (MDA) and myeloperoxidase (MPO) levels were determined in renal tissue homogenates by high-performance liquid chromatography (HPLC), and apoptotic cell assesment using TUNEL. The mRNA level of iNOS in renal tissue was determined by Real-time PCR (RT-PCR).

RESULTS: Our results indicate that MDA and MPO levels were increased in the I/R group than those in the control group. Both Tadalafil and Sildenafil treatment decreased the MDA levels in I/R groups, whereas this effect was more potent with Sildenafil. RT-PCR results showed that, iNOS gen expression increased in the I/R group but decreased in the PDE5 inhibitory drugs treated group. Apoptotic cells also were decreased in PDE5 inhibitory drugs-treated group.

CONCLUSIONS: Our results indicate some beneficial effects of PDE5 inhibitory drugs on oxidative I/R injury in rat renal tissue.

Keywords: kidney, ischemia-reperfusion, tadalafil, sildenafil, apoptotic cells, RT- PCR, rat

Effects of alcoholic extract of *Achilea millefolium* flowers on fertility parameters of male rats

Rahmatollah Parandin¹, Rostam Ghorbani²

¹Dept. of Biology, Faculty of Sciences, Payame Noor University of Kermanshah, Kermanshah, Iran

²Center of Fertility and Infertility, Kermanshah University of Medical Sciences, Kermanshah, Iran

OBJECTIVE: Fertility regulation with plant preparations has been reported in ancient literature of indigenous systems of medicine. In this research the effects of administration of alcoholic extract of *Achilea millefolium* flowers on fertility indices, body weight and reproductive organs weight was evaluated in male rats.

METHODS: Eighteen rats were randomly divided into 3 groups, control, group A and group B. Each group was comprised of six rats. Animals in control group received 1 ml of distilled water (vehicle) and test groups (A and B) received graded doses of 200 and 400 mg/kg body weight of alcoholic extract of *Achilea millefolium* flowers respectively on daily basis for 50 days. At the end of 50 days of treatment period, fertility indices such as body and reproductive organs weight, sperm motility and viability, epididymal sperm reserve (ESR), daily sperm production (DSP), blood testosterone concentration and fertility percentage were measured.

RESULTS: There was a significant decrease in GSI (Testes weight/body weight ratio), epididymis weight, sperm count, ESR, DSP, blood testosterone concentration and fertility rate in both the lower dose group (0, $p<0.05$, 0, 0, $p<0.05$, $p<0.05$, and 0) and the higher dose group ($p<0.05$, $p<0.01$, $p<0.01$, $p<0.5$, $p<0.001$, $p<0.001$ and $p<0.05$) as compared to the control group.

CONCLUSIONS: The results of this study showed that alcoholic extract of *Achilea millefolium* flowers in higher doses could decrease fertility in male rats.

Keywords: *Achilea millefolium*, Fertility, Testosterone, male Rat

Effects of alcoholic extract of *Nigella sativa* seed on pituitary-testicular axis and fertility in male rats

Rahmatollah Parandin¹, Namdar Yousofvand², Rostam Ghorbani³

¹Department of Biology, Payame Noor University of Kermanshah, Kermanshah, Iran

²Department of biology, Razi university of Kermanshah, kermanshah, Iran

³Center of Fertility and Infertility, Kermanshah University of Medical Sciences, Iran

OBJECTIVE: To evaluate the role of alcoholic extract of *Nigella sativa* on pituitary-testicular axis and fertility in male rats.

METHODS: Twenty-four male rats were randomly divided into 3 groups; control, group A and group B, and each group consisted of 8 rats. Animals in control group received 1 ml of normal saline (vehicle) and treatment groups (A and B) received graded doses of 200 and 400 mg/kg body weight of alcoholic extract of *Nigella sativa* seeds on a daily basis for 60 days. At the end of treatment period, fertility indices such as body and reproductive organs weight, sperm motility, viability and count, epididymal sperm reserve (ESR), daily sperm production (DSP), blood testosterone concentration, gonadotrophin levels and fertility rate were measured.

RESULTS: There was a significant increase in testes and epididymis weight, sperm count, ESR, DSP, blood testosterone concentration, FSH, LH and fertility rate in both the lower dose group (respectively 0, 0, $p<0.05$, 0, $p<0.05$, $p<0.05$, 0, $p<0.05$ and $p<0.05$) and the higher dose group (respectively $p<0.05$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$, $p<0.001$, 0, $p<0.05$ and $p<0.01$) as compared to the control group.

CONCLUSIONS: The results of this study showed that alcoholic extract of *Nigella sativa* seeds especially in higher doses could increase fertility in male rats.

Keywords: *Nigella sativa*, fertility, male rat

3 beta-hydroxysteroid dehydrogenase activity associated with progesterone production in bovine granulosa cells cultured under different serum and gonadotrophin conditions

Ozkan Simsek¹, Monika Mihm²

¹Department of Physiology, Faculty of Veterinary Medicine, University of Kirikkale, Turkey

²School of Veterinary Medicine, University of Glasgow, United Kingdom

OBJECTIVE: Three- β -hydroxysteroid dehydrogenase (3 β -HSD) is the enzyme responsible for progesterone production. This study aimed to determine whether 3 β -HSD can be shown to reflect progesterone production by cultured granulosa cells under different serum, gonadotrophin and IGF-I conditions.

METHODS: Large bovine follicles were dissected from abattoir ovaries for granulosa cells recovery. Cells were washed, stained for viability, and plated for 48 hours in basic medium with or without 5 % fetal calf serum (FCS). Subsequently, cells were exposed to FSH, LH or FSH + IGF-I in serum-free medium for another 96 hours, predicted to cause different degrees of luteinisation. Before and after incubation, granulosa cells were stained for 3 β -HSD activity using a previously published staining solution (Payne et al., 1980) [0.1M phosphate buffered saline (PBS) containing 0.1% BSA, 1.5mM NAD, 0.25mM nitro blue tetrazolium and 0.2mM 5 α -androstene-3 β -ol-17 one].

RESULTS: In cells pre-incubated with FCS, the high dose of IGF-I increased ($p < 0.05$) progesterone secretion over 3-fold compared with FSH alone or the low dose of IGF-I. This was reflected in the degree of 3- β HSD staining, with cells exposed to high IGF-I stained much darker. In addition, 3- β HSD staining showed that cells pre-incubated with FCS became firmly attached with the typical phenotype of luteinised granulosa cells.

CONCLUSIONS: Measuring 3- β HSD activity before and after in vitro culture appears to be an accurate indicator of progesterone producing capacity of live bovine granulosa cells. If quantifiable, this method has potential to detect the in situ luteinisation status and luteinising effect of hormones used in granulosa cell culture.

Keywords: 3 β -hydroxysteroid dehydrogenase, bovine granulosa cells, progesterone, FSH, LH, IGF-I

Evaluation of Citric Acid (E330) as Food Additives by Using Frog Embryo Teratogenesis Assay (FETAX)

Ayper Boga¹, Secil Binokay¹, Kubra Akillioglu¹, Yasar Sertdemir²

¹Department of Physiology, Faculty of Medicine, Cukurova University, Adana, Turkey

²Bioistatistics, Faculty of Medicine, Cukurova University, Adana, Turkey

OBJECTIVE: The developmental toxicity of citric acid (E330) was investigated using the frog embryo teratogenesis assay-Xenopus (FETAX).

METHODS: *Xenopus laevis* embryos were exposed to 11 different concentrations of E330, from stage 8 to 11, for 96 h under static renewal test conditions. The median lethal concentration (LC50), malformation (EC10), non-observed adverse effect concentration (NOAEC), and the lowest observed adverse effect concentration (LOAEC) and the Minimum Concentration to Inhibit Growth values (MCIG) were calculated.

RESULTS: The corresponding LC50 and EC10 values determined for E330 exposure were 0.0124 and 0.015 g/L, respectively. Since EC50 value could not be determined, the TI (LC50/EC50) value of citric acid (E330) was not calculated. The NOAEC, LOAEC, MCIG values were calculated as 0.001, 0.01 and 0.0107 g/L, ($p < 0.001$), respectively.

CONCLUSIONS: Abnormality rates of citric acid were not high on *Xenopus* embryos. Because the embryos of *Xenopus* were seen either alive or death. Based on these results, we conclude that using as 'Not Limited' Citric Acid may result essential preterm birth or abort on human being, and must be reevaluated. Moreover, our results confirm that the FETAX assay can be a useful pretest for integrated biological hazard assessment of chemical agents used in food industry.

Keywords: Citric Acid, *Xenopus*, FETAX, Teratogenicity

Localization of Gat1 Protein In Testes of Stress-Exposed Rats: An Immunohistochemical Study

Tuba Demirci, Elvan Özbek

Department of Histology and Embryology, Atatürk University, Faculty of Medicine, Erzurum, Turkey

OBJECTIVE: Gammaamino butyric acid (GABA) is an inhibitory neurotransmitter that leads to hyperpolarization of the postsynaptic membrane. This increased membrane potential is named as inhibitory postsynaptic potential (IPSP). GABA transporter 1 (GAT1) plays an important role in GABA reuptake from the synaptic cleft. GAT1 is found in the testes besides central nervous system and participates in the regulation of testicular function. Recently, it has been shown that GAT1 overexpression affects both testicular morphology and fertility. This study aims to investigate the effects of stress on the GAT1 localization in rat testes.

