POSTER SESSION D

D01: Cardiac physiology

D01-1
Serotonergic 5-HT2B receptors in mitral valvulopathy: bone marrow mobilization of endothelial progenitors

R. Lawson[1], E. Ayme-Dietrich[1], L. Maroteaux[2], L. Monassier[1]
[1]*University of Strasbourg, Department of Pharmacology, Strasbourg, France*
[2]*University Pierre Marie Curie, Paris, France*

Valvular heart disease is highly prevalent in industrialized countries. Chronic use of anorexigens, amphetamine or ergot derivatives targeting the serotonin system has been associated with valvular heart disease.
Here, we investigated the contribution of serotonin receptors in a mouse model of valve degeneration induced by nordexfenfluramine the main metabolite of the anorexigens dexfenfluramine and benfluorex.

Chronically activated 5-HT2B receptors by nordexfenfluramine in mice mimicked early steps of mitral valve remodelling attested by increased valve thickness, and cell density in a thick extracellular matrix. Lesions were totally prevented by inhibition of both 5-HT2A and 5-HT2B receptors by antagonists, in transgenic Htr2B-/-, or Htr2A/2B-/- mice. Surprisingly, we found that valve lesions are mainly formed by numerous non-proliferative CD34+ endothelial progenitors. We show that these progenitors originate from bone marrow as revealed by bone marrow transplantation. Initial steps of mitral valve remodelling involve bone-marrow derived CD34+CD31+ cells mobilization by 5-HT2B receptor stimulation. Moreover, the analysis of human mitral valve prolapse, showing spontaneous degenerative lesions highlights the presence of non-proliferating CD34+/CD309+/NOS3+ endothelial progenitors expressing 5-HT2B receptor.This work reveals a crucial contribution of bone-marrow derived endothelial progenitor cells in valve tissue remodelling and highlights the contribution of this new mechanism involved in human valvular heart disease.

D01-2
Cardioprotective effect of Aqueous Viscum album extract on isoproterenol induced myocardial infarction in rats .

E. SUVEREN[1], H. Y. CINPOLAT[2], F. YILMAZ[3]
[1]*ABANT IZZET BAYSAL UNIVERSITY, HEALTHCARE SCIENCES,NURSING DEPT., BOLU, Turkey*
[2]*ABANT IZZET BAYSAL UNIVERSTY, CLINICAL BIOCHEMISTRY, Bolu, Turkey*
[3]*ABANT IZZET BAYSAL UNIVERSTY, CLINICAL PATHOLOGY, Bolu, Turkey*

Viscum album has prosperious cardiovascular effects such as antihypertensive and vasodilator activity increased by NO synthase and antioxidant. However, little is known about its effects on myocardial injury. The current study was designed to investigate the cardioprotective effect of *Viscum album* L.aqueous extract (AVa) against ISO induced acute myocardial injury in male rats by demonstrating the changes in hemodynamics, bichemical parameters and histopatological architecture. Sprague Dawley rats were randomly divided 6 groups(n=8); control, ISO, Sham, AVa (0.1,1,10 mg/kg). AVa was administered intraperitonally at a dose 0.1,1,10 mg/kg for 10 days. Acute myocardial infarction was induced by subcutaneous injection of 150 mg/kg-1 of ISO at an interval of 24 h to the groups on 9 th and 10th day.Blood and tissue samples were taken for chemical and histopathological evaluations following electrocardiography recording on day 11th.ISO caused ST segment elevation (0.348 ± 0.03 mV compared control P < 0.01) and increase heart rate 351±11.6 bmp compared control P< 0.01. Tissue MDA significantly decreased in all pretreatment with AVa groups compared ISO induced group (7.69±1.25, p<0.01).Viscum album significantly increased in endogenous antioxidant level by alone (1.23±0.4, p < 0.05) compared to the other groups.Plasma nitrate level significantly increased in highest dose of AVa (10mg/kg) compared ISO and control groups (126.1±42.2, p< 0.05) ISO induced hearts revealed significant extensive myonecrosis, myocellular edema and inflammation. Pretreatment with Va (0.1 and 10 mg/kg) significantly eliminated (p < 0.01) ISO induced histopatological changes and decreased the myocardial necrosis to a greater extent. Present study provides first scientific report on protective effect of aqueous Viscum album crude extract given intraperitonally against ISO induced myocardial damage in rats.

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D01-3
EFFECTS OF THE NITRIC OXIDE DONOR S-NITROSOGLUTATHIONE AND ACUTE LOCAL VENTRICULAR STRETCH ON ISOLATED RABBIT HEART.

L. Such-Miquel[1], I. Del Canto[2], M. Zarzoso[1], L. Brines[3], C. Soler[3], G. Parra[3], A. Tormos[4], A. Alberola[3], L. Such[3], F. J. Chorro[2,5]
[1]*Universitat de València, Physiotherapy, Valencia, Spain*
[2]*INCLIVA, Valencia, Spain*
[3]*Universitat de València. , Physiology, Valencia, Spain*
[4]*Universitat Politècnica de València, Electronics Engineering, Valencia, Spain*
[5]*Universitat de València. , Medicine, Valencia, Spain*

Questions
Myocardial stretch is a proarrhythmic factor. Little is known of the effects of nitric oxide (NO) upon mechanoelectric feedback. We used isolated and perfused rabbit hearts to study the changes in mechanoelectric feedback produced by the NO donor S-Nitrosoglutathione (GSNO).

Methods
32 WNZ male rabbits were used, and anaesthetized to euthanasia, to extract the hearts. Epicardia multielectrodes were used to record myocardial activation at baseline, during and after stretching of the left ventricular free wall produced by an intraventricular device. Series: 1) control (n=10); 2) GSNO 10 μM (n=11) and 3) GSNO 50 μM (n=11). The changes in ventricular fibrillation (VF) pattern caused by stretching were analyzed.

Results
GSNO 10 μM did not modify VF at baseline but attenuated acceleration of the arrhythmia (dominant frequency was 15.6±1.7 vs 21.3±3.8Hz) and reduced the percentile 5 (P5) of the activation intervals (42±3 vs 38±4ms) caused by stretching, although it did not prevent from achieving complexity indexes of VF similar to controls. In contrast, GSNO at 50 μM concentration shortened P5 at baseline (40±7 vs 52±10ms) and increased the complexity index (1.77±0.18 vs 1.27±0.13). During stretching we obtained the lowest P5 values (34±3ms) and the highest levels of complexity (1.84 ± 0.17). A correlation between complexity index and P5 was found (r=-0.592). The stepwise regression model only admitted P5 (constant=2.4, slope=-0.02, R=0.6).

Conclusions
S-nitrosoglutathione 10 μM attenuates the effects of mechanoelectrical coupling, while at 50 μM the drug alters the baseline VF pattern and accentuates the increase in complexity of the arrhythmia induced by myocardial stretch.

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D01-4

Extracellular diadenosine tetraphosphate affects contractility and cytoplasm calcium level via proteinkinase C pathway

K. Pustovit[1,2], N. Pakhomov[1], V. Kuzmin[1,2]
[1]*Lomonosov Moscow State University, Department of Human and Animal Physiology, Moscow, Russian Federation*
[2]*Pirogov Russian National Research Medical University, Department of Physiology, Moscow, Russian Federation*

Diadenosine polyphosphates (Ap(n)A) are recently considered as endogenous signaling compounds which are present in numerous mammalian tissues, including heart. Extracellular Ap(n)A cause suppression of working myocardium contractility, induce inhibitory effects in cardiac pacemaker and alter bioelectrical activity via P1- or P2-purine receptors. However, the particular intracellular mechanisms which underlie Ap(n)A cardiac effects remain unknown. In the present study we show the role of P2Y-associated regulatory pathway in mediation of Ap4A effects in the rat heart.

Effects of Ap4A on myocardial contractility were estimated in isolated Langendorff-perfused paced (4 Hz) hearts of male Wistar rats. Action potentials (APs) were recorded with sharp microelectrodes in isolated multicellular preparations. Alteration of cytosolic calcium ([Ca2+]i) transients was measured using Fluo-4 fluorescent dye in enzymatically isolated rat ventricular myocytes.

Diadenosine tetraphosphate (10 μM, n=7) induced significant decrease in left ventricular developed pressure, maximal rate of contraction and relaxation in isolated rat heart. Inhibitory effects of Ap4A were significantly suppressed by proteinkinase C (PKC) blocker chelerythrine (5 μM, n=7). Also, Ap4A (10 μM, n=12) produced AP shortening in both atrial and ventricle myocardial preparations. Chelerythrine (5 μM, n=7) application significantly reduced effect of Ap4A on AP duration. In addition, substantial suppression of cytoplasm [Ca2+]i transients was observed in the presence of Ap4A (10 μM, n=6). PKC inhibitor (5 μM) significantly restored the amplitude of [Ca2+]i transients (n=7).

Thus, we suggest that negative effects of Ap4A in the rat heart are mediated by PKC dependent pathways.

This study was supported by Russian Science Foundation [grant no. 14-15-00268].

D01-5

Effect of anti-HMGB1 protein in experimental myocardial infarction

M. Cebova[1], A. Barta[1], M. Kosutova[1], O. Pechanova[1]
[1]*Institute of Normal and Pathological Physiology Slovak Academy of Sciences, Laboratory of Neuro-cardiovascular Interaction, Bratislava, Slovakia*

Introduction: High mobility group box 1 (HMGB1) is a DNA-binding protein associated with various pathological conditions such as cardiovascular disease, cancer, and ischemia/reperfusion injury. The aim of this study was to evaluate the effects of HMGB1 protein on biochemical and morphological parameters after experimental myocardial infarction (MI).

Methods: 12-week-old WKY male rats used for the study were divided into following groups: shame operated WKY without MI, WKY with MI, WKY + IM+ anti-HMGB1 protein (10 in each grop). In vivo model of experimental MI was induced by ligation of the left descending coronary artery and lasted 20 min. Before reperfusion anti HMGB1 protein was administrated i.v. Animals survived 7 days after MI. NOS activity was determined by conversion of 3[H] Arginine to 3[H] Citrulline in the aorta and ischemic, border and non-ischemic region of the heart. For morphological parameters, the hearts were excised and used for TTC-staining procedure. Cytokine levels were investigated using the Bio-Plex Pro Cytokine kit in the plasma. Concentration of CD was measured spectrophotometrically.

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Results: Anti-HMGB1 protein increased NOS activity in both ischemic and border part of the heart, as well as in the aorta. It significantly decreased TNF-alpha and IL-6 level in plasma. Simultaneously, anti HMGB1 protein decreased MI part as well as border region of the heart.

Discussion: Considering the results, HMGB1 protein is a promising molecule for reduction the negative effects of the myocardium infarction, as well as a promising agent for the treatment of cardiovascular diseases.

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Keywords: myocardial infarction, HMGB1 protein

D01-6

IMPACT OF SIMVASTATIN ON LIPID AND NON-LIPID BIOCHEMICAL RISK FACTORS IN DIET-INDUCED HYPERHOMOCISTEINEMIA IN *WISTAR ALBINO* RATS

V. Jakovljevic[1], T. Nikolic[2], V. Zivkovic[1], N. Jeremic[2], J. Jeremic[2], I. Stojic[2], I. Srejovic[1], D. Djuric[3]
[1]*Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Serbia, Kragujevac, Serbia*
[2]*Faculty of Medical Sciences, University of Kragujevac, Departmet of Pharmacy, Kragujevac, Serbia*
[3]*School of Medicine, University of Belgrade, Department of Physiology, Belgrade, Serbia*

Questions
The objective of our study was to evaluate the association between long-term administration of simvastatin and body weight, food intake, plasma total homocysteine (tHcy), cholesterol (tCHOL), low-density lipoprotein (LDL), high-density lipoproteins (HDL), triglycerides (TRY) levels, as well as pro-oxidative (superoxid anion radical, hydrogen peroxide, index of lipif peroxidation) and antioxidative markers (reduced glutathione, catalase and superoxide dismutase) in Wistar albino rats.

Methods
Study was conducted on adult male *Wistar albino* rats (n=30; 4 weeks old; 100±15g body mass) in which HHcy was achieved by dietary manipulation. For 4 weeks, the animals were fed with one of the following diets: standard rodent chow (n = 10) (control fed); diet enriched in methionine with no deficient in B vitamins (folic acid, B6 and B12) (n = 10); diet enriched in methionine and deficient in B vitamins (folic acid, B6 and B12) (n = 10). Simvastatin was administrated daily for 4 weeks, 5mg/kg i.p.

R**esults**
We found significant differences between the body weights and food intakes among all groups ($p<0.05$) and significant strong positive correlation between Hcy levels, prooxidative and lipid parameters, and negative correlation with antioxidant parameters in blood after administration of simvastatin ($p<0.05$). Also, blood concentrations of the antioxidant SOD in blood were significantly affected, as well as CAT activity and all lipoproteins ($p<0.05$).

Conclusions
These data support the association between higher methionine intake and increasing of lipid and non-lipid biochemical risk factors for cardiovascular disease, with antioxidative protective role of HMG-CoA reductase inhibitors (simvastatin) in these cascade reactions.

Key words: HMG-CoA reductase inhibitors, homocysteine, rat.

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D01-7

Investigation of the Effects of Some Calcium Channel Blockers on in vivo, in vitro andIschemia / Reperfusion Injured Rat Heart Acetylcholinesterase Enzyme

E. ERASLAN[1], M. senturk[2], A. tanyeli[1], M. C. güler[1], D. ekinci[3]
[1]*Atatürk University, Physiology, Erzurum, Turkey*
[2]*ibrahim cecen university, chemistry, agri, Turkey*
[3]*ondokuz mayis university, agricultural biotechnology, samsun, Turkey*

AIM: Some calcium channel blockers were tested on Iacetylcholinesterase (AChE) enzyme in vitro. Nifedipine was found to be the most effective one among the drugs tested. In order to investigate the effect of nifedipine drug on heart tissue AChE enzyme in Ischemia/reperfusion (I/R) and I/R injured rats.

METHODS: Control, sham, I / R and nifedipine + I / R are as follows: A total of 24 male Wistar albino rats weighing 250300 g were equally and randomly divided into four groups. In the last group, nifedipine was administered at 4 mg/kg dose before intraperitoneal ischemia period. Heart tissues were removed after the bilateral I/R process. Enzymatic activity was measured by using the spectrophotometric method of Ellman. The reaction mixture include TrisHCl buffer (1 M, pH 8.0), 5,5´dithiobisbis
nitrobenzoic acid (DTNB, 0.5 mM), acetylthiocholine iodide (ATChI, 10 mM) and AChE (0.28 units/mL).

RESULTS: Different calcium channel blockers were tested in vitro on AChE enzyme and nifedipin was observed to be the most effective one among the tested drugs. Specific activity values were determined for AChE enzyme at four different experimental groups. Groups were determined as follows: Control group 0.239 ± 0.012 EU/mg protein, sham group 0.162 ± 0.005 EU/mg protein, I/R group 0.134 ± 0.004 EU/mg protein, and nifedipine + I/R group 0.128 ± 0.004 EU/mg protein.

CONCLUSION: As a result, the activity of the AChE enzyme was determined to exist mostly in ischemia/reperfusion+nifedipine group. This study has been supported by Ataturk University Scientific research project unit (2014/146).

D01-8

Association of α-adrenoeceptor Polymorphisms with Cardiac Autonomic Control

Z. Turianikova[1], B. Czippelova[1], J. Krohova[1], Z. Lazarova[1], Z. Snahnicanova[2], Z. Lasabova[2], M. Javorka[1]
[1]*Comenius University, Jessenius Faculty of Medicine in Martin, BioMed, Department of Physiology, Martin, Slovakia*
[2]*Comenius University, Jessenius Faculty of Medicine in Martin, Biomed, Department of Molecular Biology, Martin, Slovakia*

As important components of the sympathetic nervous system, α-adrenergic receptors (α-AR) modulate cardiovascular (CV) function and the changes in their function may play a role in the initiation and progression of CV diseases.

The aim of present study was to evaluate the relation between α-AR genetic polymorphisms and autonomic nervous system (ANS) function at rest or during orthostatic stress in young healthy adolescents. Subjects were genotyped for α1A-AR (rs1048101) and for α2A-AR (rs1800544) polymorphisms and underwent evaluation of ANS function using basic cardiovascular and hemodynamics measures: R-R interval, systolic, diastolic and mean blood pressure (SBP, DBP, MBP, respectively), stroke volume, cardiac output, total peripheral vascular resistance, pre-ejection period and left ventricular ejection time. All measures were recorded noninvasively, continuously and simultaneously at each heart beat in supine rest, head-up tilt and supine recovery. Reactivity was

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evaluated by subtracting the mean value for pre-stress resting period from the mean for head-up tilt period.

Results revealed significant associations between α1A-AR and α2A-AR polymorphisms and several basic CV measures. Reactivity of SBP, DBP and MBP to head-up tilt were found to be associated with α1A-AR polymorphism.

Our findings demonstrate that genetic variations in the alpha-adrenergic receptors are associated with the alterations in sympathetic cardiac control indicating their potential role in the association between AR genetic variations and CV diseases initiation and progression.

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D01-9

Influence of thioacetamide administration on autonomic control of the heart atria in rats

E. Mistrova[1], D. Jarkovska[1], M. Bludovska[2], D. Kotyzova[2], V. Krizkova[3], T. Kubikova[1], M. Chottova Dvorakova[1]
[1]*Faculty of Medicine in Pilsen, Charles University, Biomedical Center, Pilsen, Czech Republic*
[2]*Faculty of Medicine in Pilsen, Charles University, 3Department of Pharmacology and Toxicology, Pilsen, Czech Republic*
[3]*Faculty of Medicine in Pilsen, Charles University, Department of Histology and Embryology, Pilsen, Czech Republic*

Cardiac dysfunction is one of the complications of liver cirrhosis. During chronic heart failure persistent sympathetic hyperactivity was observed. Its effect is supported by sympathetic cotransmitters, e.g. neuropeptide Y (NPY) and its Y1receptor (Y1R). The calcitonin receptor-like receptor (CRLR) forms with RAMP molecules specific receptor to bind other heart neuropeptides. Release of additional neurotransmitters can have deleterious consequences for cardiac function.

Adult female Wistar rats (n=7) received thioacetamid (TAA, 200 mg/kg i.p), three times a week for 12 weeks. The rats were decapitated in 4 weeks after the end of TAA application. Blood, heart and liver samples were taken and compared with controls (n=7). In the serum enzymes were estimated, such as alanine transaminase (ALT), aspartate transaminase (AST) and glutamate dehydrogenase (GLDH) to evaluate the hepatic injury. Lipid peroxidation (LP), reduced glutathione (GSH) and activities of glutathione peroxidase (CPx), glutathione reductase (GR) and catalase (CAT) were determined in liver and kidney tissue homogenates. Relative expression of NPY, Y1R and CRLR were measured in in the left and right atrium.

Administration of TAA significantly increased liver enzymes activity. The markers of oxidative damage were significantly decreased in liver. Significant increase of LP was found in kidneys. Expression of NPY an Y1R remained unchanged. In TAA induced hepatic fibrosis the expression of CRLR in the left atrium was upregulated, which could be one of cofactors contributing to autonomic dysregulation.

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D01-10

Effects of Sertraline in Healthy and Damaged Rat Aorta

A. Koç[1], Z. I. SOLAK GORMUS[1], H. Solak[1], R. Ozen Koca[1], Z. Sahin[1], N. Gormus[1], S. Kutlu[1]
[1]*Necmettin Erbakan University, Physiology, Konya, Turkey*

AIM: Selective serotonin reuptake inhibitor group anti-depressant drugs are commonly used in patients with cardiovascular disorders. However, their effects on rat aorta are not investigated particularly experimentaly. We aimed to investigate the effect both on healthy and damaged rat aorta.

