POSTER SESSION C

C01: Cardiac physiology

C01-1
The cardioprotective remote ischemic preconditioning in SHR rats : role of age and activation of RISK signaling pathway.

V. Farkašová[1], L. Griecsová[1], M. Muráriková[1], S. Čarnická[1], Ļ Lonek[1], M. Ferko[1], A. Adameová[2], T. Ravingerová[1]
[1]*Institute for Heart Research, Slovak Academy of Sciences, Department of cardiovascular physiology and pathophysiology, Bratislava, Slovakia*
[2]*Faculty of Pharmacy, Comenius University, Department of pharmacology and toxicology, Bratislava, Slovakia*

Remote ischemic preconditioning (RIP) represents a novel form of innate cardioprotection conferred by short episodes of ischemia applied in a distant organ/tissue. RIP has been shown to exert its cardioprotective effect by activating intrinsic pro-survival signaling cascades such as reperfusion injury salvage kinase (RISK) pathway in healthy animals, however, there is no evidence on this effect of RIP in hearts from SHR animals. The aim of this study was to investigate the role of RISK pathway in effect of RIP on cardiac tolerance to I/R in SHR rats of different ages.

Rats of age three, five and eight months (3/5/8m) were anesthetized and RIP was performed on the right hind limb. Its protocol consisted of three cycles of 5min non-invasive limb occlusion followed by 5min reperfusion. Subsequently, hearts were excised, Langendorff-perfused and exposed to 30min global I and 2h R for the evaluation of reperfusion-induced ventricular arrhythmias, infarct size and recovery of contractile function.

Enhanced resistance to myocardial infarction after RIP was observed in all experimental groups. Moreover, in 3m and 5m animals RIP exhibited antiarrhythmic effect, while in 8m SHR rats its effect was either proarrhytmic. Protective effect of RIP was accompanied with increased Akt and GSK-3β activation as well as with decreased proapoptotic signaling only in hearts from 3m and 5m animals, while in 8m rats the Akt and GSK-3β activity and apoptotic signaling were not changed after RIP.

Cardioprotective effects of RIP in SHR rats show partial age-dependency, since in older adult animals, RIP decreased size of lethal injury but worsened arrhythmogenesis compared to younger individuals. These effects of RIP may be attributed to differences in activation of RISK pathway.

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C01-2
Remote ischemic preconditioning: protection of myocardial energetics

M. Ferko[1], I. Kancirová[1], M. Jašová[1], J. Kucharská[2], O. Uličná[2], O. Vančová[2], M. Muráriková[1], T. Ravingerová[1], I. Waczulíková[3]
[1]*Institute for Heart Research, Slovak Academy of Sciences, Biochemistry, Bratislava, Slovakia*
[2]*Pharmacobiochemical Laboratory, Third Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia*
[3]*Division of Biomedical, Physics, Department of Nuclear Physics, Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia*

The effect of noninvasive remote ischemic preconditioning (RIP) on the functional remodelation of heart mitochondrial membrane and its cardioprotective contribution to ischemic-reperfusion injury was observed.

Methods: RIP was induced by short-term occlusion of the artery supplying the lower limb. Heart mitochondria were isolated and forwarded to biochemical and biophysical investigation performed after 15 minute stabilisated perfusion, 30 minute ischemia and 40 minute reperfusion. Activity of mitochondrial Mg2+-ATPase was determined spectrophotometrically as the concentration of anorganic phosphate formed by ATP splitting. Mitochondrial membrane fluidity was determined by fluorescence anisotropy. Content of oxidised isoforms of coenzyme Q (CoQ9ox a CoQ10ox) was measured by HPLC method.

Results: We noticed the significant ($p<0.05$) 5.05% increase in mitochondrial membrane fluidity of RIP group in comparison with the control group after reperfusion. RIP caused 6.95% increase in total mitochondrial Mg2+-ATPase activity after reperfusion compared to the control group. The nonsignificant increase in oxidised isoforms of coenzyme Q (CoQ9ox, CoQ10ox) during stabilisation induced by RIP reflects the moderate increase of free radicals having just a signal character and iniciates the protective mechamisms. In RIP group after ischemic-reperfusion load, the content of oxidized isoforms CoQ9ox was nonsignificantly reduced by 5.62% compared to control group after reperfusion phase of myocard.

Conclusions: Functional remodelation of mitochondrial membrane triggered by RIP effectively contributed to improved recovery of myocard after ischemic-reperfusion injury. Grants: APVV-15-0119; VEGA 2/0133/15, 2/0201/15.

C01-3
Hypertension and oxidant stress: Effects of angiotensin II receptor antagonists and calcium-channel antagonists on oxidant status in Algerian hypertensive men.

N. Malti[1], C. El Hassar[1], H. Merzouk[1], S. A. Merzouk[1], A. Meziane[1]
[1]*Laboratory of PPABIONUT, University of Tlemcen, Tlemcen, Algeria*

Questions: Oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and their detoxification by antioxidants, is involved in atherosclerosis and HTA. ROS are responsible for membrane lipid peroxidation, nitrosation / nitro-sylation of proteins, and increased intracellular calcium, impairing endothelial cell function. The effects of calcium antagonists (amlodipine) and angiotensin II receptor antagonists (telmisartan) on oxidative markers were investigated in Algerian hypertensive patients.