METHODS: Twelve adult male Sprague Dawley rats were grouped randomly as the control (n=6) and stress-exposed (n=6) groups. Stress-exposed group performed “chronic-mild-stress” (CMS) procedure for four weeks. At the end of the experiment, testes were removed from sacrificed rats, and fixed in Bouin's solution. After processing the samples for histological evaluation, paraffin sections of the testes were stained with immunoperoxidase method to assess GAT1 localization and then, examined under a light microscope.

RESULTS: By light microscopy, a prominent positive immunoperoxidase reaction in a dark brown color was observed in the testis sections processed with the GAT1 antibody. Any positive immune reaction was not observed when nonimmune serum was used instead of the primary antibody. Testicular GAT1-specific immunohistochemical reaction in the stress-exposed group was more widespread and dense than that in the control group.

CONCLUSIONS: In conclusion, we thought that stress might cause an increased GAT1 expression in the testes and this might be an important risk factor regarding male infertility.

Keywords: GAT1 protein, immunohistochemistry, male infertility, rat, testis.

Endocrine and local regulation of ovarian function of the fat sand rat, *Psammomys obesus*, immunohistochemical preliminary study

Boubekri Amina¹, Exbrayat Jean Marie², Khammar Farida¹, Gernigon Spsychalowicz Thérèse¹

¹Université des Sciences et de la Technologie Houari Boumédiène, Faculté des Sciences Biologiques, Laboratoire de Recherche sur les Zones Arides, Alger, Algérie.

²Université de Lyon, Laboratoire de Biologie Générale, Université Catholique, Laboratoire de Reproduction et Développement Comparé, Lyon, France.

OBJECTIVE: The sand rat (*Psammomys obesus*) constitutes a model for studying several metabolic disorders as Mellitus diabetes. In order to perform breeding laboratory conditions, the reproductive function of this diurnal species living in North Occidental Sahara was studied. After histological, cytological and follicular dynamic study, we investigate several regulatory processes in the gonads by immunohistochemistry localization of progesterone, androgen, estrogen and aromatase in the ovaries; FSH and LH in the pituitary gland and the specific receptors of FSH, LH, progesterone and estradiol in the different component of the ovaries.

METHODS: Ovaries were removed and fixed in 10% formaldehyde or 4% paraformaldehyde or frozen in liquid nitrogen and preserved at -80°C. For immunohistochemistry, we used the LSAB 2 System, Peroxidase DAKO Kit (Biomeda): an indirect method using streptavidin-biotin complex that allows amplification of marking.

RESULTS: We identified progesterone, androgen, estrogen and aromatase in the ovaries. Anti 17 β -estradiol antibody presents strong immuno-reactivity in both the oocytes and granulosa cells; theca cells were totally devoid of label. Progesterone label was observed in thecal and granulosa cells. FSH and LH were visualized in gonadotropic pituitary cells. FSHR are found only on granulosa cells. LHR were viewed in theca interna, they ensures the production of androgen substrate for aromatase detected only in granulosa, estrogen producing cells. ER α predominate in the theca interna; RE β in the granulosa. The RPs were marked in the theca and granulosa. The cell fraction label was specific to estrous phase.

CONCLUSIONS: Cyclic estrous variations were observed and functional correlations were established.

Keywords: ovarian local regulation, estrogen receptors, progesterone receptors, FSH receptor, LH receptor.

Apelin induces contractions in rat myometrium in calcium free condition

Zubeyde Ercan¹, Emine Kacar¹, Mete Ozcan², Sinan Canpolat³, Selim Kutlu¹, Ahmet Ayar³

¹Firat University, Faculty of Medicine, Department of Physiology, Elazığ, TURKEY

²Firat University, Faculty of Medicine, Department of Biophysics, Elazığ, TURKEY

³Karadeniz Technical University, Faculty of Medicine, Department of Physiology, Trabzon, TURKEY

OBJECTIVE: Apelin is a novel peptide hormone related to some metabolic and physiological processes including reproductive system. We have previously demonstrated for the first time that apelin has a stimulatory effect on myometrial contractility in rats. In this study, we investigated possible stimulatory effect of apelin on myometrial contractility in calcium free condition.

METHODS: Adult Wistar rats in dioestrus were decapitated and myometrium strips were removed (n=6). All strips were placed in a jacked tissue bath containing Krebs solution at 37 oC and pH 7.4, constantly bubbled with 95% oxygen and 5% carbon dioxide and were allowed to contract under 1g tension and isometric contractions were measured by force displacement transducer.

RESULTS: After equilibration of spontaneous contractions, calcium free condition was set and contractions were disappeared immediately because of lack of extracellular calcium source. This silent period (no contraction) was recorded for 20 mins and apelin was added to the organ bath. Apelin was induced myometrial contraction at 10uM concentration in calcium free medium.

CONCLUSIONS: According to the results of this study and our previous data, apelin can stimulate uterus contractions by inducing calcium release from intracellular storages. Further detailed investigations are needed to clarify the mechanism(s) of apelin induction on myometrium contractility and the role of apelin in pregnancy and parturition processes.

Keywords: Apelin, myometrium, contraction, rat

Effects neonatal LPS injection and IL-1 beta and NOS inhibition on timing of preputial separation in rats

Tuba Tapan, Sedat Yildiz, Suat Tekin, Süleyman Sandal, Alaadin Polat
Department of Physiology, Faculty of Medicine, Inonu University, Malatya, Turkey

OBJECTIVE: Neonatal injection of bacterial cell wall constituents or lipopolisaccarides (LPS) appears to program reproduction in adulthood. These effects might be mediated by increasing levels of IL-1beta and NO. We, therefore, hypothesized that neonatal LPS injection may affect puberty and prevention of rise in IL-1beta and NOS following LPS injection may prevent this delay in male rats.

METHODS: Male pups were injected with LPS (n=11; 50 ug/kg LPS), LPS + IL-1beta inhibitor (n=16, 1 mg/kg Q-Vd-OPh), LPS + NOS inhibitor (n=13, 40 mg/kg L-NAME) or saline (n=14) on postnatal day 7. All pups were kept under 12h light: 12 h dark cycle. However, in order to increase signal intensity in terms timing of puberty, all pups were kept at 16 h light: 8 h dark cycle between postnatal days 18 and 42. From postnatal day 40 onward, rats were observed daily for preputial separation.

RESULTS: Neonatal LPS injection and inhibition of IL-1beta and NOS did not affect days to preputial opening.

CONCLUSIONS: The results obtained so far suggest that neonatal LPS injection was not sufficient to produce a sufficient signal to affect days to preputial opening.

Keywords: Rat, Lipopolysaccharide, male, caspase, L-NAME

Efficacy of anti-TNF-alpha therapy in preventing uterine adhesion formation in rats

Bulent Uysal¹, Suzi Demirbag², Mehmet Ozler¹, Tarik Purtuloglu³, Yavuz Poyrazoglu⁴, Tuncer Cayci⁵, Turgut Topal¹, Sukru Oter¹, Ahmet Guven², Ahmet Korkmaz¹

¹Department of Physiology, Gulhane Military Medical Academy, Ankara, Turkey

²Department of Pediatric Surgery, Gulhane Military Medical Academy, Ankara, Turkey

³Department of Anesthesiology and Reanimation, Gulhane Military Medical Academy, Ankara, Turkey

⁴General Surgery, Elazig Military Hospital, Elazig, Turkey

⁵Department of Clinical Biochemistry, Gulhane Military Medical Academy, Ankara, Turkey

OBJECTIVE: Postoperative adhesion formation is a consequence of wound healing and sometimes can result in severe complications. Despite all the preventive measures and agents, this problem has still not been eliminated completely. The aim of this study was to evaluate the effect of TNF-alpha inhibition on the development of postoperative uterine adhesions.

METHODS: Twenty one female Wistar-Albino type rats were used in this study. The rats were randomly divided into three groups as sham-operated, adhesion and adhesion plus the TNF-alpha inhibitor etanercept. A lower midline abdominal incision was performed and a standard uterine lesion was created by electrocauterization in the adhesion control and etanercept treated groups. Starting immediately after adhesion induction, the treatment group received daily intraperitoneal injections of 2 mg/kg etanercept for a total of 3 days. At day 7, peritoneal washing fluids of rats were obtained for the determination of TNF-alpha levels. Then all of the rats were sacrificed and uterine adhesions were staged according to the Leach scoring system. Uterine tissue samples were harvested for biochemical and histopathological evaluation.