MATERIAL AND METHODS: 24 Wistar albino rats in 2 groups (n=24). Group 1 aorta-intact endothelium (n=12), group 2 aorta-damaged endothelium (n=12). Descending thoracic aorta was isolated after cervical dislocation. Aorta tissues were cleaned sectioned into 3-4 mm long rings. Rings were placed in organ baths containing Krebs solution, thermoregulated at 37 0C and aerated (95 % O2 and 5% CO2). Changes in isometric tension of aorta rings were recorded using a four channel force displacement transducer. Phenylephrine (PE 10-6M) was applied contractions were recorded in both groups. Then sertraline (SE 50 mg) was given cumulatively (10-9 -10-4 M) to group 1. In group 2 aorta damage was achieved by tearing endothelium with needle. After controlling endothelial damage by applying acetylcholine (Ach 10 -6 M), damaged strips were washed for one hour and second dose of PE was administered, then SE was given cumulatively to group 2, contractions were recorded.

RESULTS: After SE was given cumulatively to group 1, significant inhibition of spontaneous contractions was noticed in SE 10-9, 10-8, 10-7 doses ($p<0.05$), inhibition of contractions kept on in the rest of SE doses. SE 10-6 - -4 made significant inhibition in contractions according to 10-9 -8 -7 . Group 2 inhibition of second PE contractions continued after SE doses, but it was less significant when compared with group 1 ($p<0.05$).

CONCLUSION: Sertraline may be safely used in patients with aorta disease.

D01-11

Possible Effects of Sertraline on Human Heart Muscle Contractility: An in vitro experimental study

H. SOLAK[1], Z. I. SOLAK GORMUS[1], R. OZEN KOCA[1], A. KOC[1], A. KARAIBRAHIMOGLU[1], S. KUTLU[1], N. GORMUS[1]
[1]*NECMETTIN ERBAKAN UNIVERSITY, Meram Faculty of medicine, Physiology Department, KONYA, Turkey*

AIM: Patients who underwent cardiac surgery may have depression in the early postoperative period and they may need treatment with anti-depressant drugs. In this study we aimed to investigate the in vitro effects of sertraline on human atrium muscle contractility.

METHODS: Human atrium tissues (n=28) are taken from the cardiac surgery performed cases for coronary bypass surgery during the venous cannulation through right atrium appendage before the initiation of cardiopulmonary bypass. The patients age were between 47 to 72. All atrium tissues are placed into isolated organ bath and washed for 3 hours in order to diminish the effects of anaesthetic agents. Adrenaline 10-1 was administered in tissue cabs for producing isometric contractions. Contraction width measurements were used as contraction parameters. Cumulative sertralin (10-9 to 10-4) doses were added in organ baths. The contractions were recorded accordingly. Friedman and Kruskal Wallis tests were used for statistical evaluation.

RESULTS: Inhibition of contraction was statistically significant at the 10-7 M, 10-6 M, 10-5 M, 10-4 M doses of sertralin following the initial administration of adrenaline. Also statistically significant inhibition of contraction occured at 10-6 , 10-5 , 10-4 M doses when compared with 10-9, 10-8, 10-7 M doses of sertralin.

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CONCLUSIONS: Results of this study showed that sertralin which is a selective serotonin re-uptake inhibitor agent, may cause negative inotropic effects on human atrium muscle. This negative effect is markedly seen when sertralin doses are augmented. Therefore, sertralin may be carefully used in cardiac surgery performed patients , especially in the early postoperative period.

D01-12

Cerebral oxygenation in Metabolic Syndrome patients during mental task and muscles metaboreflex activation: a preliminary study

S. Roberto[1], A. Doneddu[1], V. Pinna[1], R. Lecis[1], M. Guicciardi[1], F. Velluzzi[1], S. Vanni[1], G. Sainas[1], S. Magnani[1], A. Crisafulli[1]
[1]*University of Cagliari, Dipartment of Medical Sciences and Public Health, Cagliari, Italy*

Introduction.
Muscles metaboreflex activation increases sympathetic tone and it is a well established method to study cardiovascular adjustments to exercise as well as to reveal cardiovascular diseases[1,2]. Our hypothesis was that, in patients suffering from Metabolic Syndrome (MS), cerebral oxygenation decreased during the metaboreflex elicited by post-exercise muscle ischemia (PEMI) method, ought to an exaggerated increase in sympathetic tone in these patients[3]. Furthermore, we hypothesized that, in MS patients, during the metaboreflex cerebral oxygenation was further impaired by superimposing a mental task (MT).

Methods.
15 subjects (7 females, age 33-58 yrs.) suffering from MS took part in this investigation. They were free form any known sympathetic dysfunction. Patients underwent 5 different tests each lasting 12 min: PEMI, control exercise recovery (CER), PEMI+MT, CER+MT, and MT alone. During each sessions, cerebral oxygenation was detected by Near Infrared Spectroscopy (NIRS) by applying sensors to the skin of the forehead.

Results.
Data analysis showed that during MT test, cerebral oxygenation was significantly reduced with respect to the other tests. No reduction was found in cerebral oxygenation during PEMI. The MT test added to the PEMI test did not induced any significant decrease in cerebral oxygenation.

Conclusions.
From our results it can be concluded that in patients with MS adding MT to PEMI does not impair cerebral oxygenation. It remains to ascertain whether the response to this tests is similar in healthy subjects.

[1]Roberto et al. J Appl Physiol 2012 113: 1323-31.

[2]Roberto et al. J Appl Physiol 2017 122: 376-385.

[3]Crisafulli and Roberto Curr Diabetes Rev 2017 (at press)

D01-13

A method for isolation of functional human ventricular myocytes from fresh epicardial biopsies

J. Marinovic[1], C. Bulat[2,1], M. Cavar[1], D. Bakovic[3,1], M. Ljubkovic[1]
[1]*University of Split School of Medicine, Physiology, Split, Croatia*
[2]*Split University Hospital, Cardiac Surgery, Split, Croatia*
[3]*Split University Hospital, Cardiology, Split, Croatia*

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Most of basic research findings in the field of cardiac cell physiology rely on data gathered in cells obtained from various animal models or tissues other than human ventricle (atrial myocardium or derived from iPS cells). Although these approaches offer possibilities of mechanistic investigations, they still have many limitations leading to difficult translatability to real-life human and clinical situations. We developed a protocol for isolation of functional cardiac myocytes from small epicardial biopsy samples of human left ventricle obtained during open-chest coronary artery-bypass grafting surgery.

Biopsy was performed in patients undergoing such surgery who gave their informed consent. Biopsy samples weighing 5-10 mg were immersed in ice-cold BDM-supplemented cardioplegia solution, cut to 350 mm pieces and incubated in Joklik medium containing trypsin and Liberase TM enzymes, and gently shaken for approximately 60 min at 37 °C. Viable rod-shaped cardiomyocytes appear after 45 min of digestion. Afterwards, 1 ml of cell suspension is transferred to enzyme-blocking solution and settled for 10 min. This step is repeated every 5 min until tissue pieces become completely dissolved. Joklik medium is then replaced with Tyrode solution and calcium is gradually added to 1.2 mM. In the end, calcium-tolerant cardiomyocytes were loaded with Fura-2AM and were able to contract upon electrostimulation and exhibit stable calcium transients.

Cardiomyocytes obtained by the described procedure could be used for other purposes, such as immunocytochemistry, patch clamping, contractility assessment, etc. The procedure of sample collection is straightforward, safe for patients and is not limited to specific type of cardiac surgery.

D02: Vascular physiology

D02-1
Crowding stress results in long-term vascular and behavioral alterations of in prehypertensive rats

I. Bernatova[1], A. Puzserova[1], P. Balis[1], N. Sestakova[1], M. Kluknavsky[1]
[1]*Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia*

This study determined long-term influence of social stress on vascular function and behavior in young male borderline hypertensive rats (BHR) and Wistar-Kyoto rats (WKY). Five-week rats were exposed to crowding stress for two weeks and then returned to control conditions for two weeks. Systolic blood pressure (BP) of five-week BHR was significantly increased vs. WKY (127±3 vs. 104±3 mmHg, $p<0.01$). Crowding significantly accelerated BP increase and elevated plasma corticosterone vs. controls only in BHR, which persisted two weeks post stress. Maximal acetylcholine-induced relaxation of the femoral artery was unchanged by stress yet a significantly reduced nitric oxide (NO)-dependent component of relaxation was detected in BHR two weeks post stress in agreement with reduced aortic NO synthase activity. Stress reduced NO production in the cerebellum brain stem and hypothalamus in both rat strains, which persisted during post stress period. In stressed BHR, open arm distance traveled in elevated plus maze was increased vs. age-matched controls without changes in total distances traveled, which was not seen in WKY. In conclusion, data showed that exposure to social stress in peripubertal period led to long-term elevation of plasma corticoserone, reduced NO production, acceleration of the early development of hypertension and delayed behavioral alterations only in rats with borderline elevated blood pressure. Study was supported by tgrants Nos. APVV-0523-10 and VEGA 2/0160/17.

D02-2
Enhanced inhibition of endothelial cell proliferation and migration by multikinase inhibitor and blocking of metabolism

J. Horvathova[1], R. Moravcik[1], M. Zeman[1]
[1]*Faculty of Natural Sciences, Comenius University , Department of Animal Physiology and Ethology , Bratislava 4, Slovakia*

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Angiogenesis contributes to pathophysiological processes such as inflammation and cancer and therefore several antiangiogenic therapies have been established. Blocking of angiogenesis signals has become an attractive strategy. Metabolism of endothelial cells is an important regulator of angiogenesis and therefore glycolysis represents another promising target. We investigated the efficiency of combined approach for inhibition of glucose metabolism together with blocking of angiogenesis by multikinase inhibitor. Effects were evaluated on the basis of proliferation and migration of endothelial cells and postreceptor signaling pathways underlying both processes.

Human umbilical vein endothelial cells (HUVEC) were treated with multikinase inhibitor (sunitinib) and inhibitor of glucose metabolism 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) alone or in combination. Inhibition of HUVECs proliferation was tested by MTT assay and migration was evaluated by wound healing assay. Signaling pathways were analyzed by western blot analysis.

3PO had an inhibitory effect on cell migration and proliferation and sunitinib suppressed both processes in a dose dependent manner. Combined treatment with sunitinib and 3PO had additive suppressive effect on HUVECs migration and proliferation as compared to cells treated with individual inhibitors.

We showed stronger effects of simultaneous inhibition of glucose metabolism together with multikinase inhibitor treatment on reduction of migration and proliferation of endothelial cells *in vitro*. Results provide a basis for identification of new pathways, which are promising for effective inhibition of angiogenesis.

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D02-3
CHOLINESTERASES IN RAT AORTA

K. Szmicseková[1], Z. Kiliánová[1], D. Dingová[1], J. Vetešková[1], J. Šranková[1], L. Piváčková[1], P. Křenek[1], A. Hrabovská[1]
[1]*Faculty of Pharmacy of Comenius University, Dpt. of Pharmacology and Toxicology, Bratislava, Slovakia*

QUESTIONS. The endothelium-dependent vasodilatory effect of acetylcholine (ACh) is well described, but only little is known about ACh-hydrolyzing cholinesterases (ChE) in blood vessels. Therefore, the aim of our project was to examine the presence of ChE in rat aorta, and to describe their possible physiological functions. **METHODS.** Thoracic aortas isolated from 12 weeks old male Wistar rats were used. Modified Karnovsky and Roots method was used for staining of ChE in the present of selective inhibitors for acetylcholinesterase (AChE), BW284C51 or butyrylcholinesterase (BChE), iso-OMPA. Immunohistological experiments were performed with polyclonal anti-AChE and monoclonal anti-BChE antibodies prepared in our laboratory. Commercially available specific markers of smooth muscle (anti-MLCK) and endothelium (anti-e-NOS) were used. Expression of AChE and BChE were analyzed using qRT-PCR. Effect of BChE on the ACh – induced vasodilatation in isolated aorta was observed by adding iso-OMPA. **RESULTS.** Activity staining, immunohistochemistry and qRT-PCR methods confirmed presence of AChE and BChE in rat thoracic aorta, with precise localization in the smooth muscle. Interestingly, BChE was clearly predominant in rat aorta. Selective inhibition of BChE by iso-OMPA lead to decrease of ACh-induced vascular relaxation in comparison to control aorta. **CONCLUSION.** We conclude that BChE is the dominant ChE in rat aorta and plays an important role in the regulation of the vascular tone.

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D02-4

Levamisole, a cocaine adulterant, impairs acetylcholine dependent relaxation in the rabbit renal artery.

S. Guerra-Ojeda[1], P. Marchio[1], M. Gimeno-Raga[1], M. Aldasoro[1], S. L. Valles[1], A. Jordá[1], C. Aldasoro[1], M. D. Mauricio[1], J. M. Vila[1]
[1]*University of Valencia, Department of Physiology, Valencia, Spain*

Questions: Cocaine induces endothelial dysfunction mainly by reducing nitric oxide (NO) production. Levamisole, increasingly used as cocaine adulterant, produces vasculitis, but its effects on vascular tone are not fully investigated.

Methods: Rabbit renal rings were mounted for isometric tension recording in organ baths. Concentration-response curves to acetylcholine (Ach) were obtained in the absence and presence of levamisole, cocaine and the combination of both drugs. Furthermore, Ach curves were performed in the presence of L-NG-Nitroarginine methyl ester (L-NAME, 10^{-4}M) and superoxide dismutase (SOD, 200 U/L) to study NO-dependent vasorelaxation and ROS production.

Results: Relaxant response to Ach was reduced by both cocaine (10^{-5} and 10^{-4}M) and levamisole (10^{-3}M). Levamisole blockade was higher than that induced by cocaine (pD2 6.6±0.1 for levamisole 10^{-3}M vs 7.5±0.2 for cocaine 10^{-4}M, $p<0.05$). Cocaine plus levamisole did not potentiate the response of levamisole. Reduced Ach response in the presence of L-NAME was not modified by cocaine whereas levamisole further potentiated the blockade. SOD completely prevented the effects induced by cocaine but partially those induced by levamisole.

Conclusions: Cocaine impairs endothelium-dependent relaxation by reducing NO bioavailability that could be related to increased ROS production. Decreased Ach-response to levamisole is independent of NO and could be in part mediated by oxidative stress.

D02-5

Acute adrenergic effects of levamisole, a cocaine adulterant, in rabbit carotid artery.

S. Guerra-Ojeda[1], P. Marchio[1], M. Gimeno-Raga[1], M. Aldasoro[1], S. L. Valles[1], M. D. Mauricio[1], C. Aldasoro[1], A. Jordá[1], J. M. Vila[1]
[1]*University of Valencia, Department of Physiology, Valencia, Spain*

Questions: Cocaine effects on vascular tone are primarily mediated by an enhancement of the sympathetic activity. Levamisole, a cocaine adulterant, blocks the reuptake of noradrenaline in cell cultures, potentiating the actions of cocaine. However, the synergistic effects of cocaine and levamisole in vessels have not yet been evaluated.

Methods: Rabbit carotid rings were mounted for isometric tension recording in organ baths. Concentration-response curves to phenylephrine (Phe) (10^{-9}-$3x10^{-5}$M) were obtained in the absence and presence of levamisole (10^{-5}-10^{-3}M), cocaine (10^{-5}-10^{-4}M) and the combination of both drugs. Electrical field stimulation response was obtained at 8 Hz in the absence and presence of levamisole (10^{-6}-10^{-3}M), cocaine (10^{-6}-10^{-4}M) and the combination of cocaine (10^{-4}M) plus levamisole (10^{-6}-10^{-3}M).

Results: Cocaine 10^{-4}M potentiated the contractile response to Phe (pD2=5.7±0.1 vs 6.0±0.1, control vs cocaine $p<0.05$). Levamisole 10-3 M reduced the contractile response to Phe (pD2= 5.7±0.1 vs 5.1±0.1, control vs levamisole $p<0.05$) that was not modified in the presence of cocaine. Both cocaine (10^{-5}-10^{-4}M) and levamisole (10^{-6}-10^{-4}M) produced a concentration-dependent potentiation of EFS (26±2% for control, 43±3% for cocaine (10^{-4}M) and 52±5% for levamisole (10^{-4}M). This effect was further increased by combination of cocaine 10^{-4}M plus levamisole 10^{-4}M (60±3%, $p<0.05$). Levamisole 10^{-3}M abolished the contractile response to EFS either alone or in the presence of cocaine.

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Conclusions: Levamisole potentiates the cocaine sympathetic response. However, at higher concentrations, levamisole acts as an alpha1-adrenergic antagonist and abolish adrenergic neurotransmission, pointing to a toxic effect.

D02-6

Protein expression of HIF-1 alpha, VEGF and cyclooxygenases in cerebral blood vessels of Sprague-Dawley rats on a short-term high salt diet

Z. Mihaljević[1], A. Ćosić[1], N. Bilić-Dujmušić[1], L. Prenek[2], P. Engelmann[2], M. Baus Lončar[3], I. Drenjančević[1]
[1]*Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Department of Physiology and Immunology, Osijek, Croatia*
[2]*University of Pecs, Institute of Immunology and Biotechnology, Pecs, Hungary*
[3]*Ruđer Bošković Institute, Department of Molecular Medicine, Zagreb, Croatia*

Question: Our previous study showed increased oxidative stress and impaired vascular reactivity in cerebral blood vessels of rats on high salt diet (HSD). Increased oxidative stress can affect HIF-1α expression and its downstream proteins, such as COX-2. Purpose of this experiment was to determine effect of HS diet on protein expression of HIF-1α, its target protein VEGF and COX-1 and COX-2 in cerebral blood vessels.

Methods: 11 week old Sprague-Dawley rats (N=8 per group) were divided in three groups: CTRL (control group of rat); HSD (rats on HS diet for 7 days, 4% NaCl) and HSD+TEMPOL (rats on HS diet and 1mM TEMPOL in drinking water for 7 days). Prior to decapitation and surface cerebral blood vessels collection, rats were anesthetized with ketamin-chlorid (75 mg/kg) and midazolam (0.5 mg/kg). Protein levels of HIF-1α, VEGF and COX-1 and COX-2 were assesed. One-way ANOVA or student t-test was used when appropriate (SigmaPlot v11.2, Systat Software, Chicago, USA), $p<0.05$ was considered significant. All experimental procedures conformed to the European Guidelines for the Care and Use of Laboratory Animals (directive 86/609) and were approved by the local and national Ethical Committee.

Results: Results show significantly increased expression of HIF-1alpha transcriptional factor and VEGF in HSD groups compared to CTRL and HSD+TEMPOL group. There was no differences in COX-1 expression. Expression of COX-2 was increased in HSD group compared to HSD+TEMPOL group.

Conclusion: Increased protein levels of HIF-1alpha and COX-2 in brain blood vessels may be related to increased oxidative stress caused by high salt diet and reveresed by TEMPOL in vivo.

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D02-7

REMODELING OF CORONARY ARTERY NETWORK DURING QUERCETIN SUPPLEMENTATION

F. Lonyi[1], A. Monori-Kiss[1], G. Pasti[1], E. Monos[1], G. Nadasy[2]
[1]*Semmelweis University, Institute of Clinical Experimental Research, Budapest, Hungary*
[2]*Semmelweis University, Department of Physiology, Budapest, Hungary*

Objective: Polyphenols, including quercetin, present in human diet, have various physiological effects. Short term vasodilatory actions on coronary arterioles have been demonstrated by us, but no information is available concerning their long term effects on microvascular networks.

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Methods: Male Wistar rats were divided into two groups. In group Q, animals were treated with 30 mg/kg quercetin per os (n=9), while group C was kept in parallel (n=10) for 8 weeks. Animals were sacrificed, and left descending coronary artery network was prepared down to 80 μm inner diameter. Orifice was cannulated, and continuous saline flow and input pressure were maintained during recording. Pictures of the network were analyzed digitally.

Results: Quercetin treatment reduced hemodynamically disadvantageous components in the network: branching angles under 45° or over 105° were less frequent in group Q (9% vs. 23%, $p<0.05$). Segmental tortuosity decreased (11.0±0.1% vs. 9.4±0.1%, $p<0.01$), similar to the frequency of broken courses (13.5±0.6 vs. 1.0±0.5 pcs, $p<0.01$). At bifurcations, asymmetry of daughter branches decreased (2.3±0.2 vs. 1.7±0.1, $p<0.03$). Vessel wall thickened beyond 400 μm arterioles ($p<0.05$). Network tortuosity, an indicator of order in coronary networks, was decreased as an effect of quercetin treatment (11.5±0.2% vs. 9.7±0.1%, $p<0.01$).