RESULTS: The total adhesion score and tissue malondialdehyde levels were found to be increased whereas superoxide dismutase and glutathione peroxidase activities decreased significantly in the adhesion control group ($P < 0.05$ vs. sham-operated animals). TNF-alpha inhibition, significantly reversed these findings ($P < 0.05$ for all vs. adhesion control group). Additionally, in the etanercept-treated group, TNF-alpha levels of the peritoneal washing fluid were also significantly lower than the adhesion control group.

CONCLUSIONS: Anti-TNF-alpha therapy reduces the prevalence of peritoneal and uterine tube adhesion formation in rats possibly by suppressing TNF-alpha production as well as limiting oxidative stress.

Keywords: Etanercept; Oxidative stress; TNF-alpha; Uterine adhesion

Role of vasoactive intestinal peptide in experimentally induced ischemia-reperfusion of the urinary bladder

Reni Kalfin¹, Anna Tolekova², Giampietro Sgaragli³, Federica Pessina³

¹Department of Synaptic Signaling and Communications, Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

²Department of Physiology, Pathophysiology and Pharmacology, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

³Department of Neuroscience, Siena University, Italy

OBJECTIVE: The participation of vasoactive intestinal peptide (VIP) in various normal and pathological processes is under intensive investigations lately. It was shown that diabetes mellitus is associated with a decrease in VIP content of gastrointestinal tract. The role of VIP in diabetes is not clear, and will be studied by us in the near future. VIP possesses a potent antioxidant, anti-inflammatory and neuroprotective activity, suggesting that this neuropeptide is a prospective protective peptide.

METHODS: Guinea-pig detrusor strips isolated from urinary bladder were mounted for tension recording in small organ baths. The strips were subjected to electrical field stimulation (5 s trains, 0.05 ms duration, 40 Hz, 50 V) with 15-min intervals between stimulations. The nerves were subjected to 60 min of ischemia followed by 150 min of reperfusion. VIP was added to the perfusing Krebs solution at 0.1, 0.3 and 1 microM concentrations and kept during the whole ischemia plus the first 30 min of reperfusion. Inhibition of lipid peroxidation was assessed based on the oxidation of linoleic acid initiated by ABAP. Five increasing concentrations VIP were added to the peroxidising system.

RESULTS: VIP improved the neurogenically-induced contractions during ischemia and reperfusion as compared to the untreated controls. The antioxidant activity of VIP assessed as its capability to scavenge peroxy radicals during linoleic acid oxidation was 6.42 ± 0.13 pIC₅₀ M.

CONCLUSIONS: VIP counteracts the damage suffered by neurons in the urinary bladder, exposed to ischemia-reperfusion conditions.

Acknowledgement: Supported by Grant DDVU-02-24/2010 from the National Science Fund, Sofia, Bulgaria.

Keywords: Vasoactive intestinal peptide, urinary bladder, ischemia, reperfusion, diabetes, neuroprotection

Role of oxytocin in deceleration of early atherosclerotic inflammatory processes in adult male rat model

Marwa Ahmed¹, Gehan Elosaily²

¹Physiology Dept., Faculty of Medicine, Assiut University, Assiut, Egypt

²Pathology Dept., Faculty of Medicine, Assiut University, Assiut, Egypt

OBJECTIVE: The study aimed to examine the effect and mechanism of oxytocin (OT) administration on the inflammation and atherosclerosis.

METHODS: Thirty adult male rats equally divided into three groups. Control group fed regular diet; group II fed control diet supplemented with L-methionine (10gm/Kg) for 10 weeks. Group III received L-methionine and oxytocin treatment (1 mg/kg/day) intraperitoneally for 10 weeks. Blood samples were evaluated for total homocysteine, interleukin-6(IL-6), monocyte chemoattractant protein-1(MCP-1), C-reactive protein (CRP), lipid profile, nitric oxide (NO), malondialdehyde (MDA) and reduced glutathione (GSH). RT-PCR for oxytocin receptor mRNA was done. Specimens from aortas were processed for immunohistochemical staining for (NF- κ B) p65 protein.

RESULTS: RT-PCR analysis showed that OT administration increased oxytocin receptor mRNA (2-folds, $P < 0.05$). OT administration to group III decreased the plasma levels IL-6, MCP-1 and CRP levels, which were elevated in group II. Moreover, there were increased plasma levels of NO and GSH of group II and decreased plasma levels of MDA. Marked increased expression of NF- κ B in the aorta of group II was detected. However, OT administration restored the histological structure of the aorta and decreased the expression of NF- κ B in the aorta of group III.

CONCLUSIONS: Oxytocin has anti inflammatory pathway in atherosclerosis, as it decelerates atherosclerosis by decreasing the proinflammatory responses via up-regulation of its receptors.

Keywords: oxytocin, atherosclerosis, inflammation, methionine.

Sildenafil has a protective effect on rat testis after using antituberculosis drugs

Harun Alp¹, Namık Kemal Hatipoğlu², Osman Evliyaoglu³, Mehmet Yucel⁴, Necmettin Pembegül⁵, Aysegül Kucuk⁶

¹Dicle University, Faculty of Veterinary, Department of Pharmacology, Diyarbakir, TURKEY

²S.B. Diyarbakir Educational and Research Hospital, Department of Urology, Diyarbakir, TURKEY

³Dicle University, Faculty of Medicine, Department of Biochemistry, Diyarbakir, TURKEY

⁴Dumlupınar University, Faculty of Medicine, Department of Urology, Kutahya, TURKEY

⁵Dicle University, Faculty of Medicine, Department of Urology, Diyarbakir, TURKEY

⁶Dumlupınar University, Faculty of Medicine, Department of Physiology, Kutahya, TURKEY

OBJECTIVE: The aim of the present study was to evaluate the effects of anti-tuberculosis (anti-TB) drugs on testicular tissue and to evaluate the protective effects of the sildenafil on the testicular damage treated with anti-TB drugs.

METHODS: Eight groups were designed. Group I (control), group II given sildenafil (1.5 mg/kg p.o. for 45 days), group III given isoniazid (INH) (30 mg/kg p.o. for 45 days), group IV streptomycin (STR) (20 mg/kg i.m. for 45 days), group V (INH+STR), group VI (Sildenafil+INH), group VII (Sildenafil+STR), and group VIII (Sildenafil+STR+INH). Left testis was excised and histopathological examination was performed. Groups were analyzed with one-way ANOVA followed by Tukey.

RESULTS: All groups were compared according to the Johnsen testicular biopsy score. Compared with the control group (group I), group III, group IV, and group V scores were significantly decreased. Histopathologic examination of testicular tissue in groups III and groups II, a little bit more obvious signs of oedema were observed than the other groups in general. At seminiferous tubules, varying degrees of increased spermatogenesis was observed in sildenafil given groups. INH was decreased more in the STR according to the Johnsen testicular biopsy score. Sildenafil had a protective effect on the testis in rats which were treated with INH and STR.

CONCLUSIONS: Our results indicate that sildenafil reduced the testicular damage in rats which were treated with antituberculosis drugs.

Keywords: antituberculosis drugs, sildenafil, rat, testis, histopathological examination, seminiferous tubules, tuberculosis

Hormone replacement therapy as a protector of arteriosclerotic complications in postmenopausal women

Sunchica Marko Petrovska, Beti Dejanova, Jasmina Gligorovska, Elizabeta Sivevska
Medical Faculty, Institute of Physiology, Skopje, Republic of Macedonia

OBJECTIVE: Menopause is often accompanied by degenerative processes such as arteriosclerosis that suggests an acceleration of aging triggered by estrogen deficiency. Therefore, hormone replacement therapy (HRT) has been considered the most suitable treatment for the above symptoms and processes. This study was aimed at evaluating the influence of HRT on some of the arteriosclerotic risk factors such as the serum lipids (total cholesterol, HDL-CH, LDL-CH tryglycerydes), inhibitor of fibrinolysis - plasminogen activator inhibitor type 1Ag (PAI-1 Ag), and factor VII of coagulation in postmenopausal women.

METHODS: A total number of 32 women at post-menopausal phase with application of HRT for six months were examined. Lipid concentrations were determined with standard colorimetric-spectrophotometric method. The concentration of PAI-1 Ag was determined by using immuno-enzymatic method and the concentration of factor VII of coagulation with the method of deficient plasma.

RESULTS: Statistical analysis has shown that HRT applied in post-menopausal women within a six-month period significantly decreased the concentration of LDL-CH ($p < 0.05$), of PAI-1 Ag and factor VII of coagulation ($p < 0.001$) but significantly increased the HDL-CH serum concentration ($p < 0.05$). However, there was no statistical significance in the level of tryglycerides and total cholesterol.

CONCLUSIONS: There was a significant decrease of atherogenic risk factors (LDL-CH, PAI-1 Ag and factor VII of coagulation) as well as significant increase of protective factors (HDL-CH) in post-menopausal women treated with HRT. Thus, the present findings justify the application of this therapy in post-menopausal women.

Keywords: hormone replacement therapy, arteriosclerosis, postmenopause.

Complement system activation and antioxidant system changes in pregnant rats' offspring treated with ethanol by gavage

Sibel Akyol¹, Erdal İnce², Turgut Ulutin², Halil Tunali¹

¹Department of physiology, Cerrahpasa Medicine Faculty, University of Istanbul, Turkey

²Department of medical Science Biology, Cerrahpasa Medicine Faculty, University of Istanbul, Turkey

OBJECTIVE: We investigated relationships between the complement system activation (CH50, AH50), a humoral system component CD19 lymphocytes and antioxidant status (GSH, GPX, GR) in ethanol-administered pregnant rats and their offsprings.

METHODS: Twenty female Wistar rats (12 week old) were randomly allocated into a non-alcoholic pregnant (CP) and alcoholic pregnant groups (AP) (n=10 each). A 30% ethanol in water solution was administered 6 g/kg daily by gavage for 4 weeks before mating and then throughout the pregnancy. Blood samples were obtained at day 18 of pregnancy and also taken of the offspring (n=10 CPO, n= 10 APO) on the day lactation ceased (day 10). The flow cytometric method was used for determining CD19. Microplaque haemolytic determination method was used for CH50 and AH50. Antioxidant status was examined in liver, lymph node and spleen.

RESULTS: Compared with CP, we obtained significant decreases in CH50 and significant increases AH50 in AP. The comparison between CPO and APO exhibited no significant changes while CH50 and AH50 increased significantly. CD19 showed a significant decrease in the CP and AP, and groups of CPO and APO displayed a considerable increase. GSH levels and GR enzyme activities decreased, whereas increased GPX activities were observed in liver, spleen, lymph node tissues in the AP than in the CP. In parallel with their offsprings, GSH levels and GR activities were decreased and GPX activity was increased in APO more than in CPO.

CONCLUSIONS: Our findings suggest that activation of the complement system may be associated with progression of ethanol-induced organ injury.

Keywords: complement system, CD19 lymphocyte, ethanol, oxidative stress, pregnancy

Status of magnesium in diabetic subjects

Ahmed Ghouini¹, Khelidja Larbaoui², Djamel El Harb Djoghla¹, Khireddine Khelfat²

¹Laboratory of Physiology department of medicine, Blida, Algeria

²Hospital N.Hamoud, Algiers, Algeria

OBJECTIVE: The role of magnesium in diabetes is still controversial in many studies. Therefore, we investigated the status of magnesium in normal versus diabetic subjects and patients with elevated concentration of blood glucose (non-diabetic). It seems interesting to know that diabetic subjects have a decrease in magnesium level in order to introduce an appropriate alimentation in these patients to reduce glucose metabolism disorder.

METHODS: Eighty subjects (women and men) aged 35 to 60 years were examined. All subjects underwent to clinical evaluation, blood sample measurements. The blood glucose concentration, HbA1C and the concentration of urine glucose were measured. The lipidic profiles of all subjects were also identified. The patients were classified in groups according the Body Mass Index (BMI), the blood glucose, age and sex.

RESULTS: No statistically significant difference was found between patients with diabetic disease, patients with increased blood glucose or normal subjects. These results were found after studying magnesium concentration in each group according to each parameter (BMI, blood glucose, age and sex).

CONCLUSIONS: Our prospective study has permitted to show that the blood concentration of magnesium is not a definite factor in diabetic illness and nor an important parameter in complications of diabetic diseases or in glucose metabolism imbalance. It is possible that the measurement of cellular glucose concentration gives a way to answer at the hypothesis that the magnesium has a role in the glucose metabolism.

Keywords: magnesium, diabetic disease, glucosemetabolism.

Comperative investigation of the effects of long term cigarette smoking on blood mineral levels

Fazile Nur Ekinci Akdemir¹, İsmail Meral²

¹School of Nursing, Ibrahim Çeçen University, Ağrı, Turkey

²Department of Physiology, Medicine School, Yüzüncü Yıl University, Van

OBJECTIVE: In this study, we investigated the possible negative effects of long term smoking on some blood mineral levels.

METHODS: Twenty five non-smokers (control group) and fifty long-term cigarette smokers (for at least fifteen years; smoker group) have participated in the study. Subjects were aged between 25-40 years old. Control and smoker groups were matched for age, sex and body mass index status. The blood samples were taken from smokers and non-smokers after twelve hours of fasting period. Student's t-test was used to compare the control and smoker groups, and $P < 0.05$ indicated a significant difference. Pearson's correlation coefficient was used to demonstrate the relationship among parameters in smoker and control groups.

RESULTS: Although there was not any statistical difference ($P > 0.05$) between the groups regarding to the levels of K, P, Mg, Na, Cl, Zn, Fe, Ca and Cu, some positive correlations, which was not observed in smokers, were observed in controls.

CONCLUSIONS: It was concluded that smoking may negatively affect some important positive correlations among minerals observed in healthy individuals.

Keywords: Smoking, minerals, blood

Assosiations Of Hyperthyroidism and Hypothroidism With Insulin Like Growth Faktor-I And Insulin Like Growth Factor Binding Protein-3

Raziye Kursunluoglu, Sebahat Turgut, Fulya Akın, Mehmet Baştemir, Bünyamin Kaptanoğlu, Osman Genç
Department of Physiology, Pamukkale University, Denizli, Turkey

OBJECTIVE: Thyroid hormones have an important role on normal growing and developing of skeletal muscle. Insulin like growth factor-I (IGF-I) is important growing factors and it is necessary for produced and developing of thyroid cells. IGF-I stimulates fibroblast, follicular cells and endothelial cells in thyroid glands. It was shown that thyroids hormones have an important role on regulation of IGF-I and insulin like growth factor binding protein-3 (IGFBP-3). In present study, we investigated the frequencies of IGF-I (CA)19 and IGFBP-3 -202 A/C gene polymorphisms in thyroid diseases with hyperthyroid or hypothyroid states and it may be the role of this polymorphisms in thyroid functions.

METHODS: This study was performed of thirty-seven patients with hyperthyroidism, seventy-six patients with hypothyroidism and fifty healthy controls. Genomic DNA from the patients and controls was prepared from peripheral blood samples. Investigated genomic areas were studied using specific primers by PCR methods. Amplified fragments were separated agarose gel electrophoresis and identified using the UV gel documentation system.

RESULTS: The frequencies of IGF-I (CA)19 gene polymorphism were found statistically significant among the patients with hypothyroids, hyperthyroids and healthy controls. Although we did not find any difference between control and patient groups compared to the frequency of IGF-I (CA)19 gene polymorphism, the difference of genotypes between patients with hypothyroids and hyperthyroids was statistically significant. The frequency of IGFBP3 -202 A/C gene polymorphism was found statistically significant between hypothyroid and hyperthyroid patients, $\chi^2=6.24$ $df=2$ $p=0.044$.

CONCLUSIONS: In conclusion, IGF-I (CA)19 and IGFBP-3 -202 A/C gene polymorphisms may be a risk factor for hypothyroidism. This study was supported by Pamukkale University Research Fund.

Keywords: Hypothyroidism, Hyperthyroidism, Insülin like growth factor- I (IGF-I), Insülin like growth factor binding Protein-3 (IGFBP-3)

Severity of acne vulgaris is associated with an insulin-like growth factor-I gene polymorphism

Levent Tasli¹, Sebahat Turgut², Nida Kacar¹, Ceylan Ayada², Melis Coban¹, Raziye Akcilar², Seniz Ergin¹

¹Department Of Dermatology, Faculty of Medicine, University of Pamukkale, Denizli, Turkey

²Department of Physiology, Faculty of Medicine, University of Pamukkale, Denizli, Turkey

OBJECTIVE: Acne vulgaris is a multifactorial disease of the skin. Several studies have shown that elevated levels of serum insulin-like growth factor-I (IGF-I) correlate with overproduction of sebum and acne. Recently functional relationship between IGF-I (CA) polymorphism and circulating IGF-I levels in adults has been reported. The aim of our study was to investigate for the first time whether IGF-I (CA) polymorphism might be involved in the pathogenesis of acne.

METHODS: We included 115 acne patients and 117 healthy subjects to the study. The clinical grade of acne was assessed based on the Global Acne Grading System. Participants were questioned about diabetes mellitus, PCOS, and other systemic disease. We searched for the IGF-I (CA) 19 polymorphism in this study. IGF-I (CA) 19 polymorphism was performed by polymerase chain reaction.

RESULTS: We categorized the IGF-I (CA) 19 polymorphism area into 3 groups as lower than 192 bp, 192–194 bp, and higher than 194 bp. We found that the frequency of genotype IGF-1(CA)19 gene was significantly different between control and acne patients ($P=0.0002$). A significant association between IGF-I (CA) genotypes and severity of acne was found ($P = 0.015$). No significant difference was found between male and female patients ($P>0.05$).

CONCLUSIONS: Our results suggest that IGF-I (CA) 19 polymorphism may contribute to a predisposition to acne in Turkish patients.