Conclusions: Chronic administration of quercetin resulted in reduced number of hemodynamically disadvantageous sections of the coronary networks. It is assumed that quercetin can delay non-beneficial vascular remodeling caused by e.g. long term hemodynamic stress, or ageing.

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D02-8

Age-related changes in endothelial function of pulmonary arteries in an experimental model of essential hypertension

A. Puzserova[1], A. Zemancikova[1], P. Balis[1], J. Radosinska[2,3], I. Bernatova[1], M. Kluknavsky[1], M. Kvandova[1], J. Torok[1]
[1]*Institute of Normal and Pathological Physiology SAS, Bratislava, Slovakia*
[2]*Institute of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia*
[3]*Institute for Heart Research SAS, Bratislava, Slovakia*

Questions: The spontaneously hypertensive rat (SHR) is the commonly used model of essential hypertension (EH). Hypertension in SHR may progresses to heart failure (HF). Hypertrophy of the right heart ventricle (RHV) is a reliable marker of the HF. The failing SHR become less active. SHR can also serve as a model of pulmonary hypertension due to left ventricular dysfunction, but a detailed functional study of the pulmonary arteries (PA) has not been carried out. The aim of this study was therefore to analyze the endothelium-dependent relaxation (EDR) of the PA in the young adult (20-week-old) and old (52-week-old) male SHR compared with WKY rats.

Methods: Blood pressure (BP) was determined by tail-cuff and locomotor activity using the open-field. EDR of large PA and small mesenteric arteries (SMA) was investigated in the presence and absence of nitric oxide (NO) synthase inhibitor.

Results: In both SHR group, BP and locomotor activity was increased. Biometric analyses indicated left ventricular hypertrophy, but not hypertrophy of the RHV in both SHR groups. EDR was significantly reduced in SMA of adult SHR vs. WKY, which was not observed in the PA. We observed NO-related endothelial dysfunction (ED) in the PA in old SHR.

Conclusions: Results demonstrated ED in the PA of the old SHR, however without signs of HF. Thus, ED in the PA may not be associated with RHV hypertrophy and/or HF in SHR.

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D02-9

Tissue oxygenation modulates muscle compression-induced hyperaemia

A. Messere[1], W. Franco[2], D. Maffiodo[2], G. Ceravolo[2], C. Ferraresi[2], S. Roatta[1]
[1]*University of Turin, Neuroscience, Turin, Italy*
[2]*Politecnico di Torino, Mechanical and Aerospace Engineering, Torino, Italy*

The characteristic of skeletal muscles to develop a rapid hyperemia in response to muscle compression was recently pointed out by several studies. Controversial are the hemodynamic effects of repeated compressions, reported in the literature. The issue is relevant for medical treatments aimed at improving local circulation by repetitive tissue compression, e.g., intermittent pneumatic compressions (IPC).

The present study aims at investigating changes of compression-induced hyperemia upon repetitive stimulation. Leg mechanical compressions were delivered at different inter-stimulus intervals (ISI, ranging from 20 to 160 s) through a customized IPC device based on five pneumatic cuffs wrapped around the lower part of right limb of ten healthy subjects. A comprehensive approach was employed, including continuous monitoring of tissue oxygenation and blood volume in calf muscles by near infrared spectroscopy (NIRS), as well as simultaneous eco-Doppler monitoring of femoral artery inflow and vein outflow.

The results indicates that: i) a long lasting increase in local tissue oxygenation follows the compression-induced hyperemia; ii) the amplitude of the hyperemia is not associated with the amount of blood volume displaced by the compression iii) the hyperemia in response to the repetitive stimulation exhibits different patterns of attenuation depending on the ISI; iv) the extent of the attenuation of the hyperemic response to each single compression correlates with the extent of oxygenation already present in the tissue ($r=-0,78$, $P<0.05$).

Conclusions: i) increased tissue oxygenation limits the hyperemia in response to repetitive compressions; ii) NIRS is a useful tool for monitoring the outcome of IPC treatments.

D02-10

Different structural alterations in conduit arteries of spontaneously hypertensive rats compared to Wistar rats from the prehypertensive period to late adulthood

F. Kristek[1], M. Drobna[1], S. Cacanyiova[1]
[1]*Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia*

Systolic blood pressure (BP) and structure of thoracic aorta, carotid and iliac artery of spontaneously hypertensive rats (SHRs) were assessed during ontogeny. Wistar rats and SHRs aged 3, 17, and 52 weeks were used. BP was measured by plethysmography weekly. The vascular system was perfused with a fixative under the pressure of 90 mm Hg in 3 and 120 mm Hg in 17- and 52-week-old rats. The middle parts of the arteries were processed for electron microscopy. The wall thickness (intima+media) (WT), cross-sectional area (intima+media) (CSA), inner diameter (ID), and WT/ID were measured in light microscopy. The volume densities of endothelial cells (ECs), muscle cells (SMCs), and extracellular matrix (ECM) were measured in aorta using electron microscopy, and their CSAs were calculated. In 3-week-old SHR compared to Wistar rats, the BP did not differ; in the thoracic aorta, all of the parameters (WT, CSA, ID, WT/ID, CSA of SMCs, CSA of ECs, and CSA of ECM) were decreased; in the carotid artery, WT and CSA did not differ, ID was decreased, and WT/ID was increased; in the iliac artery, WT, CSA, WT/ID were increased, ID was decreased. In 17- and 52-week-old SHRs, the sBP and all of the above parameters in all arteries were increased, only ID in iliac artery in 52-week-old SHRs and CSA of ECs in the aorta in 17-week-old SHRs did not change. The study revealed that in some conduit arteries of SHRs, (i) morphological changes are present in the prehypertensive period, and (ii) there is a positive correlation but quantitative disproportional relationship between BP increase and structural alterations during ontogeny. Different modes of

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alteration can reflect the flexibility of the arterial tree to the different needs of supplied areas. Supported by VEGA 2/0048/17, APVV-15-0565.

D02-11

Quercetin supplementation moderates hypertension induced remodeling of coronary artery network

A. Monori-Kiss[1], F. Lonyi[1], L. Danics[1], E. Monos[1], G. Nadasy[2]
[1]*Semmelweis University, Institute of Clinical Experimental Research, Budapest, Hungary*
[2]*Semmelweis University, Department of Physiology, Budapest, Hungary*

Aims: Previously, we demonstrated beneficial acute and chronic effects of quercetin on coronary network properties and function. In this work, it was studied if chronic administration of quercetin could influence network remodeling of coronary vessels induced by experimental hypertension.

Methods: Male Wistar rats were divided into two groups. HQ group was treated with quercetin (30 mg/kg), while HC group was kept as parallel control. After 28 days, osmotic minipumps were implanted to infuse angiotensin II (200 ng/min/kg) for 28 days. At the end of treatment, animals were sacrificed, left descending coronary with its ramifications were prepared down to 80 µm and perfused. Using computerized videomicroscopy, network properties were analyzed offline.

Results: Quercetin treatment resulted in decreased blood pressure (161±7/122±6 vs. 137±10/110±10 mmHg, $p<0.05$). Segmental tortuosity of coronaries decreased in HQ group (1.3±0.19 vs. 0.4±0.06%, $p<0.01$), vessel wall slightly thickened over 200 µm inner diameter ($p<0.01$). Proximally, extremely widened (600-800 µm) artery segments appear in HC group, while distally more resistance size vessels were found (100-300 µm) in HQ group ($p<0.01$). Hemodinamically disadvantageous branching angles were less frequent in HQ group (24 vs. 16 % of total). Network tortuosity, an indicator of disorganization, decreased in HQ group (14±1 vs. 10±0.1%, $p<0.01$). Number of vascular anomalies decreased in HQ group (6.8±0.7 vs. 3.6 ±0.7 pcs, $p<0.01$).

Conclusion: Chronic quercetin treatment moderates angiotensin induced hypertension and disadvantageous remodeling of coronary artery network.

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D02-12

Exploring the murine microvascular response variability to hyperoxia with the wavelet transform

H. Silva[1,2], H. Ferreira[3], A. P. Gadeau[4], L. Monteiro Rodrigues[1,2]
[1]*CBIOS / Universidade Lusófona, Lisboa, Portugal*
[2]*Universidade de Lisboa / Faculty of Pharmacy, Pharmacol. Sc. Dep., Lisboa, Portugal*
[3]*Universidade de Lisboa / Faculdade de Ciências, IBEB, Lisboa, Portugal*
[4]*Université de Bordeaux & INSERM, U1034, Adaptation cardiovasculaire à l'ischémie, Pessac, France*

Oxygen inhalation is widely used to dynamically assess microcirculation in vivo, and it is known to produce variable responses. Laser Doppler flowmetry (LDF) is a reference technique to quantify in vivo microcirculation, providing an oscillatory signal with several frequency components, whose components are still, however, poorly described in murine. Our aim was to describe the components of the murine cutaneous microcirculation using the wavelet transform (WT). 16 male C57/BL6 mice (18 ± 10 weeks old) were subjected to a 100% oxygen breathing protocol under ketamine-xylazine anesthesia, consisting in three phases – 10 min room atmosphere breathing, 10 min 100% oxygen breathing, and 10 min recovery. LDF signals were recorded on both hind paws. All procedures involving animal experimentation were ethically supervised. Two different vascular responses to hyperoxia were observed – perfusion decrease (PD) in 20/32 paws and perfusion increase (PI) in 12/32 paws. WT allowed the identification of several LDF frequency ranges, compatible with the cardiac (5.3-4.6 Hz), respiratory (3.8-3.2 Hz), myogenic (0.17-0.059 Hz), sympathetic (0.052-0.020 Hz), endothelial NO dependent (0.017-0.0094 Hz) and endothelial NO independent (0.0084-0.0042 Hz). The animals that responded with PD showed a significant increase in the cardiac and myogenic activities and a significant decrease in the NO-dependent activity. The animals that responded with PI showed a significant increase in the cardiac and myogenic activities, with no significant changes in the endothelial components. These results show the murine LDF frequency ranges, and also highlight the usefulness of the wavelet transform for the characterization of microvascular reactivity to hyperoxia.

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D03: Molecular & cellular physiology

D03-1

The Role of Palmitoylation in Glutamate-Mediated Excitotoxicity in Neurodegenerative Diseases.

S. Wordie[1]
[1]*University of Edinburgh, Centre for Integrative Physiology, Edinburgh, United Kingdom*

Chronic neurodegenerative disorders such as Alzheimer's and Huntington's disease are associated with neuronal degeneration due to glutamate-mediated excitotoxicity as a result of dysfunctional proteins in the glutamatergic tripartite synapse. Palmitoylation controls several synaptic proteins in physiological glutamate neurotransmission but the role of palmitoylation in neurodegenerative diseases is unknown.

An integrated approach was taken to review the published literature and to make novel predictions to determine whether synaptic protein palmitoylation was disrupted and contributed to excitotoxicity in neurodegenerative diseases, with a focus on proteins already implicated in these disorders.

The major conclusion drawn is that disrupted palmitoylation to proteins throughout the tripartite synapse contributes to the mechanisms controlling excitotoxicity in Huntington's disease. The majority of synaptic proteins implicated in disease are predicted to be palmitoylated. Therefore, aberrant palmitoylation in each component of the tripartite synapse is predicted to affect homeostatic glutamate neurotransmission.

This research develops our understanding of the role of palmitoylation in neurodegenerative disease. Palmitoylation is disrupted in Huntington's disease which highlights a novel therapeutic target in the treatment of this disorder. But further research is necessary to establish whether aberrant palmitoylation is a common pathological mechanism in other life-limiting, neurodegenerative diseases.

D03-2

Cellular Calcium Balance in Chronic Kidney Disease

I. Lajdova[1], V. Spustova[1], A. Oksa[1], D. Chorvat[2], M. Morvova Jr.[3], L. Sikurova[3], A. Marcek Chorvatova[2,4]
[1]*Slovak Medical University, Department of Clinical and Experimental Pharmacotherapy, Bratislava, Slovakia*
[2]*International Laser Centre, Department of Biophotonics, Bratislava, Slovakia*
[3]*Comenius University, Department of Nuclear Physic and Biophysics, Bratislava, Slovakia*
[4]*University of Ss. Cyril and Methodius, Department of Biotechnology, Trnava, Slovakia*

Questions: Calcium cations (Ca^{2+}) are an important ubiquitous messenger, controlling a broad range of cellular processes. Disturbances in cellular calcium homeostasis in patients with chronic kidney disease (CKD) represent a complex process which aggravates with CKD progression. We aimed to

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investigate the state of cellular Ca^{2+}, Ca^{2+} entry via Ca^{2+}/cation channels and Ca^{2+} removal through plasma membrane Ca^{2+}-ATPases (PMCA) in the blood cells of CKD patients.

Methods: The study involved 22 healthy volunteers and 22 CKD stage 2-3 patients. Cytosolic Ca^{2+} measurements were performed by Fluo-3 fluorimetry. To examine the Ca^{2+} entry via calcium release activated calcium (CRAC) channels, an inhibitor of these channels, was applied. To determine the function of $P2X_7$ receptors, the antagonist and agonist of these receptors were used. The activity of PMCA was determined by UV-VIS spectrofotometry.

Results: Cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and Ca^{2+} concentration of intracellular stores of CKD patients was higher in comparison with healthy subjects. The Ca^{2+} entry via CRAC channels were increased in CKD patients. Activation of $P2X_7$ receptors caused an increase in $[Ca^{2+}]_i$ in both groups, but the effect was significantly smaller in CKD patients, likely by reason of higher $[Ca^{2+}]_i$ under basal conditions. Inhibition of $P2X_7$ receptors reduced $[Ca^{2+}]_i$ in CKD, but had no effect in healthy subjects. The activity of PMCA, was found to be decreased by 25 % when compared to healthy subjects.

Conclusions: Our results demonstrate that all these alterations inCa^{2+} signaling are contributing to the elevated $[Ca^{2+}]_i$ from early stages of CKD.

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D03-3

An Investigation into the Effects of Extracellular Acidification on Mouse Uterine Contractions; Are ASICs involved?

A. Almohanna[1], S. Wray[1]
[1]*University of Liverpool, ITM, Liverpool, United Kingdom*

Adequate uterine contraction is crucial to safe delivery of the foetus. Acidity can play an important role in affecting myometrial contraction but the effects, especially concerning external acidification, are unclear. This study was designed to determine the effects of acidic pHo on myometrium and any gestational differences, and to examine ASICs as possible mediators to the effects. Methods: Mouse uterus was used either on day 14 or 19 (term) of gestation. Changes in pHo to 6.9 were made for 10 minutes, by adding HCl to physiological saline. Spontaneous contractions were measured and data analysed. Statistical differences were tested with Student's t test and taken at P<0.05. Western Blotting was done following the protocol used previously in our lab. All assays. Mouse brain and urinary bladder were used as a control for ASICs expression. Results: At *14 days*, extracellular acidification to pH 6.9 significantly increased AUC, mainly due to an increased frequency and amplitude. At *19 days*, extracellular acidification to 6.9 also significantly increased AUC, frequency and amplitude (P value<0.05). Application of repetitive 10 min episodes of acidification (pHo 6.9) caused sequential increase in the amplitude of the force on term myometrium, but not in 14 days pregnant mouse. Western blotting showed ASICs 2a and 3 proteins were expressed in the mouse myometrium. Discussion: In mouse, a decrease of pHo stimulated pregnant uterine contraction. Extracellular acidification significantly increased the amplitude in term pregnant mouse more than mid-pregnant uterus which suggests a possible role of external acidification in labour. ASICs have been reported, and thus may contribute to increases in force via depolarization and increased Ca entry

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D03-4

The effects of the luteal cells cocultured with islet cells on cell viability and functionality in rats*

A. A. Yigit[1], G. Boyuk[1]
[1]*Kirikkale University/Faculty of Veterinary Medicine, Physiology, Kirikkale, Turkey*

Questions: It was aimed to evaluate the islet cell viability and functionality by coculturing the islet cells with luteal cells.

Methods: After isolation of luteal cells from the superovulated corpus luteum, they seeded to wells to attach the bottom of the wells and incubated for 24 hours. Islet cells were isolated at 24th hours of incubation of luteal cells. Then luteal cells and islet cells cultured separately or together by coculturing for 96 hours. Islets viability and glucose stimulation tests performed and stimulation index (SI) was calculated and total insulin secretion was determined in the islet medium and coculture medium at 48th and 96th hours.

Results: It was observed that islets viability increased significantly (p<0.05) at 48th h of the incubation in the cocultured group as compared to the only islet group. Islets viability was also increased at the 96th h in the cocultured group, but this increase did not found to be significantly. Islet functionality and insulin secretion were increased (P<0.05) in the cocultured group as compared to the only islet groups both 48th and 96th hours.

Conclusions: Although islet cells were incubated up to 48 h to transplant in routine procedure; in this study, viability and functionality of islet cells were maintained up to 96 hours, by coculturing with luteal cells and increased insulin secretion. These results suggest that the coculturing of islets cells with luteal cells may help the induction of insulin secretion after transplantation, by maintaining the viability and functionalities of the islets and it is extremely important for transplantation success.

Keywords: coculture, islet cell, luteal cell

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D03-5

Role and transcription profiles of α1 and β2 adrenergic receptors in tissues of yellow and silver European eels

E. Fabbri[1], P. Valbonesi[1], A. Kiwan[2], S. Franzellitti[1]
[1]*University of Bologna, BIGEA, Bologna, Italy*
[2]*Univ of Bologna, CIRI, Ravenna, Italy*

Adrenergic receptors (AR) belong to the wide family of G-protein coupled receptors (GPCR) and specifically bind catecholamines, i.e. epinephrine and norepinephrine. According to mammalian classification, there are two main groups of AR, α and β, with several subtypes: α1 (a Gq coupled receptor) and α2 (a Gi coupled receptor); β1, β2 and β3 (all coupled to Gs proteins). Pharmacological ligands were of limited help to demonstrate the presence and role of α and β subtypes in tissues from teleost fish, while further insights have recently been provided thanks to molecular approaches. In the present study, we evaluated the glucose output from isolated hepatocytes of yellow (feeding phase) and silver (sexually developing and migrating phase) European eels (*Anguilla anguilla*), treated with epinephrine. The hormone induced a time and dose-dependent glucose output, which appeared to be slightly different between yellow and silver eels. Major differences were instead observed in AR transcription profiles.The expression of α1 and β2 AR was evaluated by qPCR in different tissues of eels. Expression *α1-AR* mRNA was in the range of tens copies/ng RNA in liver, brain, gills and muscle of yellow eels, with values about 10-fold higher in the liver of silver eels. Expression of *β2-AR* mRNA in liver, brain, gills and muscle of yellow eels was in the range of hundreds of copies/ng RNA, with

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values about 5-fold higher in the liver of silver eels. Differently, expression of *α1-* and *β2- AR* mRNA in the heart was about 8-fold lower and about 2-fold higher in silver versus yellow eels, respectively.

D03-6

A new animal model for epithelial ion transport modeling (focusing on CFTR) – wild type ferrets

E. Tóth[1], J. Maléth[1], P. Pallagi[1], V. Venglovecz[2], Z. J. Rakonczay[3], P. Hegyi[4,5]
[1]*University of Szeged, First Department of Medicine, Szeged, Hungary*
[2]*University of Szeged, Department of Pharmacology and Pharmacotherapy, Szeged, Hungary*
[3]*University of Szeged, Department of Pathophysiology, Szeged, Hungary*
[4]*University of Szeged, MTA-SZTE Momentum Translational Gastroenterology Research Group, Szeged, Hungary*
[5]*University of Pécs, Institute for Translational Medicine/1st Department of Medicine, Pécs, Hungary*

Cystic fibrosis (CF) is a lethal genetic disease affecting several organs, including the pancreas. Several animal models are available to study the CF related tissue damage but they have clear limitations. Recently a cystic fibrosis transmembrane regulator (CFTR) knock out ferret model was generated, they born with a normal pancreas, but, within a short period they develop a CF related pancreatic damage. This model would be the first available one to study pharmacological prevention of the disease development.