Keywords: acne vulgaris, polymorphism, genetic, insulin-like growth factor I

Effect of Resveratrol on Selected Serum Components in A Rat Endotoxemic Model

Ramazan Çöl, Ercan Keskin

Department of Physiology, Faculty of Veterinary Medicine, Selcuk University, Konya/TURKEY

OBJECTIVE: Resveratrol (RES) exhibits a wide range of biological activities including anti-inflammatory and antioxidant effects. Lipopolysaccharide (LPS, endotoxin) and proinflammatory cytokines are involved in the pathogenesis of sepsis and plays a pivotal role in the initiation of tissue damage, multiple organ failure (MOF) and haemostatic disturbances. In this experimental study, our aim was to investigate the role of RES on inflammatory and biochemical disturbances in LPS-treated rats.

METHODS: A total of 60 adult male Wistar rats were divided into six equal groups: Group 1 served as negative control (C) and was infused 10 ml saline for 6 h via the tail vein of rats. Animals in Group 2 were continuously infused LPS (*Escherichia Coli*, 0.111:B4, 1.6 mg/100g) in 10 ml of physiological saline for 6 h. In Group 3, RES (30 mg/kg) was injected intraperitoneally and was infused 10 ml saline for 6 h. In Group 4 and 5, after RES (15 and 30 mg/kg respectively) was administrated intraperitoneally, endotoxin was infused. In group 6, before the infusion of endotoxin, RES (20 mg/kg/day) was injected intraperitoneally for 7 successive days.

RESULTS: LPS caused statistically significant increases in plasma TNF- α , IL-6 and IL1 β levels, CRP, AST, ALT, creatinine, BUN, cholesterol, triglyceride concentration, and caused statistically significant decreases in total protein and albumin levels. RES could inhibit only serum cytokine levels ($P < 0.05$) in group 5, However, it moderately suppressed both the levels of serum cytokines and biochemical parameters ($P < 0.05$) in group 6.

CONCLUSIONS: In conclusion, administration of RES may lead to potential new therapies in endotoxemia.

Keywords: Resveratrol, cytokine, biochemical parameters, endotoxin, rat

The Effects of Carbohydrate Solution to Endotoxemic Rats, Erythrocyte Deformability and Perfusion of Splanchnic Area

Faruk Metin Çomu¹, Turgut Deniz², Badagül Akpınar³

¹Department of Physiology, Kirikkale University School of Medicine, Kirikkale, Turkey

²Department of Emergency Medicine, Kirikkale University School of Medicine, Kirikkale, Turkey

³Department of General Surgery, Kirikkale University School of Medicine, Kirikkale, Turkey

OBJECTIVE: The aim of this study was to investigate effects of oral carbohydrate (maltodextrin) administration on splanchnic circulation and erythrocyte deformability in endotoxemic rats.

METHODS: Forty-eight male Wistar rats (weighing 180–200 g) were used in this study. Group SC (n=8) was designated as sham control. Group S (n=10) served as starvation only. Group S+ lipopolysaccharide [LPS] (n=10) was subjected to endotoxemia. Group CH (n=10) received carbohydrate (Preop, 240 mOsm/l, 12.5% maltodextrin, sodium 50 mg), 0.5 mL in every 4 hours for 48 hours, through orogastric lavage. Group CH+LPS) (n=10) ingested same amount of carbohydrate and was subjected to endotoxemia. Endotoxemia was induced by intraperitoneal injection of 30 µg/kg of LPS. Erythrocyte deformability index was measured using a filtration technique 5 µm in diameter polycarbonate filter with a fixed flow (1.5 ml / min.) Pyloric, ileal, portal and hepatic surfaces were measured by a laser Doppler microvascular perfusion monitor.

RESULTS: According to control group, (S + LPS) group, deformability was significantly and negatively affected (p <0.05). Deformability of (S + LPS) and (Ch+LPS) group was measured positively affected although there was no significant difference. LPS caused a significant decrease in hepatic surface blood flow (p<0.05). Ingestion of carbohydrate significantly improved (p<0.05) ileal, pyloric blood flows in endotoxemic rats. Increases in hepatic surface blood flow were found in S and Ch groups, although these changes were not statistically significant.

CONCLUSIONS: These results have shown that endotoxemia caused a significant decrease in splanchnic blood flow. We suggest that decreased blood flow with an increase in bacterial translocation can cause infection development in the gastrointestinal system.

Keywords: Erythrocyte deformability, Mesenteric blood flow, Endotoxemia, Maltodextrin

Does Vitamin C Prevent the Effects of High Dose Dexmedetomidine on Rat Erythrocyte Deformability?

Mustafa Arslan¹, Ömer Kurtipek¹, Faruk Metin Çomu², Levent Öztürk³, Metin Alkan¹, Kutluk Pampal¹

¹Department of Anesthesiology and Reanimation, Gazi University Medical Faculty, Ankara- Turkey

²Department of Physiology, Kırıkkale University Medical Faculty, Kırıkkale- Turkey

³Department of Anaesthesiology and Reanimation, Ankara Atatürk Training and Research Hospital, Ankara-Turkey

OBJECTIVE: Dexmedetomidine is an anesthetic agent frequently used for sedation in intensive care units, and during general anesthesia. The purpose of our study was to investigate whether vitamin C prevents the effect of high dose dexmedetomidine on erythrocyte deformability in rats.

METHODS: The study was performed on 21 male rats, with 7 rats in each study groups and control group. The rats in the study groups were treated with intraperitoneal dexmedetomidine (10 µg.kg-1) and intraperitoneal dexmedetomidine plus Vitamin C (ascorbic acid) (100 mg.kg-1 ascorbic acid administered 1 hour before administration of 10 µg.kg-1 dexmedetomidine), respectively. Intraperitoneal physiological saline was administered in the control group. Erythrocyte packs were prepared using heparinized total blood samples. Deformability measurements were done by erythrocyte suspensions in phosphate buffered saline (PBS) buffer. A constant flow filtrometer system was used to measure erythrocyte deformability, and the relative resistance was calculated.

RESULTS: Erythrocyte deformability was significantly higher in dexmedetomidine group than in control and vitamin C plus dexmedetomidine groups ($p=0.003$, $p=0.013$, respectively). Erythrocyte deformability indexes were found similar control group and vitamin C plus dexmedetomidine group ($p=0.383$)

CONCLUSIONS: High dose dexmedetomidine may cause functional deterioration in blood flow and tissue perfusion with negative effects in erythrocyte deformability. Vitamin C supplementation seems to reverse those negative effects and variations in erythrocyte deformability. However, our preliminary results should be confirmed in wider serious of experimental and clinical trials.

Keywords: Erythrocyte deformability, α_2 agonist, Dexmedetomidine, vitamin C, Rat

Human stem cells of different origins: similarities and differences

Florina Bojin¹, Oana Gavriiuc¹, Mirabela Cristea², Simona Anghel¹, Daciana Nistor¹, Carmen Tatu¹, Gabriela Tanasie¹, Carmen Panaitescu¹, Virgil Paunescu¹

¹Department of Physiology-Immunology, "Victor Babes" University of Medicine and Pharmacy Timisoara, Romania

²Transplant Immunology Center, Clinical Emergency County Hospital, Timisoara, Romania

OBJECTIVE: Bone marrow-derived mesenchymal stem cells (MSCs) have long been considered as prototype of stem cells with marked proliferative potential, increased plastic ability, and presence of certain surface molecules. We isolated and cultured stem cells from different sources - bone marrow (MSC), dental pulp (DSC), skeletal muscle (MuSC) and umbilical cord (UCSC) - and comparatively analyzed their characteristics.

METHODS: Tissue samples were processed using enzymatic digestion (Collagenase IA) or explant method, and adherent, fibroblastic-like cells were cultured in DMEM/F12 medium supplemented with 10% FCS. Starting with passage 2, using appropriate differentiation media, cells were induced towards the mesodermal lineages - adipocytes, osteoblasts and chondrocytes. Subsequently, differentiated cells were analyzed for presence of characteristic immunocytochemical markers: FABP4, osteocalcin and aggrecan. Phenotypical profile of undifferentiated cells was assessed by flow cytometry using characteristic surface markers, and we determined the ratio of positive cells between different populations: CD29, CD34, CD44, CD45, CD73, CD106, CD117, and CD90. Immunocytochemical staining used CD117, vimentin and Ki67 to certify presence of stem cells characteristics.

RESULTS: Although cells of each type were obtained from 10 different subjects, we could not confirm the differentiation of DSC cells towards adipocytes, compared with stem cells from the other sources, which presented differentiation ratios ranging from 50% (MSC) to 85% (MuSC). CD29 and CD90 were significantly higher expressed in DSC compared with the other cellular types, while CD44 expression was decreased in stem cells populations. Expression of CD117 was increased in all cellular types when compared with MSC.

CONCLUSIONS: Stem cells from different sources are endowed with properties which make them suitable for diverse cellular therapies.

Keywords: stem cells, MSC, adipocytes, characteristic markers

Histochemical Properties Of Gastronecmius Muscle Fibers In The Young And Aged Rats

Suna Cebesoy¹, Nesrin Özsoy¹, Dilşad Okan², Güler Öztürk³, Kazime Gonca Akbulut⁴, Şevin Güney⁴

¹Ankara University Faculty of Science Department of Biology

²Kastamonu University Faculty of Science and Art Department of Biology

³Maltepe University Faculty of Medicine Department of Physiology

⁴Gazi University Faculty of Medicine Department of Physiology

OBJECTIVE: In this study, the effects of aging on the development of muscle necrosis in male albino rats were evaluated by light microscopy.

METHODS: The animals were divided into two groups, namely the young and the old. Each group had three rats. The young group was 4 months old, while the old group was 24 months old. The animals were kept under a 12-hour light/12-hour dark period in the laboratory. The rats were fed with normal tap water and standard rat chow. The rats were decapitated under xylazine (5-10 mg/kg)- ketamine (40-50 mg/kg) anesthesia and gastrocnemius muscles were dissected free. Muscle tissue samples were fixed in 10% formaldehyde solution and were embedded in paraffin, 5µm thick sections were taken from the muscle tissue for histological examinations and were stained with hematoxylen-eosin, periodic acid Schiff and Masson's trichrome.

RESULTS: In the young group, there were no histological changes in their muscle tissue samples. On the other hand, in the aged group, some histopathological changes were determined in different degrees. In the aged group muscle fibers showed a loss of contractile proteins. There were also increased lipid tissues between muscle fibers. Increases in collagen fibers were observed in the intercellular area.

CONCLUSIONS: Generally our findings were degeneration, loss of contractile proteins, necrosis, increased lipid tissue and hypertrophy on the muscle tissue in the aged group.

Keywords: Aging, rat, skeletal muscle, histochemistry

The effects of putrescine on TNBS-induced colitis model

Hümevra Ünsal¹, Rauf Onur Ek², Cengiz Ünsal³, Serpil Çeçen⁴, Mukadder Serter⁵

¹Department of Physiology, Adnan Menderes University Faculty of Veterinary Medicine, Aydın, Turkey

²Department of Physiology, Adnan Menderes University Faculty of Medicine, Aydın, Turkey

³Department of Physiology, Adnan Menderes University Faculty of Veterinary Medicine, Aydın, Turkey

⁴Department of Biochemistry, Adnan Menderes University Faculty of Medicine, Aydın, Turkey

⁵Department of Physiology, Marmara University, Faculty of Medicine, Istanbul, Turkey

OBJECTIVE: In this study we aimed to determine the effects of putrescine on some colonic bacteria and antioxidant and inflammatory parameters.

METHODS: Twenty-four female Wistar albino rat were divided to 4 groups; control, colitis, colitis + putrescine (300 µmol/kg) and putrescine (300 µmol/kg). Experimental colitis model was induced with enema composed of trinitrobenzenesulfonic acid (TNBS) and ethanol. Putrescine was injected intraperitoneally once a day for three days shortly after colitis was induced. The numbers of lactobacilli and Enterobacteriaceae in colon content and the glutathione reductase (GR), glutathione (GSH), IL-6 and myeloperoxidase (MPO) levels of colonic tissue were determined. Results were tested using one-way ANOVA and post-hoc Tukey tests.

RESULTS: Enterobacteriaceae numbers were lower in non-colitis groups than the colitis groups and were highest in colitis putrescine group, but the differences between the mean of the groups were not statistically significant. The reduction of lactobacilli in colitis putrescine group was significant with putrescine group ($P<0.05$). GR activity was lower in colitis groups than the non-colitis groups ($P<0.001$). GSH activity was decreased in colitis and in putrescine group as compared with the control ($p<0.001$). Also, IL-6 and MPO levels were higher in colitis group than the non-colitis groups ($p<0.001$).

CONCLUSIONS: Putrescine (300 µmol/kg) could not restore the colonic tissue and luminal environment.

Keywords: Putrescine, colitis, TNBS, large intestine, microflora, rat

The role of gastric mucosal blood flow in protective effect of Orexin-A (OXA) on ischemia/reperfusion (I/R)-induced gastric mucosal injury

Ruken Tan, Burcu Gemici, Vecihe Nimet Izgüt Uysal
Department of Physiology, Akdeniz University, Antalya, Türkiye

OBJECTIVE: The aim of the study is to investigate the role of gastric mucosal blood flow in the protective effect of orexin-A (OXA) on ischemia/reperfusion (I/R)-induced gastric mucosal injury.

METHODS: Three main experimental groups were arranged: Sham, I/R and I/R+OXA. I/R was applied for I/R+saline, I/R+capsaicin, I/R+L-NAME and I/R+vagotomy subgroups while additional OXA infusion was applied for I/R+capsaicin+OXA, I/R+L-NAME+OXA, I/R+vagotomy+OXA subgroups. In sham group, saline infusion (0.01 ml/kg.min) was applied through the jugular vein simultaneously with 3.5 hours of I/R. For I/R+OXA groups, 500 pmol/kg.min of OXA was administered at the same rate. Capsaicin (125 mg/kg, s.c.) was used for sensory nerve denervation while L-NAME (30 mg/kg, i.v.) was administered as nitric oxide synthase (NOS) inhibitor. 24 hours prior to I/R and/or OXA application, subdiaphragmatic bilateral vagotomy was performed to the vagotomy group animals. Following I/R application at each group, stomachs were taken out and opened through the greater curvature. Mucosal blood flow was measured by Laser Doppler Flowmeter and lesion index was determined under stereomicroscopy.

RESULTS: : I/R-induced gastric mucosal damage was significantly decreased and blood flow was increased in OXA group. No significant difference was observed for either lesion index or blood flow in I/R+Capsaicin and I/R+L-NAME groups compared to I/R group. Lesion index was significantly increased while mucosal blood flow was decreased in I/R+Capsaicin+OXA, I/R+L-NAME+OXA and I/R+Vagotomy+OXA groups compared to I/R+OXA group. For each of these three groups, protective effect of OXA diminished and the outcomes approximated to I/R group.

CONCLUSIONS: In conclusion, the increased level of mucosal blood flow is effective in the OXA-mediated gastric mucosal protection.

Keywords: orexin-A, ischemia/reperfusion, gastric mucosal protection, gastric mucosal blood flow

Effects of Rosemary and Sage Extracts on *in vitro* Rumen Microbial Fermentation

Ahu Demirtas¹, Hakan Ozturk¹, Ilksin Piskin¹, Deniz Demirkiran², Yasemin Salgirli¹, Ulvi Reha Fidanci², Bahri Emre¹

¹Department of Physiology, Faculty of Veterinary Medicine, University of Ankara, Ankara, Turkey

²Biochemistry, Faculty of Veterinary Medicine, University of Ankara, Ankara, Turkey

OBJECTIVE: The objective of this study was to determine the effects of rosemary and sage extracts on *in vitro* ruminal fermentation using the long-term rumen simulation technique (RUSITEC).

METHODS: The RUSITEC system was equipped with six fermenters, each with a capacity of 1000 ml. Each fermenter received daily 5 g barley straw and 5 g barley. The experiment lasted 12 days. After an adaptation period of 6 days, the fermenters were divided into 3 groups, first two vessels received no additives (control), second two vessels received 250 mg rosemary extract daily, and third two vessels received 250 mg sage extract daily. During the experimental period rumen fluid pH and protozoa number were determined and samples were collected for the analysis of ammonia nitrogen (NH₃-N), volatile fatty acids (VFA) and dry matter digestibility.

RESULTS: Rosemary and sage extracts had no effect on ruminal pH, total-VFA, propionate and butyrate production, total protozoa number, NH₃-N concentration and dry matter digestibility. However, acetate production and acetate/propionate ratio decreased ($p < 0.05$) when compared with the unsupplemented control fermenters.

CONCLUSIONS: The results of this study showed that rosemary and sage extracts exerted beneficial effects on some fermentation parameters in the RUSITEC system. If these effects were also induced when these substances are added to the rations of ruminants, beneficial changes in the animals' performance may be expected.

Keywords: Fermentation, rosemary extract, rumen, sage extract

IL-12 and IFN- γ production and NK cell level comparative assessment in disc hernia tissue and peripheral blood

Sibel Akyol¹, Murat Hanci²

¹Department of Physiology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

²Department of Neurosurgery, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

OBJECTIVE: This study aims to provide the profile of the IL-12 and IFN- γ cytokines and NK lymphocytes, main components of inflammatory responses, in both degenerated disc and plasma samples of a group of patients with lumbar degenerated disk disease (DDD).

METHODS: Twenty-six patients were suggestive of lumbar DDD were included in this study. Control subjects included 14 autopsy cases. From each patient, disc tissues and peripheral blood samples for plasma were collected during the surgery. For the controls, disc samples and blood for plasma by intracardiac puncture were obtained during autopsy. Flow cytometry was used to obtain the lymphocyte CD56 (NK). The Luminex was used to obtain IL-2, IL-4, IL-10, IL-12, IFN-gamma, in both plasma and disc tissues. The results were statistically compared between the two groups.