Intra/interlobular pancreatic ducts were isolated from the WT ferret pancreas. Expression of CFTR was detected by immunohistochemistry. Resting pH, buffer capacity and Cl-/HCO3—exchange activity were evaluated by microfluorometry. Buffer capacity was calculated by measuring ΔpHi in response to different concentrations of NH4Cl-/HCO3—pulses in Na+-free solutions. Fluid secretion was examined by video microscopy.

CFTR was expressed on the luminal membrane of ferret pancreatic ducts. The resting intracellular pH of pancreatic epithelial cells is lower (7.17±0.08) in ferrets compared to mice (7.31) or to guinea pigs (7.36). Concerning the bicarbonate influx mechanisms, functionally active sodium/hydrogen exchanger and sodium/bicarbonate cotransporter were detected. Anion exchanger activity measured by NH4Cl-technique, Cl- removal and inhibitory stop methods indicated that ferret pancreatic ducts secrete similar amount of bicarbonate as mice and guinea pigs. Video microscopy revealed a significant increase in fluid secretion to HCO3— and to 5μM forskolin stimulation.

Major epithelial ion transporters are expressed in the ferret pancreatic ductal epithelial cells. Our results indicate that ferret could be a suitable model organism to study the CF style pancreatic damage.

D03-7

Interactions of cyclic adenosine monophosphate production and store operated Ca2+ entry

J. Fanczal[1], T. Madácsy[1], P. Hegyi[2], S. Muellem[3], J. Maléth[1]
[1]*University of Szeged, 1st department of medicine, Szeged, Hungary*
[2]*University of Pécs,, Institute for Transl. Med. &1st Dep. of Medicine, Pécs, Hungary*
[3]*NIDCR, NIH, Epithelial Signaling and Transport Section, Molecular Physiology and Therapeutics Branch, Bethesda, Maryland, United States*

Introduction. The cyclic adenosine monophosphate (cAMP) and Ca2+ signaling play central role in the regulation of the secretory functions of epithelial cells. The two signaling system were reported to localized to specific intracellular microdomains and have multiple synergistic interactions helping to optimize the cellular response to stimulation. One of the recently described interactions suggest connection between the store operated Ca2+ entry with adenylyl cyclase 8 (AC8) that increase cAMP production, however the molecular mechanism of this process is not known.

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Aim. We aimed to characterize the interactions of SOCE with cAMP.

Methods. Human embrional kidney (HEK) cells were transfected with plasmids encoding the proteins of interest. Cellular cAMP production was measured by fluorescence resonance energy transfer (FRET) using the cAMP reporter Epac1.

Results. The stimulation of the cells with 5μM forskolin and 100μM 3-isobutyl-1-methylxanthine (IBMX) resulted in reversible elevation in cAMP production. The expression of AC8 significantly elevated the cAMP response. The overexpression of the endoplasmic reticulum (ER) Ca2+ sensor Stim1, or the Ca2+ channel Orai1 (components of SOCE) increased the cAMP production upon stimulation. The effect of Stim1 and Orai1 was not depending on the ER Ca2+ content, or extracellular Ca2+ influx. Extended synaptotagmin 1 (E-Syt1), a recently described ER-plasma membrane tethering protein, increased the cAMP response, similarly to Stim1-Orai1.

Conclusions. Our results showed the interaction of SOCE and cAMP production that might play an important role in the regulation of cAMP production. However further studies are required to clarify the mechanisms of the interaction.

D03-8

INVESTIGATION OF PROTECTIVE EFFECT OF PARIETIN AGAINST GLUTAMATE EXCITOTOXICITY IN PRIMARY CORTICAL NEURON CULTURE

G. GUNDOGDU[1], A. TAGHIZADEHGHALEHJOUGHI[2], O. SENOL[3], B. CICEK[1], K. A. NALCI[4], A. HACIMUFTUOGLU[4]
[1]*Atatürk University , Medical Science /Physiology, Erzurum, Turkey*
[2]*Ataturk University, Veterinary Medicine, Erzurum, Turkey*
[3]*Ataturk University, Faculty of Pharmacy - Analytical Chemistry,, Erzurum, Turkey*
[4]*Ataturk University , MEdical Science - Pharmacology, Erzurum, Turkey*

QUESTION: Glutamate is one of the neurotransmitters in the central nervous system. Glutamate accumulation can excessively activate the *N*-methyl-D-aspartate receptors and cause excitotoxicity. In this study, the neuroprotective activity of parietin in primary cortical neuron cultures against glutamate excitotoxicity was evaluated.

METHOD: Primary cortical neuron cultures prepared from cerebral cortices of newborn rats. Cultures were exposed to 10^{-5} M glutamate to induce glutamate excitotoxicity. Then, different concentrations of (final concentrations in the well to be 2.5-500 μM) parietin were added into the medium. Cell survival in different parietin groups were measured by MTT assay. TAS-TOS analyses were used to evaluate reactive oxygen species generation. Obtained data were evaluated by using One-way anova with post-hoc LSD test using SPPS 20.0 software.

RESULT: In our study, IC50 (inhibitory concentration where 50% of the cells die) of parietin was determined to be 10 μM at the 24th hour cortical neuron culteres by MTT assay. TAS assay results demonstrated that 10 μM of parietin increased the antioxidant level in cells, which might help to protect neurons against glutamate induced exitotoxicity. TOS assay result exhibited that 10^{-5} M glutamate and higher doses of parietin increased oxidant level in cells inducing stress to neurons.

CONCLUSION: In this study; Parietin showed high neuroprotective effect in primary rat cortical neuron culteres against glutamate excitotoxicity. These results suggest that parietin can be used as a therapeutic agent for glutamate excitotoxicity, however, further studies are needed to clarify the mechanism/s of action of parietin.

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D03-9

Association analysis between A163G and T245G gene polymorphisms of osteoprotegerin and bone mineral density in Turkish postmenopausal women

O. PALABIYIK[1], F. OZDEMIR[2], B. TOKUC[3], T. SIPAHI[1], D. DEMIRBAG KABAYEL[4]
[1]*Trakya University Faculty of Medicine, Depertment of Biophysics, Edirne, Turkey*
[2]*Biruni Universty Faculty of Medicine, Depertment of Pyhsical Medicine and Rehabilitation,, ISTANBUL, Turkey*
[3]*Trakya University Faculty of Medicine, Public Healty, Faculty of Medicine, Edirne, Turkey*
[4]*Trakya University Faculty of Medicine, Depertment of Pyhsical Medicine and Rehabilitation, Edirne, Turkey*

Purpose: One of the most significant nominee genes for osteoporosis predisposition is Osteoprotegerin (OPG). The objective of this study was to evaluate the relationship between the A163G and T245G polymorphism in the OPG promoter with bone mineral density (BMD) of postmenopausal women.

Method and Materials: We found 194 voluntary subjects containing 109 with primary postmenopausal osteoporosis and 85 healthy people as controls. The BMD of L1, L4, total lumbar spine (L1-4), neck hip and total hip were evaluated by dual-energy X-ray absorptiometry (DEXA). The A163G and T245G polymorphisms in the OPG promoter were determined by using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Result and Diccussion: The frequencies of genotypes were AG+GG (46.8%), AA (53.2%) for A163G and GG+TG (36.7%), TT (63.3%) for T245G polymorphisms in postmenopausal women. When compared with healthy women, remarkably more women with osteoporosis were found to have AG+GG (p=0.005) and GG+TG (p=0.049). An important association of genotypes with BMD at the lumbar spine (p=0.027, p=0.019, p=0.024, and p=0.003, repectively) was observed for T245G and A163G polymorphisms in postmenopausal women and controls, respectively. Genotypes GG+TG were related with lower BMD as compared with TT genotype for T245G polymorphisms. Similarly A163G polymorphisms, genotypes AG+GG were also related with lower BMD as compared with AA genotype.

Conclusion: Our results suggest that T245G and A163G polymorphisms in the OPG promoter might make a contribution to the genetic regulation of BMD. These results will be beneficial to examine the role of OPG gene in osteoporosis in studies to be carried out in the future.

Key words osteoprotegerin, osteoporosis, gene polymorphism, bone mineral density, polymerase chain reaction.

D03-10

Cell penetrating protein C inhibitor (PCI): Internalization, nuclear translocation, and potential intracellular targets

M. Geiger[1], **H. Yang**[1], M. Furtmüller[1], B. Sokolikova[1], F. Wahlmüller[1]
[1]*Medical University Vienna, Department of Vascular Biology and Thrombosis Research, Vienna, Austria*

PCI is a secreted serine protease inhibitor (serpin) with very broad protease reactivity and tissue distribution. Binding of glycosaminoglycans and phospholipids modulates its inhibitory activity and target enzyme specificity. We have shown that PCI can be internalized by cells most likely by directly crossing the phospholipid bilayer. Inside the cell it translocates to the nucleus. Currently we are analyzing the exact mechanism of PCI internalization and its intracellular role. N-terminally cleaved PCI as well as N-terminally truncated, functionally actice PCI-mutants are not internalized by cells. Testisin, a glycosylphosphatidylinositol-anchored serine protease, which co-localizes with PCI in the testis, cleaves PCI not only at its reactive site but also at a site close to its N-terminus. This N-terminal cleavage releases a peptide rich in basic amino acids which was recognized as cell-penetrating peptide. Local proteases might therefore regulate the cellular uptake of PCI. With cultured cells we observed uptake of PCI by only 10-15% of cells. Therefore there must be also hitherto unidentified cellular mechanisms regulating its internalization.

We have shown that PCI contains a functional nuclear localization signal in its H-helix. Analysis of subcellular fractions revealed that PCI is mainly found in the nuclear envelope fraction, where it interacts with cathepsin L. Enrichment of cathepsin L in the nucleus has been observed in cancer cells and in some transformed cell lines. Several substrates of nuclear cathepsin L have recently been identified, such as histone H3, 53BP1, and the transcription factor CUX1. Internalized, nuclear PCI may therefore be involved in the regulation of epigenetic modifications and/or in cell cycle progression.

D03-11

Significance of co-expression of transient receptor potential vanilloid 4 and aquaporin5 in pregnant uterine contractility in rats

E. Ducza[1], A. Csányi[1], î Szőke[2], Z. Tiszai[1], R. Gáspár[1]
[1]*University of Szeged, Szeged, Hungary*
[2]*University of Pécs, Pécs, Hungary*

Questions: The expression of aquaporin5 (AQP5) is under multifactorial control (e.g. steroids and oxytocin). AQP5 has a crucial role in cellular osmotic changes. The increased AQP5 expression is followed by reduced uterine contractility. The transient receptor potential vanilloid 4 (TRPV4) is a Ca2+-permeable cation channel activated by osmotic changes. Our aim was to investigate the co-expression and cooperation of AQP5 and TRPV4 in pregnant uterine contractions in rat.

Methods: Reverse-transcriptase PCR and Western blot techniques were used to detect the changes in AQP5 and TRPV4 expression in pregnant rat uteri. The uterine contractions were measured in an isolated organ bath system with selective TRPV4 agonist (RN1747). Immunohistochemical method was used to determine the localization of AQP5 and TRPV4.

Results: The TRPV4 expression continuously increased from day 5 to the last day of pregnancy. The maximum of AQP5 expression was detected at day 18 of pregnancy, but it was practically disappeared at day 22. The TRPV4 and AQP5 co-expressed on pregnancy days 18 and 20. On day 22, however, TRPV expression was proved, only. The TRPV4 agonist did not influence the uterine contractions on pregnancy day 18, but it increased the activity at day 22.

Conclusion: Presumably AQP5 causes hypertonic state in the cells that inhibits the TRPV4 function. The lack of AQP5 expression at the end of pregnancy triggers a hypotonic stress which activates the TRPV4 and uterine contraction.

Key words: AQP5, TRPV4, pregnancy

D03-12

Sex, age and weight as determinants of plasma DNA: a cross-sectional study

B. Konecna[1], V. Lenka[1], L. Janovičová[1], B. Vlková[1], P. Celec[1]
[1]*Comenius University, Bratislava, Slovakia*

Questions: Extracellular DNA (ecDNA) is studied in many diseases as a possible marker but also as a trigger of an immune response important for the pathogenesis, both, in clinical studies and in animal experiments. The aim of our study was to describe the interindividual variability of ecDNA in plasma of healthy mice and to analyze potential determinants of the variability including sex, age and bodyweight.

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Methods: For this experiment 78 adult CD1 mice (41 female and 37 female) of a variable age (126-493 days) and bodyweight (22-55 g) were used. DNA was isolated from plasma. Total ecDNA concentration in plasma was measured fluorometrically. Nuclear ecDNA and mitochondrial ecDNA were quantified using real time PCR. Deoxyribonuclease activity was measured using SRED method. A simple correlation analysis was conducted with age and bodyweight.

Results: No gender differences were found in plasma ecDNA and its subcellular origin (ecDNA average values 51,68 ηg/ml of plasma for male and 52,67 ηg/ml of plasma for female) or in DNase activity (0,0398 KU/μl for male and 0,0391 KU/μl for female). Correlations were found for total, nuclear and mitochondrial ecDNA neither with bodyweight, nor with age of the mice.

Conclusions: The results suggest that the interindividual variability of ecDNA in laboratory mice is with a coefficient of variability of 138% high. Sex, age and bodyweight seem not to affect plasma ecDNA in mice. The major contributors to the variability are yet to be identified in future studies taking into account the endogenous deoxyribonuclease activity.

D03-13

The role of aquaporin-4 e isoform in the regulation of rapid cell volume changes in astrocytes

M. Lisjak[1], **M. Potokar**[1,2], B. Rituper[1], J. Jorgačevski[1,2], R. Zorec[1,2]
[1]*Laboratory of Neuroendocrinology – Molecular Cell Physiology, Institute of Pathophysiology, University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia*
[2]*Celica BIOMEDICAL, Ljubljana, Slovenia*

Water channel aquaporin 4 (AQP4) plays a key role in the regulation of water homeostasis in the brain. It is predominantly expressed in astrocytes at blood-brain and blood-liquor interfaces. In the mammalian brain several AQP4 isoforms have been identified. Two of them (AQP4a (M1) and AQP4c (M23)) have been confirmed to cluster into plasma membrane supramolecular structures, termed orthogonal arrays of particles (OAPs) and to enhance water transport through the plasma membrane. However, the role of the newly described water conductive mammalian isoform AQP4e is unknown. The focus of our study was to learn the dynamics of AQP4e aggregation into OAPs and its role in the regulation of astrocyte water homeostasis. With super-resolution structured illumination microscopy we determined, that AQP4e isoform is co-localized in OAPs in rat astrocytes. We observed that in hypoosmotic conditions which elicit cell edema, OAP formation is considerably enhanced by overexpressed AQP4e. This suggests that AQP4e may be involved in regulatory volume changes in astrocytes. We tested this hypothesis by using atomic force and confocal microscopies. Our results revealed that AQP4e is key in regulating rapid changes of volume in astrocytes.

D06: Respiratory physiology

D06-1

Exogenous surfactant enriched with anti-IL-8 antibody additionally improved lung functions in experimental meconium-induced lung injury

P. Mikolka[1], J. Kopincova[1], P. Kosutova[1], M. Kolomaznik[1], A. Calkovska[1], D. Mokra[1]
[1]*Comenius University, Jessenius Faculty of Medicine, Department of Physiology and Biomedical Center Martin, Martin, Slovakia*

Introduction
Meconium aspiration syndrome (MAS) is associated with massive neutrophils influx into the lungs and resulting inflammation and oxidation which could inactivate surfactant and induce respiratory failure. We hypothesized that addition of anti-IL8 antibody to therapeutic surfactant could suppress neutrophil influx and inflammation what can contribute to improved lung functions and enhanced surfactant therapy in MAS.

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Methods
New Zealand rabbits with meconium-induced respiratory failure were divided to: non-treated (M), exogenous surfactant treated (M+S) and treated with combined surfactant and anti-IL-8 antibody (M+S+anti-IL-8) groups. Blood gases and ventilation parameters, i.e. PaO_2/FiO_2, oxygenation index (OI), ventilation efficiency index (VEI), mean airway pressure (MAP), dynamic lung compliance (C_{dyn}), alveolar-arterial gradient (AaG) and O_2 saturation ($SatO_2$), were observed before and 30 min after meconium instillation and at 30 min, 1, 2, 3, 4, 5 h of the therapy (Th). Total and differential leukocyte counts were determined in bronchoalveolar lavage fluid (BAL).

Results
Combined M+S+anti-IL-8 therapy significantly improved PaO_2/FiO_2, OI, VEI at 2h and 5h Th compared to surfactant ($p<0.05$ *vs.* M+S) and reduced count of neutrophils and monocytes in BAL ($p<0.01$ and $p<0.05$ *vs.* M). Other lung function parameters showed comparable effects for both therapies M+S and M+S+anti-IL-8 *vs.* M.

Conclusions
Addition of anti-IL-8 antibody to surfactant therapy improved lung functions and reduced neutrophils and monocytes in BAL. Targeted inhibition of neutrophil influx and neutrophil-induced inflammation/oxidation by anti-IL-8 antibody likely stabilized surfactant and enhanced therapy of MAS.

Support: VEGA 1/0305/14; VEGA 1/0469/16; APVV-0435-11; APVV-15-0075

D06-2

Effects of inhaled roflumilast and tadalafil on airway reactivity and inflammation in ovalbumin-sensitized guinea pigs

J. Mokry[1], A. Urbanova[1], M. Kertys[1], I. Medvedova[1], P. Mikolka[2], P. Kosutova[2], D. Mokra[2]
[1]*Jessenius Faculty of Medicine, Comenius University, Biomedical Center Martin and Department of Pharmacology, Martin, Slovakia*
[2]*Jessenius School of Medicine, Comenius University, Biomedical Center Martin and Department of Physiology, Martin, Slovakia*

Question: Phosphodiesterases (PDEs) are enzymes involved in degradation of cAMP and cGMP. The clinical effectiveness of selective PDE4 inhibitors has been confirmed previously in chronic obstructive pulmonary disease associated with neutrophil inflammation. The aim of this study was to evaluate effects of roflumilast (selective PDE4 inhibitor) and tadalafil (selective PDE5 inhibitor) on *in vivo* and *in vitro* airway smooth muscle reactivity to histamine, changes in blood cells count in blood and bronchoalveolar lavage fluid, and some inflammatory mediators (IL-4 and IL-5) in ovalbumin sensitized guinea pigs with experimentally induced eosinophil inflammation.

Methods: Sensitized animals were exposed to aerosol of roflumilast, tadalafil, or both for 3 minutes once daily for 7 days.
Results: Sensitization with ovalbumin led to a significant increase in *in vivo* and *in vitro* airway reactivity. Similarly, increased plasmatic levels of IL-4, and IL-5 in blood were observed, with significant increase in differential counts of eosinophils both in blood and BAL fluid.

Conclusions: The administration of roflumilast and tadalafil suppressed significantly *in vivo* airway reactivity, *in vitro* airway reactivity in tracheal tissue strips, and IL-4 and IL-5 levels, suggesting that PDE4 and PDE5 inhibition has potent anti-inflammatory effects in guinea pigs with ovalbumin induced eosinophil inflammation.

Keywords: phosphodiesterase, inhibitors, airway reactivity, interleukin, inflammation

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D06-3

Comparison of three types of lung-protective ventilation in an experimental model of meconium aspiration syndrome

D. Mokra[1], P. Mikolka[1], P. Kosutova[1], M. Kolomaznik[1], K. Matasova[2], M. Zibolen[2], A. Calkovska[1]
[1]*Jessenius Faculty of Medicine, Comenius University, Biomedical Center Martin and Department of Physiology, Martin, Slovakia*
[2]*Jessenius Faculty of Medicine, Comenius University and University Hospital Martin, Clinics of Neonatology, Martin, Slovakia*

Questions: Ventilation support in severe meconium aspiration syndrome (MAS) is realized with lung-protective techniques: conventional mechanical ventilation (CMV), high-frequency oscillatory ventilation (HFOV), or high-frequency jet ventilation (HFJV). This experimental study compared effects of three ventilation modes on the lung functions and inflammation in a model of MAS to estimate their efficacy.