RESULTS: Comparison of the two groups regarding plasma showed that IL-1 β (pg/ml C; 6.42 ± 2.05 , P; 46.6 ± 9.03), IL-2 (pg/ml C; 7.62 ± 2.61 , P; 26.11 ± 6.39), IFN- γ (pg/ml C; 4.62 ± 1.19 , P; 8.94 ± 2.36) were significantly higher than in patients than those of the controls. Likewise, tissue levels of IL-1 β (pg/ml C; 11.45 ± 3.80 , P; 31.61 ± 4.82), IL-2 (pg/ml C; 3.92 ± 1.13 , P; 11.88 ± 2.51) IL-4 (pg/ml C; 8.37 ± 3.35 , P; 32.18 ± 6.38), IL-10 (pg/ml C; 9.09 ± 3.20 , P; 30.67 ± 7.67), IL-12 (pg/ml C; 4.09 ± 1.04 , P; 7.33 ± 1.15), TNF- α (pg/ml C; 11.80 ± 2.89 , P; 28.94 ± 4.04) were found to be significantly higher in the patients. Findings suggest that both local and systemic inflammatory responses occur in lumbar DDD.

CONCLUSIONS: Using specific cytokines either by local or systemic application may reverse the degenerative process.

Keywords: Cytokine, Degenerative disc disease, NK, Immune system, IL-12

Improving Clinician-Patient Communication for Better Recognition and Diagnosis of Tinnitus: How does Turkish population describe their tinnitus?

Ozlem Tanriover¹, Muzeyyen Doğan², Aysenur Kucuk Ceyhan², Yavuz Selim Pata²

¹Department of Family Medicine, Yeditepe University, Istanbul, Turkey

²Department of ENT, Yeditepe University, Istanbul, Turkey

OBJECTIVE: Majority of the patients may describe their perception of tinnitus according to their cultural background and pose a challenge for the clinician. Patients with tinnitus usually have sensory-neural hearing loss and audiologic properties of hearing loss may effect the perception of tinnitus. The aim of this study was to identify the correlation between the patients' description of tinnitus and objective measurements.

METHODS: One hundred and five cases with tinnitus (50.5% male and 49.5% female) were included in the study. They were identified as having pure sensory-neural hearing loss. They filled out a questionnaire including their socio-demographic features and their complaints and perceptions of tinnitus. Pure tone audiometry, high frequency audiometry, tympanometric evaluation and pitch-loudness matching were performed for each patient.

RESULTS: According to the description of tinnitus, a statistically significant difference was noted between the frequencies of hearing loss ($p < 0.01$). Patients with a frequency of hearing loss higher than 8000 Hz described their tinnitus as crickets, insects, and sounds of steam, whereas in frequencies of hearing loss between 4000-8000 Hz tinnitus was described as resonance, bell tone and transformer noise. Under 4000 Hz frequency of hearing loss, patients identified their tinnitus as the sound of wind.

CONCLUSIONS: We have demonstrated a correlation between tinnitus descriptions of patients and audiologic measurements, which may lead the clinicians to better comprehend their patients' complaints. Understanding and describing the definition of tinnitus are closely related to patients' native language and culture. Further studies are needed to validate our results in larger population-based studies in different cultures.

Keywords: Tinnitus, sensory-neural hearing loss, audiology, doctor-patient communication

Students' Life Styles and Risk Factors for Health

Smaranda Rodica Gotia¹, Smaranda Laura Gotia¹, Claudia Borza², Corina Serban², Roxana Popescu³, Nicoleta Filimon⁴

¹Department of Physiology, University of Medicine and Pharmacy, Timisoara, Romania

²Department of Pathophysiology, University of Medicine and Pharmacy, Timisoara, Romania

³Cell and Molecular Biology Department, University of Medicine and Pharmacy, Timisoara, Romania

⁴Department of Biology, West University, Timisoara, Romania

OBJECTIVE: Dopamine levels in the brain are increased by alcohol and these are associated with the pleasurable aspects of alcohol drinking. In the last years, alcohol use is a reality among teenagers and students.

METHODS: The study estimated in alimentary manners, the incidence and motivation of the alcohol use among students in Timisoara. A questionnaire about daily nutrition, alcohol consumption, life style, was distributed to 105 students, aged 18-28 years.

RESULTS: From the investigated students, 87% do not have a stable food timetable, and 53% of them eat three times daily. Most of them eat relating to their everyday timetable, but sadly the majorities eat in a hurry, in fast foods. The incidence of coffee consumption was 58.33%. A healthy nutrition prevents the young obesity and gastrointestinal diseases. The students are occasional drinkers. Youths drink alcohol to get free from their shyness, to escape from their own inhibitions and consider it as a way to be accepted in a group. The main occasions of alcohol consumption were social events (67%), going-out with friends (61%). Beer is the first choice (59%) in the top of students' preferences and they usually drink two bottles, while 13% of them drove after they drank alcohol. Unfortunately, alcohol-related automobile accident is a major cause of teen's deaths. Special programs with educational, social and psychological evaluation of the young people who use alcohol can be useful.

CONCLUSIONS: The incidence of risk factors for health in students was increased. So, we recommend discussing with students about a healthy diet and the consequences of alcohol abuse on their health.

Keywords: student life, nutrition, alcohol use, health

First aid knowledge level of students studying at a health services of a vocational college of a university

Gul Arslan, Mustafa Tozun, Alaettin Unsal, Unal Ayranci
Osmangazi University, Eskisehir, Turkey

OBJECTIVE: To determine the first aid knowledge level in a group of university students.

METHODS: This descriptive study was conducted out on the students studying at the health services of a vocational college of Eskisehir Osmangazi University between Dec 1st 2010 and Jan 31st, 2011. The study group consisted of 346 out of a total of 437 students (79.2%). Those receiving a score of 28.5 and over were accepted as “first aid knowledge level is enough”. Data were evaluated by Chi square test with a value of $p < 0.05$.

RESULTS: The study group consisted of 245 female (70.8%) and 101 male students (29.2%). Their mean age was 19.16 ± 1.63 years, ranging from 17 to 33. The most correctly known knowledge question of first aid was “telephone number of emergency ambulance services is 112” (98.5%). The least correctly known was “that people with tube gas poisoning drink salty buttermilk is an effective method” (37.9%). The mean score of students' knowledge about first aid was 30.91 ± 5.50 . The students studying at the Department of Ambulance and Emergency Care, female students, those whose fathers were working actively in a job, those who had received knowledge from any source, and those who had done first aid application in any case had a higher level of knowledge ($p < 0.05$, for each one).

CONCLUSIONS: We suggest that more informative studies are needed to be done to improve the students' knowledge level about first aid.

Keywords: University students, first aid, knowledge level

Effects of wireless internet access devices on the reproductive system of growing rats

Halil I Atasoy¹, Yalcin Gunal², Pinar Atasoy³, Serenay Elgun⁴, Guler Bugdayci⁵

¹Abant Izzet Baysal University School of Medicine, Department of Pediatrics, Bolu, Turkey

²Abant Izzet Baysal University School of Medicine, Department of Physiology, Bolu, Turkey

³Kirikkale University School of Medicine, Department of Pathology, Kirikkale, Turkey

⁴Ankara University School of Medicine, Department of Medical Biochemistry, Ankara, Turkey

⁵Abant Izzet Baysal University School of Medicine, Department of Biochemistry, Bolu, Turkey

OBJECTIVE: The aim of this study was to examine the effects of radiofrequency waves emitted from wireless fidelity internet access devices on testes.

METHODS: Ten Wistar-Albino male rats were divided into an experimental and control groups. Standard wireless internet access devices communicating at 2.437 GHz band were used as radiofrequency wave sources. Experimental group was exposed to radiofrequency energy for 20 weeks. The rats were sacrificed at the end of the study.

RESULTS: Significant increases in serum 8-hydroxy-2'-deoxyguanosine levels and 8-hydroxyguanosine stainings in the testes of the experimental group were observed. Decreased levels of catalase and glutathione peroxidase activities in the experimental group, which might be due to radiofrequency effects on enzyme activity were found.

CONCLUSIONS: These novel findings raise questions for the security of wireless fidelity internet access device radiofrequency exposure specially on testes of growing rats, potentially affecting both the fertility and the integrity of germ cells.

Keywords: Wireless technology; internet; testes; oxidative stress; infertility; mutagenesis.

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CLEMMENSEN Christoffer	S8.3	DAKKA Taoufiq	OC40
CLICHICI Simona	OC12(EYPS), PC029, PC262, PC277, PC281	DAL Uğur	PC225
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COLAK Rıdvan	OC18(W1), PC265	DANEASA Andrei	PC127
ÇOLAK Mehmet	PC225	DANIŁA Maria	PC211
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CONLON John Michael	OC44, PC064	DAYI Ayfer	PC116, PC117, PC126, PC150
CONTUK Gazi	OC11(EYPS), OC47, PC216	DAYIOĞLU Enver	PC063
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CUESTA Sara	OC16(W1), OC37, PC184	DEJANOV Petar	PC245
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CULHA Mustafa	OC49	DEL CANTO Irene	OC41
CUMBUL Alev	PC002	DELİBASÍ Tuncay	PC173, PC192
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DEMİRCİ Hasan PC137
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DEMİREL TEMEL Sernaz PC086, PC088,
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DEMİRKAYA MİLOĞLU Fatma PC252
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DOĞANAY Songül OC32, PC226, PC264
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DOLU Nazan OC29, PC100, PC111, PC112, PC113,
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ELOSAİLY Gehan PC301
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ERDEM Tamer Lütfi PC203, PC207
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FODOR Janos	PC011	GİNİS Zeynep	PC173, PC192
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GARCÍA ARÉVALO Marta	OC01(EYPS)	GOTİA Laura	PC204, PC205
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GARRY Emer M	S12.3	GOTİA Smaranda Laura	PC048, PC221, PC256, PC319
GASSMANN Max	S7.1	GOTİA Smaranda Rodica	PC048, PC221, PC256, PC319
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GENY Bernard	PC034, S11.2	GÜÇLÜER Nilay	PC143
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HAERLİN Ulrich PC028
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HUDSON Brian D. S8.2
HYNİE Sixtus PC008, PC040
IANCAU Maria PC102, PC162
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KACAR Emine PC080, PC297

KACAR Nida PC308

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KAHRAMAN Mehmet OC49

KAISER Josephine PC134

KALAY Zeynep PC288

KALE Ebru PC258

KALENDER Suna PC254

KALFİN Reni PC038, PC300

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 LUTZ Thomas Alexander S9.3
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 MACDERMOTT Amy B. S12.1
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MURESAN Adriana OC12(EYPS), OC46, PC029, PC262, PC277, PC280, PC281

NADAL Angel OC01(EYPS)

NADRĪGNY Fabien OC34

NAGY Andras OC12(EYPS), PC262, PC277

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NEGREV Negrin Nestorov PC176, PC177

NEGRĪSANU Gabriela PC205

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NGUYEN Genevieve S5.2

NĪCOLAU Cristina PC104, PC164

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NODA Mami S3.4

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NORSK Peter S13.1

NORTH Alan PL04

NOURĪZAD Shahla PC167, PC251

NOVAKOVA Monika PC055

NOVOKHATSKA Tetiana OC04(EYPS)

NURTEN Rüstem PC012

NURULLAHOĞLU ATALIK Kismet Esra OC45

NYAGOLOV Yuri Penkov PC176, PC177

OANCEA Cristian PC204

OARGA Marilena PC262

ÖGETÜRK Murat PC185

ÖĞÜNÇ Ayliz Velioğlu PC253

ÖĞÜT Serdal PC271

OGUZ Elif Kaval OC20

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OKUDAN Nilsel	OC45, PC208, PC214, PC236, PC283	ÖZDEMİR Zarife Nigâr	OC11(EYPS), PC182, PC263
OKULU Emel	PC285	OZEN Oguz Aslan	PC267
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OLÁH Tamás	PC011	OZER Cigdem	PC058, PC228, PC229, PC247, PC282, PC286
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OLTEANU Elena Diana	OC12(EYPS)	OZKAYA Ahmet	PC257
OLUK Ali İhsan	OC11(EYPS)	ÖZKAYA Yaşar Gül	PC240
ÖMEROĞLU Suna	PC282	OZKURT Mete	PC290
ONAR Musa Kazım	PC138	OZLER Mehmet	OC14(W1), PC206, PC299
ÖNDER Feyyaz	PC181	ÖZLÜ Aysun	PC230, PC231
ÖNEM İdris	OC11(EYPS)	ÖZPINAR Haydar	PC022
ONER Gulsen	PC041	OZSOY Ozlem	PC139
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OTTMANN Daniel	PC104	OZTURK Gurkan	OC20
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ÖZ OYAR Eser	PC284	ÖZTÜRK Gülyüz	PC013
ÖZAÇMAK Veysel Haktan	PC163, PC266	ÖZTÜRK Levent	PC089, PC090, PC311, W24
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ÖZAYKAN Besim	PC068, PC070	OZYUREK Hamit	PC120
OZBAL Seda	PC116, PC150	Pál Balázs	PC011
ÖZBEK Elvan	PC295	PALABIYIK Orkide	PC036
ÖZBEYLİ Dilek	OC47, PC216	PALERMO Maria	PC147
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PATON Julian F.r.	PC057	POLONARA Gabriele	PC151
PATRIZIA Ambrogini	PC082	POP JORDANOVA Nada	PC160
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PAVONE Francesco	S14.3	POROKHNYA Maria Viktorovna	OC10(EYPS)
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PINAR Lamia	PC228, PC229	RAMS Carmiņa	OC41
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PLANAS Ruben Rial	PC136, PC195	RIAL Ruben Victor	PC114
PLUNCEVIC Jasmina	PC245	RICCARDO Cuppini	PC082
POEHLKE Christine	PC134	RICHARDS Mark	S14.1
POGGESI Corrado	S14.3	RIOLOBOS Adelaida	PC105
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POHL Ulrich	OC03(EYPS)	RIZA Anca Lelia	PC187, PC201

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ROBBEN Joris S8.1		SANDAL Süleyman	OC38, PC026, PC298
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RODRÍGUEZ FERRER José Manuel	PC085	SARABÍA Juan Antonio	PC164
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RUSSO Antonella	PC146, PC147, PC156	SAYAN OZAÇMAK Hale	PC163, PC266
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SADJIA Hammouche	PC078	SCHUBERT Kai Michael	OC03(EYPS)
SáEZ MORENO José Antonio	PC085	SCHULER Beat	S7.1
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SAHİN Hilal	PC274	SEİDENBUSCH Walter	PC028
SAHİN Leyla	PC113	SEKER Fatma Burcu	OC38, PC122, PC189
ŞAHİN Arzu	PC181	SEKKİN Selim	PC248
ŞAHİN Bünyamin	PC138	ŞEN Leyla Semiha	PC263
ŞAHİN Deniz Say	PC172	ŞENEL Ebru	PC216
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SAKA TOPCUOGLU Esen	PC139	ŞENGEZER Tijen	OC33
SALATAİ Anta	PC043	ŞENTÜRK Ümit Kemal	PC067
SALCEANU Alexandru	PC045	SEO Jai W	S18.5
SALEHİ Ensieh	PC051	SERBAN Corina	PC048, PC205, PC256, PC319
SALGİRLİ Yasemin	PC316	SERGIU Batar	OC46
SALVOLİNİ Ugo	PC151	SERHATLIOGLU Ihsan	OC23, PC075, PC213, PC215, PC237, PC238
SANCHEZ CAMPİLLO María	PC164	SERMET Abdurrahman	PC174, PC175

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SEVGİLİ A. Meltem	PC120	SÖNMEZOCAK Osman Haluk	PC089
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SHABANİ Mohammad	PC167	STANCHEVA Emiliya Georgieva	PC177
SHAHROKHİ SARDOO Nader	PC167, PC251	STANZANİ Stefania	PC146, PC147, PC156
SHEİBANİ Vahid	OC07(EYPS)	STAVREVA Galya Tsvetanova	PC149
SİAHPOSHT KHACHAKİ Ali	OC07(EYPS)	STEFANO Ciuffoli	PC082
SİDA Pavel	PC008, PC040	STEFFENS Heinz	OC34
SİMON Scott	S18.2	STEPPAN Dominik Ludwig	PC020
SİMONA Stefania	PC029, PC277	STİMMELOVA Judita	PC055
SİMONE Donald	OC19	STİR Ariana	PC025
SİMSEK Gonul	PC074	STİUFİUC Rares	PC025
SİMSEK Kemal	OC14(W1)	STOZER Andraz	PC183
SİMSEK Ozkan	PC293	SUCH BELENGUER Luis	OC41
ŞİMŞEK Yasin	PC171	SUCH MİQUEL Luis	OC41
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TEKİN Hasibe Sena PC194
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TOLEDO ARAL Juan José OC17(W1)
TOLEKOVA Anna PC038, PC300
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TOPSAKAL Şenay PC169
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TOSUN Murat PC260, PC290
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TRAFFORD Andrew S14.1
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TRAPP Stefan PC009, S16.3
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TRETJAKOVŠ Peteris PC061
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TURAN Güven OC06(EYPS)
TURAN Inci PC266
TURGUT Günfer PC169, PC220
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UYAR Ruhi OC48, PC007, PC017
UYGUR Emine PC218, PC224
UYGUR Ramazan PC053, PC054, PC224, PC267
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WESTERTERP PLANTENGA Margriet S S9.2
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YERLİKAYA Emrah PC169
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YESİLYURT Ahmet PC173
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ZHANG Nan PC037
ZHANG Ying PC037
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