Methods: Model of MAS was induced in young rabbits by intratracheal instillation of meconium suspension (25 mg/ml, 4 ml/kg). After induction of respiratory distress, meconium-instilled animals were ventilated either with CMV, HFOV, or HFJV (n=7 in each group) for additional 4 h, n=6 of healthy non-ventilated animals served as controls. Parameters of ventilation and gas exchange were measured regularly. At the end of experiment, lung edema formation was determined from wet-dry lung weight ratio. Concentrations of proinflammatory cytokines (TNFα, IL-1β, IL-8) were measured in the plasma and lung tissue homogenates by ELISA methods.

Results: Meconium instillation decreased lung compliance, deteriorated gas exchange, and triggered inflammation and edema formation. All ventilations supplied sufficient gas exchange without clear differences in the lung functions, edema formation, or cytokine concentrations between the groups, with slightly better results observed in HFOV group.

Conclusions: All tested lung-protective ventilations exerted effective gas exchange and nearly comparable impact on the lung tissue, with a slight preference for HFOV.

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D06-4

Oxidative and inflammatory modifications in the extra-pulmonary organs associated with primary acute lung injury

S. Rezáková[1], P. Mikolka[1], J. Kopincová[1], P. Košútová[1], A. Čalkovská[1], D. Mokrá[1]
[1]*Comenius University, Jessenius Faculty of Medicine , Department of Physiology and Biomedical Center Martin, Martin, Slovakia*

Questions: Acute lung injury (ALI) is characterized by diffuse lung damage, inflammation, edema, and surfactant dysfunction leading to hypoxemia. Severe ALI patomechanisms could also accelerate progression of extra-pulmonary organs injury, but it´s poorly known. Therefore this study evaluated oxidative and inflammatory modifications of heart and liver.

Methods: ALI was induced in rabbits by 1) intratracheal instillation of meconium (Mec-ALI), 2) repetitive saline lung lavage (Lav-ALI) and compared with healthy controls (C), all ventilated for 5 hours. Concentrations of markers of inflammation (TNFα; galectin-3, Gal-3), oxidative damage to lipids (thiobarbituric acid reactive substances, TBARS) and proteins (3-nitrotyrosine, 3NT), vascular damage (receptor for advanced glycation end products, RAGE) in heart and liver homogenates were determined.

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Results: In ALI groups, significantly increased levels of the mentioned markers were observed compared to controls, i.e. in the heart: TBARS (p<0.05 Mec-ALI); 3-NT (p<0.05 Mec-ALI, p<0.02 Lav-ALI); TNFα (p<0.04 Mec-ALI, p<0.004 Lav-ALI); Gal-3 (p<0.03 Mec-ALI); RAGE (p<0.01 Mec-ALI, p<0.04 Lav-ALI); in the liver: TBARS (p<0.04 Mec-ALI); 3-NT (p<0.04 Lav-ALI); TNFα (p<0.03 Mec-ALI, p<0.01 Lav-ALI); Gal-3 (p<0.04 Mec-ALI, p<0.03 Lav-ALI).

Conclusions: In both ALI models, elevated markers of inflammation, oxidative and vascular damage in heart and liver were observed compared to controls. Severe ALI showed direct effect on extra-pulmonary organs.

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D06-5

Exogenous superoxide dismutase in the surfactant treatment of experimental meconium aspiration syndrome

J. Kopincova[1], P. Mikolka[1], P. Kosutova[1], M. Kolomaznik[1], A. Calkovska[1], D. Mokra[1]
[1]*Comenius University, Jessenius Faculty of Medicine, Department of Physiology and Biomedical Center Martin, Martin, Slovakia*

Production of reactive oxygen species is important pathway in meconium aspiration syndrome (MAS) pathogenesis. Oxidative stress causes bronchoconstriction, impairs endogenous and exogenous surfactant and destructs pneumocytes. Intratracheal administration of superoxide dismutase (SOD) has proved anti-inflammatory effects in MAS. However, coaction of SOD and surfactant in the treatment of MAS has not been studied yet. Thus, we wanted to verify if SOD addition would improve surfactant therapy of MAS.

MAS had been induced in young New Zealand rabbits of 2.36 ± 0.12 kg of body weight by intratracheal meconium application. One group was left without the treatment (Mec; n=8), the treated groups were administered an exogenous surfactant (Curosurf® – Surf; n=8) or surfactant combined with SOD (Surf+SOD; n=8). Animals were ventilated for 5 hours by FiO_2 1.0. Respiratory parameters were recorded, and *post mortem* IL-1β, IL-6 and TBARS in lung tissue homogenate and total and differential leukocyte count in bronchoalveolar lavage fluid (BALF) was evaluated.

SOD addition into surfactant preparation led to strong, but transient betterment in all measured respiratory parameters (PaO_2/FiO_2, OI, VEI, $PaCO_2$, AaG), with significant improvement compared to surfactant monotherapy seen in some of them. After combined therapy, IL production was decreased compared to solely surfactant, while no effect was seen in total and differential leukocytes in BALF.

Although SOD therapy of MAS had strong effect in respiratory parameters when added to surfactant, it seems that the effect is just transient and SOD is being inactivated by meconium presence.

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D06-6

Effects a phosphodiesterase-4 inhibitor on the inflammation and oxidative stres in an experimental model of acute lung injury

P. Košútová[1], P. Mikolka[1], M. Kolomazník[1], A. Čalkovská[1], D. Mokrá[1]
[1]*Comenius University, Jessenius Faculty of Medicine, Department of Physiology and Biomedical Center Martin, Martin, Slovakia*

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Questions: Acute lung injury (ALI) is characterized by loss of alveolar–capillary membrane integrity, migration and activation of inflammatory cells into the lungs, and release of pro-inflammatory mediators. Phosphodiesterase (PDE)-4 is mainly expressed in inflammatory cells and its inhibition reduces action of inflammatory cells. The main goal of our study was to evaluate if systemic administration of selective PDE-4 inhibitor roflumilast influences inflammation and oxidative damage in saline lavage-induced model of acute lung injury.

Methods: ALI was induced by repetitive saline lung lavage (30 ml/kg) until arterial PO2 reaches values <26.7 kPa in oxygen ventilation. Animals were divided into 3 groups: animals with ALI model without therapy (ALI), animals with ALI treated with intravenous roflumilast (1 mg/kg; ALI+Rof), and healthy non-ventilated controls (Control). After 4 hours of ventilation, total and differential counts of cells in bronchoalveolar lavage fluid (BAL) were measured. Lung edema was expressed as wet/dry weight ratio. Concentrations of markers of oxidative stress (TBARS, 3NT), inflammation (TNFα, IL-6 and -8) were analysed in the lung tissue and plasma.

Results: Roflumilast therapy reduced leak of cells into the lung ($P<0.05$), mainly neutrophils ($P<0.05$) and monocytes/macrophages ($P<0.01$), lung edema formation ($P<0.05$), concentrations of markers of inflammation (TNFα, IL-6, and -8, $P<0.05$) and oxidative damage of lipids (TBARS, $P<0.01$).

Conclusion: Therapy with selective PDE-4 inhibitor roflumilast positively affected the inflammation and oxidative stress in lung tissue and plasma.

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D06-7

Exogenous surfactant reduces endotoxin-induced inflammation and oxidative stress in rat lungs

M. Kolomaznik[1], I. Zila[1], P. Kosutova[1], J. Kopincova[1], P. Mikolka[1], D. Mokra[1], A. Calkovska[1]
[1]*Comenius University, Jessenius Faculty of Medicine, Department of Physiology and Biomedical Center Martin, Martin, Slovakia*

Questions: Pulmonary surfactant is a lipoprotein complex situated at the air-liquid interface that may interfere with many substances. This causes decrease of its activity, with functional effects on the respiratory system. Lipopolysaccharide (LPS) is a major component of outer membrane of Gram-negative bacteria that interacts with structures of the lungs. The aim of the study was to investigate the influence of exogenous surfactant on acute lung injury induced by intratracheal instillation of LPS.

Methods: In adult rats (Wistar, 340±30g), lung injury was induced by intratracheal instillation of LPS (100, 500 or 1000 μg/kg of b.w.). Treated group received exogenous surfactant (Curosurf®) at dose 50 mg PL/kg and control received saline (for all 2.2 ml/kg b.w.). After 5 hours of artificial ventilation IL-1β, MCP-1, ANGPT2, surfactant protein A and oxidative stress (TBARS) were evaluated in homogenized lung (HL) tissue and bronchoalveolar lavage fluid (BALF). Lung edema was expressed as wet/dry weight ratio.

Results: Instillation of LPS at dose 500 and 1000 μg/kg caused formation of lung edema ($p<0.01$), increased levels of IL-1β ($p<0.01$), ANGPT2 ($p<0.05$) in HL and BALF and oxidative stress in HL (TBARS, $p<0.05$). The administration of surfactant decreased ANGPT2 in BALF ($p<0.05$ vs. LPS 500). Decrease of IL-1β, MCP-1 in BALF was not significant ($p>0.05$). Surfactant therapy also reduced the lung edema ($p<0.05$ vs. LPS).

Conclusions: Intratracheal administration of LPS leads to changes reminiscent of bacterial infection. Administration of exogenous surfactant reduces inflammation, edema formation and oxidative stress.

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D06-8

The effects of Simvastatin on Galectin-3 and TBARS in Lung Tissue during Endotoxemia

H. Yorulmaz[1], **G. ATES ULUCAY**[2,3], E. Kaptan[4], E. Ozkok[5], S. Tamer[3]
[1]*Halic University, Medical Faculty, Department of Physiology, Istanbul, Turkey*
[2]*Istanbul Yeni Yuzyil University, Faculty of Medicine, Department of Physiology, Istanbul, Turkey*
[3]*Istanbul University, Istanbul Medical Faculty, Department of Physiology, Istanbul, Turkey*
[4]*Istanbul University, Faculty of Sciences, Department of Biology, Istanbul, Turkey*
[5]*Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Neuroscience, Istanbul, Turkey*

Questions: Galectins constitute a soluble mammalian β-galactoside binding lectin family which play homeostatic roles in regulation of cell cycle and apoptosis, as well as display inflammatory and immune modulatory activities in various pathological conditions. Previous studies have implicated galectin-3 in regulation of various inflammatory conditions including endotoxemia and airway inflammation. Statins, a key precursor inhibitors of HMG-CoA reductase,may prevent development and progression of inflammation when prior statin treatment in sepsis. Endotoxemia causes formation of Thiobarbituric acid reactive substances (TBARS), the consequence of lipid peroxidation reactions by damaging of the cell membrane, and oxidation of proteins, carbohydrates, and DNA molecules. In this study, we aimed to show the effects of Simvastatin on Galectin-3 and TBARS in lung tissue of rats treated with Lipopolysaccharides (LPS) during the early phase of sepsis.

Methods: Rats were divided into four groups: control, LPS (20 mg/kg, i.p.), Simvastatin (20 mg/kg, p.o.), and Simvastatin+LPS group. Galectin (gal-3) expression in formalin fixed and paraffin embedded lung tissue sections showed by immunohistochemistry. The levels of TBARS were determined using thiobarbituric acid (TBA) assay and measured spectrophotometrically 532 nm.

Results and Conclusions: There were decreased densities and the number of the gal-3 immunoreactivities in Simvastatin+LPS group compared to LPS group in pneumocytes and bronchial epithelium of lung tissue. However TBARS levels were significantly decreased in LPS+Simvastatin group according to LPS group ($p<0.001$). In conclusion, simvastatin protective effect on lung tissue during endotoxemia.

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D06-9

The Effect of Splenectomy on Lung Inflammation in Rats: The Protective Role of Curcumin

G. Simsek[1], Y. Altınel[2], V. Sozer[3], P. Uysal[4], M. Altan[1], O. F. Sonmez[1], A. Oruc[1], A. Caglar[5], R. Gelisgen[6], C. Simsek[7], H. Uzun[6]
[1]*Istanbul University, Cerrahpasa Faculty of Medicine, Physiology, Istanbul, Turkey*
[2]*Bagcılar Education and Research Hospital, Surgery, Istanbul, Turkey*
[3]*Yıldız Technical University, Biochemistry, Istanbul, Turkey*
[4]*Acıbadem University, Faculty of Medicine Atakent Hospital, Chest Diseases, Istanbul, Turkey*
[5]*S.B.Bagcılar Education and Research Hospital, Pathology, Istanbul, Turkey*
[6]*Istanbul University, Cerrahpasa Faculty of Medicine, Biochemistry, Istanbul, Turkey*
[7]*Istanbul University, Cerrahpasa Faculty of Medicine, Istanbul, Turkey*

Spleen derived leukocytes and their mediators may have important antiinflammatory functions. In this case splenectomy may cause lung inflammation. NF-κB plays a critical role in inflammatory response. Curcumin is suggested to be a inhibitor of NF-κB. Thus we have investigated whether splenectomy causes lung inflammation and the protective effect of curcumin. In study female Wistar Albino rats was divided into four groups containing 8 in each; splenectomy, splenectomy+curcumin, splenectomy+corn oil and control. Curcumin was given intragastrically with corn oil for 28days after splenectomy.At the

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end, anesthetized rats were sacrificed and their lungs were removed. NF-κB, VEGF, PDGF, MDA; Cu-ZnSOD activity and GPx levels are determined in samples. Also samples were examined histopathologically. Non-parametric Mann-Whitney U test was used for statistical evaluation. In splenectomy group, NF-κB, VEGF, PDGF and MDA increased significantly ($p<0.01$) compared to control group and SOD and GPX decreased significantly ($p<0.05$).A significant decrease in inflammatory parameters and a significant increase in antioxidant parameters were detected in splenectomy+curcumin group.. Histopathologic findings suggest that splenectomy induces acute and chronic inflammation, however curcumin alleviates acute inflammation and reduces the formation of tertiary lymphoid follicles. Splenectomy may cause NF-κB-mediated inflammation. Curcumin may exhibit antiinflammatory properties with inhibition of NF-κB.

D06-10

Effect of lipopolysaccharide on alveolar epithelial type II cells

Z. Nová[1,2], D. Mokrá[1,2], E. Vidomanová[2], M. Kolomazník[1,2], H. Škovierová[2], E. Halašová[2,3], A. Čalkovská[1,2]
[1]*Jessenius Faculty of Medicine, Comenius University, Department of Physiology, Martin, Slovakia*
[2]*Biomedical Center Martin, Martin, Slovakia*
[3]*Jessenius Faculty of Medicine, Comenius University, Department of Medical Biology, Martin, Slovakia*

Introduction: Alveolar epithelial type II (ATII) cells have a critical role in the production of pulmonary surfactant which is required for a lung homeostasis and normal breathing. ATII cells can be damaged during lung inflammation caused by bacterial lipopolysaccharide (LPS). Exposure of ATII cells to this endotoxin could impair cell viability and modulate their ability to produce surfactant. However, impact of LPS on ATII cells is still not well known. Therefore this study evaluates the effect of LPS on ATII cells viability and function.

Methods: Human ATII cells were cultivated under standard conditions (Cell Biologics, USA). Different concentrations of LPS (E. coli 055:B5, Santa Cruz Biotechnology) 1, 5, 10, 15 and 20 μg/ml were added to ATII cells and incubated for 1, 4 and 24 hours. The survival of ATII cells was determined microscopically and by the biochemical method (MTT assay).

Results and conclusions: Our results indicate that cell viability was not affected with LPS in time and dose-dependent manners. Even so, the surfactant production still remain to be determined to prove the LPS effect on ATII cells function.

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D08: Behavioral and cognitive neuroscience

D08-1

How the Emotional Status of the Emoji Characters Affect the Process of Response Activation?

D. D. KOYUNCU[1], T. ERGENOGLU[1], G. CEYLAN[1]
[1]*Mersin University, Faculty of Medicine, Department of Physiology, Mersin, Turkey*

Introduction: Emoji characters allow people to express their concomitant feelings or answers quickly to other side through the digital communication. It makes us to wonder about whether the emotional content of the emoji characters has an impact on event-related potentials (ERP). We aimed to evaluate the effects of emoji characters on the process of response activation by using a visual go-nogo task.

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Methods: ERPs were recorded with 30 electrodes using a visual go-nogo task from 18 male healthy volunteers. Go and nogo stimuli were randomly presented with 1.5 s inter-stimulus intervals (ISI) and probabilities of 0.6 and 0.4, respectively. We designed a go-nogo paradigm including three types of go stimuli as positive, negative, and neutral emoji characters presented in equal percentages. A yellow circle which has exactly the same radius with go stimuli is set as nogo stimuli. The amplitudes and latencies of ERPs to each go stimuli groups were analyzed by repeated measure of ANOVA.

Results: The reaction time of the positive emoji icons was found to be significantly lower than both the negative and neutral emoji icons ($p=0.001$). The amplitude of the P3 potential in response to the positive emoji icons was significantly higher than the other two go stimuli groups ($p=0.001$).

Conclusions: Our results suggest that the response activation to the positive emoji icons is higher than both the negative and neutral emoji icons. During the evaluation processes of the positive emoji icons, more brain resources were used than the other two groups. Since the amplitude of the P3 potential reflects both the amount of the attentional resources used and the information which can be gathered from the stimuli.

Keywords: Emoji characters, go-nogo task, response activation

D08-2

The Effects of Preceding Context on the Processes of Response Inhibition in Healthy Adults

G. CEYLAN[1], **D. D. KOYUNCU**[1], T. ERGENOGLU[1]
[1]*Mersin University, Faculty of Medicine, Department of Physiology, Mersin, Turkey*

Introduction: The evaluation of event related potentials (ERPs) is one of the electrophysiological assessment methods can provide information of neural networks with a high temporal resolution. Both the response activation (go condition) and inhibition processes (nogo condition) in the brain can be analyzed with the go-nogo task. In this study, we aimed to investigate the effects of preceding context on response inhibition process by a visual go-nogo paradigm.

Methods: ERP data were recorded from 17 healthy volunteers (19.94±0.83 yrs) via 30 scalp electrodes in an electrically shielded, sound attenuated room. Go and nogo stimuli were pseudo-randomly presented with 0.3 s stimulus duration and the probabilities with 0.6 and 0.4, respectively. According to stimulus type (nogo or go with three different difficulty levels) of preceding nogo stimuli, they were divided into four groups. The averaged responses to nogo stimuli were analyzed by repeated measures of ANOVA for each nogo stimulus groups.

Results: It is observed that subjects made more commission errors to nogo stimuli if the preceding stimulus is a nogo stimuli ($p=0.001$). The amplitudes of P3 potentials of nogo responses were lower if the preceding stimuli are nogo stimuli ($p=0.003$). The amplitude of P3 potentials of nogo stimuli were higher if the preceding go stimuli are easier ($p=0.049$). The latency of P3 potentials of nogo stimuli were longer if the preceding go stimuli are more difficult ($p=0.001$).

Conclusion: Our results suggest that type and the difficulty level of preceding stimuli affected the amplitudes and latencies of nogo P3 potentials by modulating the response inhibition.

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D08-3

THE EFFICACY OF AUTISM-RISK SCREENING OF YOUNG CHILDREN IN SLOVAK POPULATION SAMPLE

H. Celusakova[1], S. Hnilicova[1], A. Kubranska[1], D. Filcikova[1], M. Vidosovicova[1], K. Babinska[1], D. Ostatnikova[1]
[1]*Comenius University Faculty of Medicine, Institute of Physiology, Bratislava, Slovakia*

Questions: Autism Spectrum Disorders (ASD) are neurodevelopmental disorders characterized by deficits in social communication and restrictive, repetitive behavior. The aim of our study was to assess efficacy of screening for ASD using *M-CHAT questionnaire* in a sample of Slovak high-risk population and compare it with the objective diagnostic tools such as *Autism Diagnostic Observation Schedule - second revision (ADOS-2)* and *Autism Diagnostic Interview - Revised (ADI-R)*.

Methods: Study population included 92 children, age 1,7 - 6, who were signed up by their parents for ASD testing at our institution during years 2013 to 2016. Children were screened for ASD by M-CHAT questionnaire filled by their parent, and then examined using ADI-R and ADOS-2.

Results: Significant difference of summary score of M-CHAT between group of children with ASD and without ASD, diagnosed using ADOS and ADI-R was observed ($p<0.001$). Two out of three diagnostic domains of ADI-R: scores of Abnormalities in Reciprocal Social Interaction and Abnormalities in Communication significantly correlated with total M-CHAT score ($p<0.01$). Total M-CHAT score correlated with overall rate of symptoms in ADOS ($p<0.05$) and as well as with Social Affect score ($p<0.01$) and Restricted and Repetitive Behavior scores ($p<0.01$).

Conclusions: Our results in the sample of high-risk children demonstrated that the M-CHAT score can be used to differentiate between children with and without ASD risk and that it correlates with objective measures for diagnosis of ASD, i.e. with ADOS and ADI-R. Further research is needed to determine the sensitivity and specificity of M-CHAT in ASD screening in general population of children in Slovakia.

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D08-4

Levels of Faecal Calprotectin Correlate With Behavioural Markers in a Sample of Individuals with Autism Spectrum Disorders from Slovakia

K. Babinska[1], A. Tomova[1], H. Celusakova[1], J. Babkova[1], G. Repiska[1], M. Vidosovicova[1], D. Filcikova[1], S. Hnilicova[1], D. Ostatnikova[1]
[1]*Comenius University, Faculty of Medicine, Institute of Physiology, Bratislava, Slovakia*

Questions: Autism Spectrum Disorders (ASD) are neurodevelopmental disorders which affect social interaction, communication and behaviour. There is an evidence that intestinal inflammation is involved in aetiology of ASD, and elevated levels of inflammatory markers are associated with more aberrant behaviours. Levels of faecal calprotectin (FC) reflect local inflammation of the digestive tract. The aim of the study was to assess concentrations of FC and their correlations with behavioural markers in a sample of subjects with ASD.

Methods: Concentration of FC was determined by ELISA method in 87 individuals with ASD, and 51 controls (29 siblings and 22 non-related controls).

Results: In non-related controls significantly lower values of FC were observed than in both subjects with ASD and their siblings. In the group with ASD, significant correlations of FC with all three main domains of the diagnostic tool ADI-R (Autism Diagnostic Interview-Revised) were found: qualitative abnormalities in social interaction and communication, restrictive and repetitive patterns of behaviour. Results indicate that higher levels of FC correlate with more serious behavioural impairments.

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Conclusions: Our results suggest that intestinal inflammation may be one of the factors implicated in the pathophysiology of ASD. More studies are needed to validate the utilization of faecal calprotectin as a diagnostic marker for ASD.

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D08-5

Study of Acylcarnitine Profile in Dry Blood Sample of Children with Autism Spectrum Disorders

M. Vidosovicova[1], K. Babinska[1], S. Hnilicova[1], G. Addova[2], R. Gorova[2], I. Waczulikova[3], L. Siklenkova[1], G. Lakostikova[1], D. Ostatnikova[3]
[1]*Comenius University, Faculty of Medicine, Institute of Physiology, Bratislava, Slovakia*
[2]*Comenius University, Faculty of Natural Sciences, Institute of Chemistry, Bratislava, Slovakia*
[3]*Comenius University, Faculty of Mathematics, Physics, and Informatics, Department of Nuclear Physics and Biophysics, Bratislava, Slovakia*

Questions. Autism spectrum disorders (ASD) are disorders of neurodevelopment characterized by deficits in social communication and interactions, and restricted pattern of behaviours. The specific aetiology of ASD is only partially explained, and it was proposed that ASD can be associated with mitochondrial and fatty acid oxidation disorders. Acylcarnitines (ACC) can be diagnostic for a number of ß-oxidation defects. The aim of this pilot study was to investigate the levels of acylcarnitines in dry blood samples of children with and without ASD.

Methods.A total of 59 subjects with ASD and 96 controls without ASD aged 2-17 years were included in the study. Dry blood sample was taken during office visit. Commercial kit Chromsystems@ was used for determination of ACC levels by the method of tandem mass spectrometry. Groupswere compared using analysis of variance.

Results. All patients except one showed normal ACC profiles, or ACC profiles nonspecific for a certain disorder. However, ACC species C0, C2, C8, C10, C10:1, C12, C14:1, as well as several of their ratios were scored as statistically significantly higher, and C5 significantly lower in children with ASD in comparison with children without ASD.

Conclusions. Significant differences in dry blood ACC of children with and without ASD were found that provide a basis for their further investigation as potential markers for an early diagnosis of ASD.

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D08-6

LOW DOSE CAFFEINE PROTECTS FROM PSYCHOLOGICAL STRESS AND IMPROVES COGNITIVE FUNCTION

O. Kasimay Cakir[1], **N. Ellek**[1], N. Salehin[1], R. Hamamci[1], H. Keles[1], D. G. Kayali[2], D. Akakin[2], M. Yuksel[3], D. Ozbeyli[1]
[1]*Marmara University, Physiology Department, Istanbul, Turkey*
[2]*Marmara University, Histology Department, Istanbul, Turkey*
[3]*Marmara University, Istanbul, Turkey*

INTRODUCTION
Caffeine is an adrenergic antagonist that enhances neuronal activity. Psychological stress depresses cognitive function.

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AIM
To investigate the effects of acute and chronic low dose caffeine on anxiety-like behavior and cognitive functions of acute or chronic psychological stressed rats.

METHODS
Acute or chronic caffeine (3mg/kg) was administered to male Sprague Dawley rats (n=42) before acute (cat odor) and chronic variable psychological stress (restraint overcrowding stress, elevated plus maze, cat odor, forced swimming) induction. Anxiety and cognitive functions were evaluated by hole-board and object recognition tests. The brain glutathione and malondialdehyde (MDA) assays, myeloperoxidase (MPO), nitric oxide (NO), superoxide dismutase (SOD), luminol and lucigenin activity and histological examination were done. ANOVA and Student's t-test were used for statistical analysis.

RESULTS
The depressed cognitive function with chronic stress exposure and the increased anxiety-like behavior with both stress inductions were improved via both caffeine applications ($p<0.05$-0.001). Both caffeine pretreatments in chronic stressed rats, and chronic caffeine in acute stressed ones reduced the elevated MPO activities ($p<0.05$-0.01). The increased MDA, lucigenin and NO levels with acute stress were inhibited with chronic caffeine ($p<0.05$-0.01), MDA and NO levels were declined by acute caffeine ($p<0.001$). The induced histological damage with both stress exposures was ameliorated with chronic caffeine.

CONCLUSION
The increased anxiety-like behavior and depleted cognitive functions under stress conditions were improved with both caffeine pretreatments by decreasing oxidative damage parameters.

D08-7

Long shift hours was associated with increased attention performance in pediatric registrars

P. Çakan[1], S. Yildiz[1]
[1]*Inonu University, Malatya, Turkey*

Cakan P, Yıldız S.
Inonu University, Faculty of Medicine, Department of Physiology, Malatya/TURKEY

Introduction: Long shift hours are generally thought to negatively affect working performance. Pediatric registrars normally have long continuing shifts lasting and this thought to affect their attention. Therefore, the aim of the current study was to assess the effects of long shifts on their performance in attention tests.

Materials and methods: Pediatric registrars who had 32 hours continuous shifts (n=9) were compared to nurses who had normal work hours (0800 to 16:00 h; n=10) in terms of attention tests. The study started at 08:00 h on Day 1 and continued until 17:00 h on Day 2. On both days of the study, an attention test was filled at 08:00 and 16:00 h. The test consisted of sequential numbers dispersed on an A4 paper and participants were asked to follow these numbers as quickly as they can by starting from number 1 to number 25. The time was recorded by using a chronometer. The data was analyzed by using t tests.

Results: Mean time for completing attention test was 22±1.5 seconds for pediatric registrars and 30±2.7 seconds for nurses who had normal day time work hours (P=0.023). Additionally, performance of both groups were increased by time (28.7±2.5 and 24.6±1.9 seconds for Day 1 morning and Day 2 evening, respectively, P=0.045).

Conclusions: Unexpected better performance in pediatric registrars might be due to a work-oriented attention, which may be associated with vital interventions in a susceptible pediatric population.

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D08-8

Influence of Green tea extract and Passiflora, on heart rate and fatigue sensation, in intense mental stress

R. N. Jurcau[1], I. M. Jurcau[2], N. A. Colceriu[3]
[1]*"Iuliu Hatieganu" University of Medicine and Pharmacy, Pathophysiology, Cluj-Napoca, Romania*
[2]*Emergency Clinical Hospital for Children, Pathology, Cluj-Napoca, Romania*
[3]*University of Agricultural Sciences and Veterinary Medicine, Viticulture, Cluj-Napoca, Romania*

Questions. Green tea (GT) and Passiflora (PS) are known for their anti-stress and adaptogen effects. The aim was assessing the influence of a GT extract product (GTP) and PS product (PSP), in intense mental stress, by measuring heart rate (HR) and fatigue sensation (FS).

Methods. The chosen subjects (n = 24 males) were organized into 4 groups: 1) control (C=6) no GTP, no PSP; 2) only with GTP (GT=6); 3) only with PSP (PS=6); 4) with both GTP and PSP (GTPS=6). Stress was represented by an verbal arithmetic mental effort. The analyzed indicators were HR and FS. Statistical evaluation was done on the basis of Student test.

Results. Comparding GTP and PSP separated administration, the two parameters were significantly reduced, post mental stress: HR, more intense after GTP (p=0.002), FS more intense after PSP (p=0.003). Compared to GTP and PSP, GTPS reduced more, post mental stress, both HR (GT-GTPS,p=0.03; PS=GTPS, p=0.01) and FS (GT-GTPS,p=0.02; PS=GTPS, p=0.04).

Conclusions. 1) Under the GTP respectively PSP influence, HR and the FS were significantly reduced. 2) There are differences between the GTP and PSP treated groups, both for the HR and FS evolution. 3) It has been proven that the GTPS effect is significantly higher than the GTP and PSP effects, on HR and FS. 4) We suggest the using of GTP and PSP combination, in mental stress modulation.

Key words: green tea, passiflora, stress, mental stress, heart rate, fatigue sensation

D08-9

Evaluation of the influence of Romanian product "Emotional comfort" on facial expressions impact, in acute physical stress

R. N. Jurcau[1], I. M. Jurcau[2], N. A. Colceriu[3]
[1]*"Iuliu Hatieganu" University of Medicine and Pharmacy, Pathophysiology, Cluj-Napoca, Romania*
[2]*Emergency Clinical Hospital for Children, Pathology, Cluj-Napoca, Romania*
[3]*University of Agricultural Sciences and Veterinary Medicine, Viticulture, Cluj-Napoca, Romania*

Questions. Facial expression of the public can influence mood and functional parameters of an athlete. The objective of this study was to evaluate the influence of "Emotional comfort" product (ECP), on the facial expressions (FE) impact in acute physical stress.

Methods. Untrained volunteers (n=12 men) were divided in group: a) without ECP (C=6); b) with ECP (EC=6). Chosen FE: neutral (N), disappointment (D) and encouragement (E), were played in three short films, while the subject was running on treadmill. Stress was represented by an intense short term physical effort on a treadmill Excite+Run MD, in three successive physical sequences (PS), in PS+N/ PS+D/ PS+E. Evaluated parameters: heart rate (HR); three positive emotions (energized EG, relaxed RL, in control IC). *Parameters assessments: 24h (T1) and 15 min (T2) before PS+N; 15 min after PS+N (T3), PS+D (T4) and PS+E (T5); 24h after PS+E (T6).* Statistical evaluation was made on the basis of Student test.

Results. At EC, compared to C group, HR values were significantly reduced at: T2 (p=0.005), T3 (p=0.02), T4 (p=0.04) and T5 (p=0.004). At EC - compared to T1- EG, RL and IC were: a) almost unchanged at T2 and T3; b) moderately significantly reduced, at T4 (EG,p=0.05; RL,p=0.04; IC,p=0.04); c) significantly increased at T5 (EG,p=0.02; RL,p=0.001; IC,p=0.01).

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Conclusions. 1) Compared to C, influence of ECP+N/D/E was significantly intense in EC, both for HR and positive emotions. 2) Compared to C, ECP: decreased the effect of FE-D, and increased the effect of FE-E. 3) ECP influence on EG, RL and IC was almost similar. 4) ECP could be used as a modulator of FE impact on acute physical stress.

Key words: phytotherapeutic product, facial expression, heart rate, positive emotions

D11: Blood

D11-1

The effects of long-term and short-term water and food deprivation on blood antioxidant defense system

I. Matiulko[1], E. Khirazova [1], A. Bayzhumanov [1]
[1]*Lomonosov Moscow State University, Biology, Moscow, Russian Federation*

Malnutrition and temporal dehydration can lead to the intensification of free-radicals oxidation that contributes to the progression of physiological and metabolic dysfunctions in response to stress. The aim of this study was to investigate the effects of long-term and short-term water and food deprivation on blood antioxidant defense system. Adult male Wistar rats were divided into four experimental (48- and 96-hours water and food deprivation) and one control groups. Measurements of blood hemoglobin level, the concentration of protein in blood plasma, general antioxidant activity, catalase activity in blood and activity of extracellular (SOD3) and cytoplasmic (SOD1) forms of superoxide dismutase as well as the levels of non-enzymatic antioxidants (non-protein thiols and ceruloplasmin) and thiobarbituric acid-active products in blood were performed to identify the changes in blood antioxidant defense system. Short-term 48-hours food deprivation led to an increase in activity of SOD3. Water deprivation of the same duration demonstrated more profound effect on blood antioxidant defense system via an increase in hemoglobin level and general antioxidant activity along with the decrease in the level of nonprotein thiols in the blood. Long-term 96-hours food deprivation resulted in an increase of hemoglobin level whereas general antioxidant activity and activity of SOD1 decreased. Dehydration caused by long-term 96-hours water deprivation led to an increase in the hemoglobin level, concentration of ceruloplasmin, general antioxidant activity and catalase activity in blood. Water deprivation in comparison to food deprivation caused more significant changes in blood antioxidant defense system with more profound effects of long-term water deprivation. Short-term food deprivation during 48 hours resulted in smaller changes in the response of blood antioxidant defense system.

D11-3

Investigation of the effects of major autohemotherapy ozone application on erythrocyte deformability and aggregation

I. H. AKBUDAK[1], V. KUCUKATAY[1], O. KILIC-ERKEK[1], Y. OZDEMIR[1], M. BOR-KUCUKATAY[1]
[1]*Pamukkale University Faculty of Medicine, physiology, denizli, Turkey*

Question: Currently, with reappraisal of ozone therapy especially for the protection of health, it has been utilized worldwide in research. Hemorheological conditions (*ie.* Erythrocyte deformability and aggregation) basically influence tissue perfusion, oxygen and nutrient supply. Limited number of studies exploring effect of ozone on red blood cell (RBC) deformability and aggregation reported conflicting results depending on the application way of ozone, dose and the species the experiments were carried out. The effect of ozone therapy on hemorheological parameters of healthy humans was not demonstrated before. Thus, the results of the current study are expected to provide new insights into the ozon therapy field.

Methods: 10 and 50 µg/ml doses ozone were applied for 20 minute to 15ml venous blood samples obtained from 10 healthy male volunteers (age 20-25). Erythrocyte aggregation, and deformability at 9

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shear stresses were measured by an ektacytometer. Shapiro–Wilk"s test was used for determination of normal distribution. Continuous variables were defined by mean ± standard deviation. Repeated Measures ANOVA, Friedman Tests were used for comparing three dependent groups. For post hoc analysis Bonferroni method was used.

Results: Ozone application of 10 and 50 µg/ml doses did not alter erythrocyte aggregation. On the other hand, 50 µg/ml ozone increased RBC deformability measured at 0,53 Pa (p=0.045).

Conclusion: Our results demonstrate that although 10 µg/ml concentration of ozone has no effect on hemorheology, ozone therapy at 50 µg/ml concentration may have beneficial effects on circulation through reducing RBC deformability.

Keywords: ozone therapy, RBC deformability, erythrocyte aggregation

D11-4

Enhancement of erythrocyte deformability after dark chocolate ingestion in healthy humans.

J. Radosinska[1,2], M. Horvathova[3], K. Frimmel[2], J. Muchova[3], M. Vidosovicova[1], R. Vazan[1], I. Bernatova[4]
[1]*Faculty of Medicine, Comenius University in Bratislava, Institute of Physiology, Bratislava, Slovakia*
[2]*Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovakia*
[3]*Faculty of Medicine, Comenius University in Bratislava, Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Bratislava, Slovakia*
[4]*Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia*

The deformability of erythrocytes is important property of erythrocytes that considerably affects blood flow and hemodynamics in general. The high content of polyphenols present in dark chocolate has been reported to play a protective role in functionality of erythrocytes. We hypothesized that chocolate might influence erythrocytes not only after repeated chronic intake, but also immediately after its ingestion. Thus, we determined the acute effect of dark chocolate and milk (with lower content of biologically active substances) chocolate intake on erythrocyte deformability. We were also focused on selected factors that may affect erythrocyte deformability, specifically nitric oxide (NO) production in erythrocytes and total antioxidant capacity of plasma. We determined post-treatment changes in mentioned parameters 2 hours after consumption of chocolate compared with their levels before consumption of chocolate. In contrary to milk chocolate intake, the dark chocolate led to significantly higher increase in erythrocyte deformability. NO production in erythrocytes was not changed after dark chocolate intake, but significantly decreased after milk chocolate. The plasma total antioxidant capacity remained unaffected after ingestion of both chocolates. We may conclude that our hypothesis was confirmed. Single ingestion of dark chocolate improved erythrocyte deformability despite unchanged NO production and antioxidant capacity of plasma. Increased deformability of erythrocytes may considerably improve rheological properties of blood and thus hemodynamics in humans, resulting in better tissue oxygenation.

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D11-5

The effects of hyperbaric oxygen therapy on the erythrocyte osmotic deformability (Osmoscan) parameters in patients with various disorders.

N. Ertan[1,2], B. Mirasoglu[3], M. Sinan[2], M. Koc[4], O. Yalcin[4]
[1]*Istanbul University, Istanbul Faculty of Medicine, Physiology, Istanbul, Turkey*
[2]*Aydin University, Faculty of Medicine, Physiology, Istanbul, Turkey*
[3]*Istanbul University, Istanbul Faculty of Medicine, Department of Underwater and Hyperbaric Medicine, Istanbul, Turkey*
[4]*Koc University, Faculty of Medicine, Physiology, Istanbul, Turkey*

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Aim: Hyperbaric oxygen therapy (HBOT) is applied using 100% oxygen under high pressure condition, which increases the amount of dissolved oxygen in plasma. Previously, it was shown that high dissolved oxygen level damaged the erythrocytes. However recently we have shown that HBOT did not affect any hemorheological parameters. Osmotic gradient ektacytometry is a very sensitive method for detecting early erythrocyte morphology, structure and cell surface/volume ratio changes. Therefore, we aimed to measure osmotic deformability parameters in patients undergoing HBOT.

Method: After the approval of local ethical committee, the venous blood samples were collected from 29 patients (19 M/10 F) before the first and after 20th HBOT at 2.4 ATA. Erythrocyte deformability in osmotic gradient conditions was measured using osmotic gradient ektacytometry (Osmoscan). Minimal elongation index (EI) at low-osmotic environment (EImin), maximal EI (EImax), half of the maximal EI at high-osmotic environment (EIhyper), osmolality at EI min (Omin), osmolality at EImax (Omax), osmolality at EIhyper (Ohyper) and the area under the individual EI (Area) were measured.

Results: EImax and EIhyper decreased after treatment ($p<0.004$ and $p<0.01$ respectively), whereas the differences in other parameters were not significant.

Our findings pointed out that the HBOT affects erythrocytes and they become less deformable at the high osmotic environments.

D11-6
In Vitro Effects of Some Pesticides on Some Human Carbonic Anhydrases

M. C. guler[1], E. eraslan[1], A. tanyeli[1], M. senturk[2]
[1]*ataturk university , physiology , erzurum, Turkey*
[2]*ibrahim cecen university, chemistry, agri, Turkey*

AIM: Some pesticides (Carbofuran, propoxur, simazine, and atrazine) were tested on human carbonic anhydrase (CA) I and II enzymes *in vitro*. A better inhibitory activity has been observed with atrazine investigated here for the inhibition of the rapid cytosolic isozyme hCA II.

METHODS: Human hCA I and hCA II isoenzyme were isolated by means of affinity chromatography. CA activity was determined by the esterase method which follows the formation of 4-nitrophenylacetate to 4-nitrophenol at 348 nm. Activit%-[Inhibitor] graphs were drawn and IC50 values were calculated. KI values were calculated by using the Chenge-Prusoff equation.

RESULTS: Carbofuran, propoxur, simazine, and atrazine exhibited important inhibitory features with KI values in the range of 4.95 to 16.125 nM for CA I and II. Atrazine was observed to be the most effective one among the tested compounds.

CONCLUSION: As a result, activity of the CA enzyme was determined to be mostly inhibited in atrazine among all tested pesticides.

D11-7
Investigation of the Effects of Gossypin on *in vivo*, *in vitro* and Ischemia / Reperfusion Injured Rat Erythrocytes Carbonic Anhydrase Enzyme

A. tanyeli[1], E. eraslan[1], M. C. guler[1], M. senturk[2], L. katagöz[3]
[1]*ataturk university , physiology , erzurum, Turkey*
[2]*ibrahim cecen university, chemistry, agri, Turkey*
[3]*marmara university, biochemistry, istanbul, Turkey*

AIM: Some phenolic compounds (Resorcinol, catechol, curcumin, and gossypin) were tested on carbonic anhydrase (CA) I and II enzymes *in vitro*. Gossypin was found to be the most effective one among the phenols tested. Is to investigate the effect of gossypin on erythrocytes CA enzyme in Ischemia/reperfusion (I/R) and I/R injured rats.

METHODS: Control, sham, I / R, and gossypin + I / R are as follows: A total of 32 male Wistar albino rats weighing 256 ± 5.3 g were equally and randomly divided into four groups. In the last group, gossypin was administered at 400 µg/kg dose before intraperitoneal ischemia period. Enzymatic activity was measured by using the spectrophotometric method of Verpoorte.

RESULTS: Resorcinol, catechol, curcumin, and gossypin exhibited important inhibitory features with KI values in the range of 6.08 to 4014 µM for CA I and II. Gossypin was observed to be the most effective one among the tested compounds. Specific activity values were determined for total erythrocytes CA enzyme at four different experimental groups. Gossypin + I / R group was found to be the most effective one among the tested group. Groups was determined as 400 µg/kg dose gossypin + I/R group 0.149 ± 0.005 EU/mg protein.

CONCLUSION: As a result, activity of the CA enzyme was determined to be mostly inhibited in gossypin + I/R group among all applications.

This study was supported by Atatürk University SRP (Project No: 2016/052)

D11-8
Investigation of the Effect of Carbonic Anhydrase of Ischemia/Reperfusion Injured Rat

E. eraslan[1], M. C. guler[1], A. tanyeli[1], M. senturk[2]
[1]*ataturk university , physiology , erzurum, Turkey*
[2]*ibrahim cecen university, chemistry, agri, Turkey*

AIM: The purpose of this study is to investigate the effect of erythrocytes carbonic anhydrase (CA) enzyme in ischemia / reperfusion (I / R) and I / R injured rats.

METHODS: Control, sham, I / R, are as follows: A total of 24 male Wistar albino rats weighing 256±5.3 g were equally and randomly divided into three groups. Erythrocyte levels of enzyme activity of carbonic anhydrase (CA) were measured. Enzymatic activity was measured by using Verpoortes method by using a spectrophotometer. The assay system contained 0.05 M Tris-SO4 buffer pH: 7.4, including 3 mM 4-nitrophenylacetate.

RESULTS: Specific activity values were determined for CA enzyme at three different experimental groups. Groups were determined as follows: Control group 0.194 ± 0.008 EU/mg protein, sham group 0.180 ± 0.005 EU/mg protein, I/R group 0.246 ± 0.011 EU/mg protein.

CONCLUSION: As a result, the activity of the CA enzyme was determined to be mostly inhibited in sham group among all applications. Activity value of sham group decreased by about 8% compared to controls was observed that $p \leq 0.05$ is as meaningful.

D11-9
Nurses have higher blood leucocyte counts following night-shift works

P. Cakan[1], S. Yildiz[1]
[1]*Inonu University, Malatya, Turkey*

Aims: Shift works place a great pressure on metabolism and this, in turn, may affect body"s defense and immune system. Whole blood counts provide information about the level of immune system activation. Therefore, the aim of the current study was to evaluate whole blood counts in nurses following night-shift works.

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Materials and methods: Nurses having night-shift works (n=13) were compared to age-matched post-graduate students who did not have night shifts (control, n=10). They were followed for three days and blood samples were collected on the last day in the morning immediately after the commencement of the normal workday. Blood samples were collected into vacutainer tubes with EDTA as anticoagulant. As soon as taking the blood samples, they were analyzed by automated whole blood counter. The data was analyzed by GLM models of MINITAB statistical package.

Results: White blood cell counts were higher in nurses having night-shift works than the control group (P=0.053) but red blood cell counts, sedimentation rate, platelet counts, hemoglobin concentration and hematocrit values did not differ between the groups. In general, differential leucocyte counts were also similar except basophils which were higher in nurses which had night-shift works (P=0.036).

Conclusion: The results suggest that immune system is activated in night-shift nurses and this might be due to increased workload, perturbed sleep or increased exposure to microorganisms or immune activating substances.

D11-10

Evaluation of effects of hyperthermic intraperitoneal chemotherapy treatment on erythrocyte deformability

D. Kalaycı[1], A. Küçük[2], O. Şen[1], F. M. Çomu[3], **M. Arslan**[4], Y. Ünal[4]
[1]*Abdurrahman Yurtaslan Oncology Training and Research Hospital, Department of Anaesthesiology and Reanimation, Ankara, Turkey*
[2]*Dumlupınar University, Medical Faculty, Department of Physiology, Kütahya, Turkey*
[3]*Kırıkkale University, Medical Faculty, Department of Physiology, Kırıkale, Turkey*
[4]*Gazi University, Medical Faculty, Department of Anaesthesiology and Reanimation, Ankara, Turkey*

Aim: Cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (HIPEC) is a major surgical procedure that is being used increasingly frequently in therapeutic option for selected patients with peritoneal surface malignancies. Our purpose in this experimental study is to evaluate effects of intraperitoneal chemotherapy on erythrocyte deformability.

Materials and Methods: 24 New Zealand rabbits included and divided four groups. Proper laryngeal mask was placed and they were connected to mechanical ventilation. Two cathaters were placed in to abdominal cavity one to upper quadrant another to lower quadrant. Perfusion was applied as closed abdomen technique. Perfusate was given intraperitoneal cavity following heated up to 42 C. Perfusion was applied for 90 minutes. Group 1: normal saline+ HIPEC+ ketamine Group 2: Cisplatine 7mg/kg + HIPEC+ ketamine Group 3: Cisplatine 7mg/kg+HIPEC+%3 sevoflurane (2 hours) Group 4: Cisplatine 7mg/kg+HIPEC+%6 desflurane (2 hours) 24 hours after anaesthesia procedure, all rabbits were euthanasia under anesthesia and heparinized total blood samples were used to prepare erythrocyte packs. These erythrocyte suspensions were used for the measurement of deformability.

Results and Discussion: HIPEC application is determined as it increases resistance relatively more than control group (p< 0.0001). Erythrocyte deformability index is determined as increased significantly in all of the groups more than control group (p< 0.0001, all groups). Sevoflurane and desflurane use in rabbits which were predisposed to HIPEC is determined as it does not change erythrocyte deformability (p=0.846, p=0.734).

Conclusion: HIPEC application is determined as it decreases erythrocyte deformability in our study. Also we observed that inhalation agents sevoflurane and desflurane has no effect on deformability during HIPEC.

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D11-11

The effects of HES 130 / 0,4 application on erythrocyte deformability in ureteral obstructed rats

I. Güneş[1], A. Küçük[2], F. M. Çomu[3], V. Şıvgın[4], M. Alkan[4], **M. Arslan**[4], Y. Ünal[4]
[1]*Erciyes University, Medical Faculty, Department of Anesthesiology and Reamination, Kayseri, Turkey*
[2]*Dumlupınar University, Medical Faculty, Department of Physiology, Kütahya, Turkey*
[3]*Kırıkkale University, Medical Faculty, Department of Physiology, Kırıkale, Turkey*
[4]*Gazi University, Medical Faculty, Department of Anaesthesiology and Reanimation, Ankara, Turkey*

Aim: The administration of plasma expanders is crucial for managing critically ill patients across a range of clinical conditions, including major surgery, hemorrhagic shock, and trauma. However, the effects of hydroxyethyl starch (HES) on erythrocyte aggregation remain controversial. For this reason, we aimed to investigate the effects of HES 130/0.4, performed renal insufficiency by experimentaly unilateral ureteral obstruction, on erythrocyte deformability.

Materials and Methods: 24 rats were divided into 4 group Control, HES 130/0.4, Ureter Obstruction (UO) and UO-HES130/0,4. Urethral obstruction groups were treated with ketamine anesthesia with a low abdominal incision and reached to the distal of right ureter and sutured with 2.0 mersilen then waited for 3 weeks for late term renal insufficiency. 20 ml/kg of HES 130/0.4 (Voluven) were infused intravenously to the HES 130/0.4 and UO-HES130/0,4 group. After 24-hour, rats were sacrificed. Deformability measurements were performed using 5% haematocrit in a phosphate-buffered saline (PBS) buffer.

Results and Discussion: Relative resistance was increased in all groups compared to the control group ($p < 0.0001$). In all groups, erythrocyte deformability index was found to be significantly higher than the control group (Group C-Group HES, p=0.023, Group C- Group UO, p<0.0001, Group C-Group UO-HES, p<0.0001). UO-HES group had significantly increased erythrocyte deformability index compared with the HES group (p=0.031, p=0.021, respectively). It was determined that HES 130/0,4 application did not change erythrocyte deformability in ureteral obstructed rats (p=0.785).

Conclusion: The use of HES 130/0,4 has no negative effects on erythrocyte deformability in ureteral obstructed rats. We think that indications should be used correctly when using HES 130/0,4.

D11-12

IκB Kinase 2 impairs Platelet Activation

M. Salzmann[1], M. Mussbacher[1], W. C. Schrottmaier[1,2], J. B. Kral-Pointner[1,3], B. Hoesel[1], A. Assinger[1], J. A. Schmid[1]
[1]*Centre of Physiology and Pharmacology/Medical University of Vienna, Institute of Vascular Biology and Thrombosis Research, Vienna, Austria*
[2]*Umeå University, Department of Clinical Microbiology, Umeå, Sweden*
[3]*KTH Royal Institute of Technology, Department of Proteomics, Stockholm, Sweden*

QUESTION: Megakaryocytes can sense inflammatory signals, but little is known how this might change platelet function. Most inflammatory signaling pathways converge at the kinase IKK2 (IκB kinase 2) activating the transcription factor NF-κB. The aim of this study was to determine the effect of persistent inflammation on platelet function, by altering NF-κB activity in megakaryocytes with a constitutively active IKK2.

METHODS: Mice with a megakaryocyte-specific constitutively active IKK2 were compared to littermate controls. Platelet count and lifespan was determined. Platelet function was tested *in vivo* by tail bleeding and induction of thrombi via mechanical injury and via ferric chloride, together with intravital imaging. *In vitro* assays included agonist-induced degranulation and aggregation.

RESULTS: Platelet count and lifespan is unaltered, however bleeding time increased and *in vivo* thrombus formation is impaired in mice with constitutively active platelet IKK2. Consistently, *in vitro*

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platelet aggregation, degranulation and GPIIb/IIIa activation were decreased in platelets with constitutively active IKK2 upon stimulation with ADP and PAR4 receptor agonist peptide.

CONCLUSIONS: Our data indicates that active IKK2 or NF-κB interferes with platelet activation, either directly through kinase activity in platelets or via constitutively active NF-κB signaling in megakaryocytes.

D15: Other

D15-1

The physiological reaction by interaction of human body anatomical axes results in tissue function normalization - a feature of human body axis sensation

M. C. Ou[1,2], D. Ou[3], C. C. Pang[4]
[1]*Taipei City Hospital, Obstetrics & Gynceology, Taipei City, Taiwan*
[2]*Taipei Mecial University, Obstetrics & Gynecology, Taipei City, Taiwan*
[3]*Carnegie Mellon University, Pittsburgh, United States*
[4]*Su Women Hospital, Taipei City, Taiwan*

Question: Does human body anatomical axis (HBAA) impart the polarity of individual cell as embryonic axes do (Proc Physiol Soc, 2016)?

Methods: Ou MC decrescendo phenomenon treatment (OuDPt) was performed by patients themselves for neoplastic diseases treatment, the contralateral hand is placed over the lesion along HBAA of left-right, dorsoventral or vertical axis (Proc Physiol Soc, 2015). Three patients separately with endometrial cancer, ovarian cancer with carcinomatosis and suspected pancreatic cancer had been improved by OuDPt via 2 HBAAs (2 dimensions) but progressed clinically later (Cancer Res, 2016). Thus, all these 3 patients performed OuDPt with all 3 HBAAs which formed a 3 dimensional (3D) polarity system.

Results: The endometrial cancer regressed from vagina to uterine cervix as stage IIIB to II by treatment for about 3 months. The top of the ovarian tumor shrank from the third to the level beneath the fourth lumbar spine, with trapped intestinal loop detaching from the tumor after treatment for about 3 months. The size of the pancreatic isodense tumor decreased from 1.6 × 1.7 cm to 1.02 x 0.96 cm with measurement at its greatest dimension by treatment about 4 months, and no tumor was visible by sonography about 1 year later.

Conclusions: Three dimensional tissue organization has shown to be a potential non-canonical tumor suppressor, which is hypothesized as via normalizing polarity of mutant cells and, ultimately, suppressing tumor development and progression (J Cell Sci, 2008). Our study shows that 3D human body polarity system formed by HBAA interactions suppresses the development of the neoplasm more efficiently than OuDPt with 2D body polarity system (TJOG, 2017), which may indicate HBAA imparts cell polarity.

D15-2

Hypoglycemic and hypolipidemic effects of apple cider vinegar in Tunisian type 2 diabetic patients.

H. Ben Hmad [1], S. Khlifi[1], H. Ben Jemaa[1], H. Jemmousi[1,2], F. Ben Slama[1], A. Abdallah[1]
[1]*1Research Unit on nutrition, regulation of metabolic systems and atherosclerosis, High School of Health Sciences, University of , Tunis, Tunisia*
[2]*Institute of Nutrition and Alimentary Technology, Obesity, Tunis, Tunisia*

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Type 2 diabetes is one of the most prevalent endocrine disorders worldwide. Traditionally, herbal plants and their derivatives are used to lessen complications of type 2 diabetes. In the current study the hypoglycemic and hypolipidemic properties of apple cider vinegar have been reported for vinegar in type 2 diabetic patients were investigated. In this trial study, sixty patients from both sex (30 females and 30 males) with type 2 diabetes were divided into two groups. The first group took 15 ml of apple cider vinegar with their middle meal for one month. The second group received water as placebo. At the beginning and end of the study, blood samples were collected and biochemical factors including fasting blood sugar (FBS), triglycerides (TG), total cholesterol, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), very low density lipoprotein (VLDL) and atherogenic index were evaluated. Findings showed that FBS, TG, TC, LDL-C, VLDL and atherogenic index decreased where HDL-C conentrations increased significantly in the first group. There was no significant difference in the studied parameters in placebo group. Based on the results of this study, it can be concluded that apple cider vinegar is a hypoglycemic and hypolipidemic agent that can be applied for treatment of type 2 diabetes.

D15-3

The BMI1 inhibitor PTC-209 is a potential compound to halt cellular growth in biliary tract cancer cells

C. Mayr[1], A. Wagner[2], M. Löffelberger[1], D. Bruckner[3], M. Jakab[1], F. Berr[1], P. Di Fazio[4], M. Ocker[5], D. Neureiter[6], M. Pichler[7], T. Kiesslich[2,1]
[1]*Paracelsus Medical University Salzburg, Physiology and Pathophysiology, Salzburg, Austria*
[2]*Salzburger Landeskliniken - SALK, Paracelsus Medical University, Department of Internal Medicine I, Salzburg, Austria*
[3]*Salzburger Landeskliniken - SALK, Paracelsus Medical University, Research Program for Experimental Ophthalmology and Glaucoma Research, University Clinic of Ophthalmology and Optometry, Salzburg, Austria*
[4]*Philipps-University Marburg, Department of Visceral, Thoracic and Vascular Surgery, Marburg, Germany*
[5]*Bayer Pharma AG, Experimental Medicine Oncology, Berlin, Germany*
[6]*Salzburger Landeskliniken - SALK, Paracelsus Medical University, Institute of Pathology, Salzburg, Austria*
[7]*Medical University of Graz, Division of Oncology, Department of Internal Medicine, Graz, Austria*

Questions
Biliary tract cancer (BTC) is a deadly disease with limited therapeutic options. BMI1 is a core component of the polycomb repressive complex 1 (PRC1), a major epigenetic regulator. Current literature indicates that aberrant PRC1 activity contributes to BTC. Recently, a specific BMI1 inhibitor called PTC-209 was developed. The aim of this study was to investigate the effect of PTC-209 using a comprehensive BTC cell model.

Methods
Expression of BMI1 in BTC cells was measured by quantitative real-time PCR and western blot. Cytotoxic effects of PTC-209 were evaluated by Resazurin assay, confluence measurement, Caspase-Glo® 3/7 Assay, immunostaining and cell cycle analysis. Effects of PTC-209 on cancer stem cell characteristics were investigated by sphere formation and ALDEFLUOR™ Kit. RT2 Profiler PCR Array was used for comprehensive gene expression analysis. Synergistic effects of PTC-209 treatment with standard chemotherapeutic cisplatin were evaluated according to combosyn.com.

Results
BMI1 was expressed in BTC cells. Treatment with PTC-209 resulted in diminished BMI1 protein levels and cell cycle arrest at G1/S. Gene expression analysis revealed diminished expression of cell-cycle promoting genes as well as of DNA synthesis initiation factors following PTC-209 treatment, accompanied by up-regulation of cell cycle inhibitors CDKN1A and CDKN2B. In addition, PTC-209 caused reduction of cancer stem cell characteristics: tumor spheres as well as percentage of cells positive for stem cell enzyme aldehyde-dehydrogenase were reduced. Lastly, specific combinations of PTC-209 with cisplatin showed a synergistic cytotoxic effect.

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Conclusion
PTC-209 is a promising drug for future *in vitro* and *in vivo* studies in BTC.

D15-4

The effect of Napabucasin on cancer stem cells in biliary tract cancer

M. Beyreis[1], K. Helm[1], H. Dobias[1], M. Jakab[1], M. Ritter[1], D. Neureiter[2], T. Kiesslich[1,3], C. Mayr[1,3]
[1]*Paracelsus Medical University Salzburg, Institute of Physiology and Pathophysiology, Salzburg, Austria*
[2]*Salzburger Landeskliniken - SALK, Paracelsus Medical University, Institute of Pathology, Salzburg, Austria*
[3]*Salzburger Landeskliniken - SALK, Paracelsus Medical University, Department of Internal Medicine I, Salzburg, Austria*

Questions:
Napabucasin (BBI608) is a newly developed anti-cancer stem cell drug and currently used in several clinical trials. Involvement of cancer stem cells (CSCs) has been demonstrated for biliary tract cancer (BTC) - a deadly disease with poor chance of survival and limited therapeutic options. Targeting CSCs in BTC might be a beneficial and very promising therapeutic route. Up to now, scientific data regarding Napabucasin and cancer are very sparse – for BTC, no data are available. Therefore, the aim of the present study is to investigate the cytotoxicity of Napabucasin in an *in vitro* BTC cell model as well as the effect on expression of stem cell genes, pathways and functional CSC characteristics.

Methods and Results:
Cytotoxic effect of Napabucasin on BTC cell lines was evaluated by Resazurin assay. Time course experiments for 72h to investigate the proliferation and cytotoxic rate have been performed. Napabucasin showed a cell line and concentration-dependent cytotoxic effect on BTC cell lines. Time course experiments indicate cell death rather than proliferation stop as mode of cytotoxicity.

Outlook:
Further experiments in the present study will clarify the potential of Napabucasin as a possible therapeutic drug in BTC. Specifically, changes in CSC specific gene expression will be analyzed using RT^2 Profiler PCR Array and western blot following Napabucasin treatment. Moreover, the effect of Napabucasin on functional CSC characteristics such as migration, invasion, colony formation and aldehyde-dehydrogenase positivity will be investigated. Lastly, the potential of Napabucasin as an adjuvant substance with conventionally applied drugs cisplatin and gemcitabine will be determined.

D15-5

The histone-modification complex G9a and its role in biliary tract cancer/cholangiocarcinoma

K. Helm[1], C. Mayr[1,2], M. Beyreis[1], M. Ritter[1], M. Jakab[1], E. Klieser[3], T. Kiesslich[1,2], D. Neureiter[3]
[1]*Paracelsus Medical University Salzburg, Institute of Physiology and Pathophysiology, Salzburg, Austria*
[2]*Salzburger Landeskliniken - SALK, Paracelsus Medical University, Department of Internal Medicine I, Salzburg, Austria*
[3]*Salzburger Landeskliniken - SALK, Paracelsus Medical University, Institute of Pathology, Salzburg, Austria*

Questions:
Biliary tract cancer (BTC) is a deadly malignancy with limited therapeutic options, making the identification of new therapeutic targets a primary scientific goal. The histone-methyltransferase G9 specifically dimethylates lysine 9 at histone 3 (H3K9me2) and is overexpressed in cancer. However, the role of G9a in BTC is not described yet. Therefore, the aim of this study is to test G9a expression in BTC specimens and cell lines and to investigate the effect of G9a inhibition in BTC cells.

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Methods:
G9a expression in FFPE BTC specimens (n=78) was determined via immunohistochemistry (IHC) and correlated with clinicopathological data. In BTC cell lines (n=9), G9a, as well as H3K9me2 expression were examined on mRNA and/or protein level. Cytotoxicity of G9a inhibition was evaluated via the resazurin assay using established small-molecular G9a inhibitors BIX01294, BRD4770 and UNC0642.

Results:
G9a was detectable in approximately 50% of BTC specimens. G9a expression significantly positively correlated with higher tumor grading. Additionally, G9a expression was diametrically associated with Vimentin (positive) and E-Cadherin (negative) protein expression. In BTC cells, G9a and H3K9me2 are expressed in a cell line-dependent manner. Statistical analysis revealed significant correlation between G9a mRNA, G9a protein and H3K9me2 expression levels. G9a inhibition resulted in drastic reduction of viable cells.

Conclusion:
The expression of G9a in BTC specimens and BTC cells as well as the cytotoxic effect of G9a inhibition provides first evidence that G9a may be an important regulator for BTC development and progression, making it an attractive, potential therapeutic target for epigenetic-related treatment of BTC in future.

D15-7

Development and validation of body composition prediction equations for the prediction of total body water and fat-free mass in North African Arabic children

H. Ben jemaa[1], A. Mankaï [1], S. Khlifi[1], R. Minaoui [1], D. Ghozzi [1], B. Kortobi [1], F. Ben Slama[2], A. Benzarti [1], H. Aguenaou [3], K. El Kari [3], A. Aouidet [1]
[1]*Higher School of Health Sciences and Technics, Nutrition Department, Tunis, Tunisia*
[2]*Institute of Public Health, Tunis, Tunisia*
[3]*CNESTEN-Ibn Tofaïl University, Joint Unit of Nutrition and Food Research (URAC39), , Rabat, Morocco*

Background/Objectives: Accuracy of the impedance analysis depends on population-specific prediction equations. The aim of this study was to propose new prediction equations to estimate body composition using impedance analysis for healthy Tunisian children and validate them using the deuterium oxide dilution (D2O) technique.

Subjects/Methods: The body composition was estimated by the Tanita impedance analyzer. The validity of this system was investigated in 134 school children aged between 8 and 11 years. Total body water (TBW) and Fat Free Mass (FFM) were determined using the D2O technique. Participants were divided equally into development (n=67) and validation groups (n=67) to develop prediction equation using linear regression models.

Results: The comparison between body composition obtained by Tanita system and by D2O technique illustrated a significant difference ($p<0.01$). Compared to D2O technique, the impedance analysis underestimates fat mass and overestimates FFM and TBW. The prediction equations for TBW and FFM were developed with sex, age, weight and resistance index as possible predictor variables. The selected equations presented the highest adjusted coefficient of determination (R2), the lowest standard error of the estimate (SEE) value and the lowest P values. The pure error was 1.263 for the TBW equation and 1.646 for the FFM. The Bland Altman plot illustrated the good level of concordance between the TBW and FFM predicted by the new equations and measured by isotope dilution.

Conclusions: Our study provides valid prediction equations for estimation of TBW, FFM from impedance analysis measures for Tunisian children. These equations are applicable to children aged between 8 and 11 years.

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D15-8

The effect of kisspeptin fragments in late pregnant uterine function *in vitro*

J. Hajagos-Tóth[1], Z. Tiszai[1], E. Ducza[1], R. Gáspár[1]
[1]*University of Szeged Faculty ofPharmacy, Pharmacodynamics and Biopharmacy, Szeged, Hungary*

Questions: Oxytocin (OT) has an important role in the regulation of smooth muscle contractility. Several adipokines are known which can activate the OT neurons in the central nervous system such as kisspeptin (KISS1). Our aims were to clarify the myometrial effects of KISS1 58-65 and KISS1 94-121 and to determine their receptors in the pregnant rat uteri throughout gestation.

Methods: Contractions of uterine rings from non-pregnant and 22-day-pregnant rats were measured in an organ bath. The contractions were stimulated with 25 mM KCl and cumulative-dose response curves were elicited in the presence of the kisspeptin fragments (10-12 − 10-7 M), and the kisspeptin antagonist Kisspeptin-234 trifluoroacetate (10-9 M) and after removing the endometrium. The KISS1 receptor expression was determined by RT-PCR and Western blot analysis.

Results: The KISS1 receptors were expressed both in the non-pregnant and pregnant uteri. The highest expression (P=0,017) was found on the 5th day of pregnancy, which refers to the effect of kisspeptin in implantation. The expression of the receptors was higher in the endometrium than in the myometrium. Both kisspeptin fragments caused myometrial relaxation (50 %), however after removing the endometrium the relaxing effect of kisspeptin was ceased, as in the presence of kisspeptin antagonist.

Conclusions: The expression of kisspeptin was decreased throughout gestation. The effect of KISS1 58-65 fragment is endometrium-dependant while the effect of KISS1 94-121 fragment in the endometrium might be independent from the receptor. We hypothesize that the adipocyte-produced peptides in the uterus have a crucial role in the process of early or prolonged delivery.

Key words: gestation, kisspeptin, uterus

D15-9

Interaction of alpha-tocopherol and cyclooxygenase-inhibitors on smooth muscles of rats: the significance of cyclooxygenase-activity in uterus and trachea

A. Kothencz[1], J. Hajagos-Tóth[1], R. Gáspár[1]
[1]*University of Szeged Faculty of Pharmacy, Pharmacodynamics and Biopharmacy, Szeged, Hungary*

Key words: COX-inhibitor, antioxidant, smooth muscle

Questions: The use of antioxidant supplements is popular. However, the effects of these materials on various medications have only been scarcely investigated. The cyclooxygenase enzymes (COX) liberate reactive oxygen species. Our aim was to investigate how the antioxidant tocopherol influences the effects of COX inhibitors (COXi) in the smooth muscles in rats *in vitro*.

Methods: Contractility of smooth muscle tissues from 22-day-pregnant and non-pregnant Sprague-Dawley rats were measured in isolated organ bath *in vitro*. α-Tocopherol-succinate (10^{-7} M) was applied as antioxidant, while non-selective diclofenac (10^{-9} – 10^{-5} M) and COX-2 selective rofecoxib (10^{-10} – 10^{-5} M) were used in cumulative doses as COXi. The COX activities of the samples were measured by enzyme-immunoassay kit.

Results: In the presence of tocopherol, the uterus relaxant effects of the diclofenac and rofecoxib are increased on the pregnant uteri along with COX-activity. The increase in COX-2 activity was higher than that of COX-1. The diclofenac action was reduced after rofecoxib pre-treatment. Tocopherol influenced neither COX-activity nor the effect of COXi in the non-pregnant uteri. Tocopherol slightly intensified the tracheal tone-reducing effects of rofecoxib and diclofenac without alteration of COX-activity. The lowest COX-activity was found in non-pregnant uteri while the highest one was in the trachea.

Conclusions: Tocopherol modifies the smooth muscle effect of COXi. Its effect is the most intensive in smooth muscle with medium COX activity. Tocopherol itself can induce the COX activity (especially COX-2) in pregnant uteri. The relaxing effect mainly belongs to COX-2 inhibition.

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D15-10

The effects of the amoxicillin, fosfomycin and doxycycline on the aquaporin 5 expression in rat uterus before delivery.

A. Csányi[1], E. Ducza[1], J. Hajagos-Tóth[1], R. Gáspár[1]
[1]*University of Szeged Faculty of Pharmacy, Department of Pharmacodynamics and Biopharmacy, Szeged, Hungary*

Questions: The aquaporin (AQP) water channels are expressed in the female reproductive tissues and they play important role during pregnancy. Earlier we proved that AQP 1, 2, 3, 5, 8 and 9 are detectable in the late-pregnant rat uterus and the AQP5 expression showed a dramatic down-regulation on the last day of pregnancy that is regulated by oxytocin and may play a role in delivery. Since antibiotics are among the drugs used to stop preterm labor, therefore our aim was to study the changes the AQP5 expression and uterine contractility after antibiotic treatment.

Methods: 3 groups of pregnant rats were used for the study. Amoxicillin (Group 1, 40 mg/kg) or doxycycline (Group 2, 30 mg/kg) was given orally for 1 week from day 16 of gestation. Fosfomycin (Group 3, 40 mg/kg) was given orally on day 21 of gestation. On pregnancy day 22 uterine samples were collected. We used reverse-transcriptase PCR and Western blot techniques for the detection of the changes in AQP5 expression. Uterine contractility was investigated in an isolated organ bath system.

Results: Fosfomycin and amoxicillin pretreatment caused a significant increase of AQP5 mRNA and protein levels on the last day of pregnancy. Doxycycline pretreatment caused a significant decrease of AQP5 mRNA and protein levels. The fosfomycin and amoxicillin pretreatment significantly reduced the uterine contractions both to potassium chloride and oxytocin treatment.

Conclusion: We suppose that the AQP5 expression is inversely related to the uterine contractility because the increased AQP5 expression is accompanied with decreased of the contractions. Fosfomycin and amoxicillin treatment during pregnancy may be favorable in the therapy of preterm labor.

D15-11

Uterine expressions and pharmacological influences of RhoA and Rho-kinases during pregnancy in rats

D. Domokos[1], E. Ducza[1], R. Gáspár[1]
[1]*University of Szeged, Pharmacodynamics and Biopharmacy, Szeged, Hungary*

Question: Activations of RhoA and Rho- associated kinases (ROCK I,II) play a pivotal role in the regulation of smooth muscle contraction via phosphorylation of myosin-light chain and myosin phosphatase. There are only few data on the RhoA and ROCKs expression levels in rat uteri. Our aim was to investigate the mRNA and protein level of RhoA and ROCKs during pregnancy, parturition and post-partum period and to evaluate the effects of ROCK (Y-27632, fasudil and RKI 1441) and RhoA inhibitors (simvastatin) on uterine contractility.

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Methods: The mRNA and protein expressions of RhoA and ROCKs were measured in non-pregnant, on days 5, 15, 18, 20, 22 of pregnancy, during parturition and on days 1, 3, 5, 7 after delivery by Real-time PCR and Western blot analysis. Furthermore, the effects of RhoA and ROCK inhibitors were investigated on oxytocin induced uterine contractions.

Results: The mRNA and protein levels of RhoA did not change significantly from pregnancy day 15, while a sharp increase was detected during parturition. The ROCKs were down-regulated in the early stage of pregnancy, while it sharply increased in delivery. Simvastatin relaxed the myometrial contractions, although its inhibitory effects were not followed by the alteration of RhoA. The strongest inhibitory effect of fasudil was found on non-pregnant uteri. The maximum relaxing effects of Y-27632 and RKI 1441 were altered in a proportional way with the target protein expressions.

Discussion: The lower expression of ROCKs during pregnancy may contribute to the maintenance of relative quiescence of pregnant myometrium and the sudden increase of RhoA and ROCKs during labour suggests that they may contribute to the enhanced contractility and the initiation of delivery.

Key words: RhoA/ Rho-kinases, uterus, pregnancy, rat

D15-12

The Relation between Heavy Metals and Lipid Peroxidation Marker in Laryngeal Cancer

A. Yoldas[1], S. Toplan[1], D. Saribal Kanber[1], O. Aslan[2], B. Aydemir[3]
[1]*Istanbul University Cerrahpasa Medical Faculty, Biophysics, Istanbul, Turkey*
[2]*Istanbul University İstanbul Medical Faculty, Head and Neck Surgery, Istanbul, Turkey*
[3]*Sakarya University Medical Faculty, Biophysics, Sakarya, Turkey*

Background: Heavy metals are present in the environment in greater or lesser amounts it is the concentration and their dosage which cause mutagenic and carcinogenic effect. Numerous investigations have revealed an association between heavy metal exposure and the incidence and mortality of cancer. Larynx cancer is one of the most frequently seen cancer type between head and neck cancers. In present study we investigated the relation between heavy metals such as zinc (Zn), copper (Cu), lead (Pb), cadmium (Cd) levels and malondialdehyde (MDA) concentration as lipid peroxidation marker in laryngeal cancer.

Method: In present study, considered element measurements and malondialdehyde concentration determinations were realized in blood samples of larynx cancer patients (n: 20) and healthy controls (n: 15). Element concentrations were determined by atomic absorption spectrophotometer. MDA measurements were done according to Stokes Dormandy method in erythrocytes and serum

Findings: Serum Cu levels found to be higher in cancer group ($p<0.001$) than controls, but Zn levels were determined at lower level ($p<0.01$). Lead and cadmium levels in cancer group were statistically higher compare to control values in blood samples (respectively, $p<0.01$; $p<0.001$). On the other hand MDA concentrations were found higher both in plasma and blood samples of cancer group than control group (respectively, $p<0.001$; $p<0.001$).

Results: Our results show that higher MDA levels may lead to development of malign tumors. Possible reason of such a situation could explained be with the inhibitory effect of heavy metals on antioxidant defense of body.

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D15-13

Can projects-based learning in medical biophysics create precondition for better understanding clinical teaching subjects?

E. Kralova[1], M. Trnka[1], E. Ferencova[1], Z. Balazsiova[1]
[1]*Faculty of Medicine, Comenius University, Institute of Medical Physics, Biophysics, Informatics and Telemedicine, Bratislava, Slovakia*

Questions
A specific project-based learning model for Medical Biophysics is applied at the Comenius University Faculty of Medicine in Bratislava in order to acquaint first-year students with the physical principles of the modern diagnostic and therapeutic methods.

Methods
Students identify the topics of the projects at the beginning of semester and work on them with timetable agreed by the teacher. Final stage involves the presentation of the results in students auditorium followed by discussion and finally submission.

The content analysis of semester projects was undertaken with the aims: to compare obtained results with analysis done in previous academic years; to summarize the wide spectrum of physical and biophysical applications in medical diagnostic and therapeutic methods, safety problems related to physical principles of medical equipment; to confirm usefulness of project-based learning.

Results
We analyzed 234 semestral projects submitted by first-year medical students in the interval 2013/2014–2016/2017 according to their content. 18,8% of them dealt with the physical topics, 50% belonged to diagnostic applications and 32% described applications of medical biophysics in therapy. We have created a database of semestral projects containing a wide spectrum of partial physical topics connected with medical applications reflecting actual needs of medical practice.

Conclusions
Based on obtained results we have confirmed the relevance of project-based learning in medical biophysics. It represents an effective teaching tool that is positively evaluated by students. We also believe that project-based learning represents important motivating factor.

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Key words: medical biophysics, project-based learning, semester projects

D15-14

Prevalence of underweight, thinness, overweight and obesity according to WHO standards, in a group of 100 female Tunisian students

R. Serairi[1,2], K. Ayed[2], D. Sahnoun[1,2], S. Ennaifer[2], S. Jameleddine[2], R. Ksouri[1,2]
[1]*LPAM, Tunis, Tunisia*
[2]*Tunis El Manar, Nutrition, Tunis, Tunisia*

Introduction and purpose of the study: Today, around the world, obesity affects nearly 650 million adults, or 13% of the world's adult population. This problem also seems to affect the students' milieu. The objective of our study is to assess the prevalence of leanness, overweight and obesity in a group of students aged 19-25 years in Tunis (Tunisia) in 2016 using WHO references .

Subjects and methods: A cross-sectional descriptive study of 100 students, volunteers and motivated to be part of the study. They all underwent clinical examination, anthropometric measurements, impedance measurements and food surveys.

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Results: According to WHO references, 10.5% of female students are meager, 15.9% are overweight and 9% are obese. These results are confirmed by the impedancemetry which shows that 37% of the population studied has a high percentage of fat mass. The analysis of the food survey shows that the contributions of 56.5% of the students are unbalanced both quantitatively and qualitatively. Meals were also de-structured with absence of breakfast 2 to 4 times per week in 64.7% of the students. A predominance of meals taken at fast food was also noted in particular, the "melawi", a preparation made from dough made with oil and stuffed with cheese, mayonnaise and tuna.

CONCLUSION: These results highlight the existence of high prevalences of overweight and obesity in a sample of the Tunisian female student population. These results are confirmed by the impedance measurement and analysis of the food survey. More efforts are strongly recommended to target this population in terms of nutritional education.

Keywords: Obesity, students, eating behavior, imbalance.

D15-15

Treatment with estrogen receptor agonist ERß, but not ERα, improves torsion-induced oxidative testis injury in rats

S. Arabaci Tamer[1], S. Arabaci[1], S. Ciftci[1], S. Akin[1], E. Sari[1], H. Ahmetoglu[1], M. K. Koroglu[1], F. Ercan[1], M. Yuksel[1], A. Yildirim[1], B. Yegen[1]
[1]*Marmara university, Istanbul, Turkey*

Torsion of spermatic cord impairs testicular function. In order to examine the effect of estrogen receptor (ER) agonists and 17ß-estradiol (E_2) on torsion-induced injury, male Sprague-Dawley rats (6-8 week-old; n=40) underwent testicular torsion for 2 h or had scrotal incision (sham) under anesthesia. Before and after torsion, testicular blood flow was monitored by a Doppler flowmeter. Following detorsion, vehicle (oil) or E_2, ERß (DPN) or ERα (PPT) agonist was given subcutaneously (each 1mg/kg/day) for 3 days. At 72nd h of torsion, rats were decapitated and serum testosterone levels were measured. Myeloperoxidase activity (MPO), malondialdehyde (MDA), superoxide dismutase (SOD) levels, and reactive oxygen species (ROS) in testes were measured. Scoring of seminiferous tubules was made microscopically. Data were analyzed using ANOVA and Student"s t-test. Blunted testicular blood flow upon 2-h torsion was recovered following reperfusion, while recovery was significantly greater in E_2 or ER agonist-treated groups. MPO and ROS levels were increased in vehicle-treated torsion group ($p<0.05$-0.001), but elevations in SOD and MDA were not different than sham group. Elevated ROS levels were depressed by DPN or E_2, while PPT further elevated ROS levels ($p<0.05$). Number of seminiferous tubules was significantly lower in vehicle- or PPT-treated rats ($p<0.001$), but the tubule count was higher in testes of DPN- or E_2-treated rats. Although reduction in serum testosterone level of sham group was not significant, PPT and E_2 further depressed testosterone level ($p<0.01$). The results demonstrate that E_2 or ERß, but not ERα, reduced testicular injury by inhibiting generation of ischemia-reperfusion-induced release of ROS.

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