Methods: In this study, we included adult men patients with essential HTA. This study was a stratified, randomized, investigator-blinded trial that evaluated the effects of telmisartan monotherapy or amlodipine monotherapy in hypertensive adults was treated for the period of 1 year. At the beginning and after 1 year of antihypertensive therapy, adult patients with essential HTA were followed and oxidative markers (nitricoxide, superoxide anion, malondialdehyde and carbonyl proteins) were determined.

Results: The results of this study indicate that telmisartan and amlodipine are effective antihypertensive agents in the treatment of hypertension because a significant reduction in systolic and diastolic blood pressure was observed in all hypertensive patients after 1year of treatment. Our results show also that telmisartan and amlodipine treatments counteracted hypertension-dependent and oxidative stress. All hypertensive patients present high levels of pro-oxidant markers.

Conclusion: It seems reasonable to consider therapeutic agents with beneficial effects on blood oxidative stress markers, such as telmisartan and amlodipine. In addition, telmisartan, which reverses all redox changes associated with HTA, should be prescribed, especially in hypertensive patients with severe oxidative stress and its damages.

C01-4

Role of altered Ca^{2+} homeostasis during adverse cardiac remodeling after ischemia and reperfusion

A. Dominguez-Rodriguez[1], I. Díaz[1], E. Sánchez de Rojas-de Pedro[1], I. Mayoral-Gonzalez[1], A. Hmadcha[2], E. Calderón-Sánchez[1], J. Avila-Medina[1], A. M. Gomez[3], J. P. Benitah[3], A. Ordóñez[1], T. Smani[1]
[1]*Institute of Biomedicine of Seville, Seville, Spain*
[2]*CABIMER, Department of Stem Cells, Seville, Spain*
[3]*UMR S1180, Inserm, Univ. Paris-Sud, Université Paris-Saclay, Châtenay-Malabry, France*

Acute myocardial infarction (AMI) due to coronary artery occlusion represents a major cause of morbidity and mortality in humans. Increasing evidences demonstrated that despite successful reperfusion therapies, heart failure (HF) appears in ~ 10% of patients due to adverse ventricular remodeling. HF is characterized by dysfunction and abnormalities of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) handling with altered disturbed excitation-contraction coupling (EC-coupling). $[Ca^{2+}]i$ alteration is also involved in activation of Ca^{2+}-dependent transcription factors related to adverse cardiac remodeling.

Methods: $[Ca^{2+}]i$ handling was studied in rat model of I/R subjected to transient (40 minutes) ligation of left descending coronary artery. Changes in cytosolic ($[Ca^{2+}]cyto$) and intranuclear ($[Ca^{2+}]nuc$) were studied in a cardiac myocyte isolated from remote and infarted zone 1 week after surgery.

Results: Using echography and nuclear magnetic resonance we observed that rat undergoing I/R protocols have depressed cardiac contractile capacity as soon as one week after surgery. I/R treatment produces a decreased cytosolic ($[Ca^{2+}]cyto$) and intranuclear ($[Ca^{2+}]nuc$) transients in adult cardiomyocytes, not only in the risk zone but also in the remote zone. I/R treatment also induces significant reduction in sarcoplasmic reticulum Ca^{2+} content in both. These alterations were associated with changes in the expression of several ion channels both in remote and ischemic zones.

Conclusion: The calcium homeostasis undergoes significant changes during I/R, not only in the ischemic area, but also occurs in the remote area. These calcium changes may contribute to the development of adverse cardiac remodeling and further heart failure.

C01-5

Fluoxetine Attenuates Remote Myocardial Ischemia Reperfusion Injury

M. O. Yaman[1], I. Guner[1], H. Erman[2], O. E. Tok[3], M. Pala[4], M. Esrefoglu[3], R. Gelisgen[5], H. Uzun[5], N. Yelmen[1], G. Sahin[1]
[1]*University of Istanbul, Cerrahpasa Medical Faculty, Physiology, Istanbul, Turkey*
[2]*Medeniyet University, Istanbul, Turkey*
[3]*Bezmialem University, Histology and Embryology, Istanbul, Turkey*
[4]*Biruni University, Physiology, Istanbul, Turkey*
[5]*University of Istanbul, Cerrahpasa Medical Faculty, Biochemistry, Istanbul, Turkey*

Questions: Aortic ischemia reperfusion is an important factor in development of postoperative acute cardiac injury following abdominal aortic surgery. Reactive oxygen species has been implicated as a corner stone of reperfusion injury. The aim of the study is to answer the questions; what are the antioxidant effects of fluoxetine (Flx) in the context of ischemia – reperfusion (IR) injury and what are its effects on cardiac function and cellular integrity?

Methods: Male Wistar rats were divided into 3 groups (n=7 per group): 1) control; 2) IR by occlusion of infrarenal abdominal aorta (60-min ischemia and 120-min reperfusion); 3) Flx+IR (20 mg/kg/d, i.p. for 3 d before surgery). The serum creatine kinase (CK) and creatine kinase-MB (CK-MB) levels were considered as cardiac function markers. Lipid hydroperoxide (LOOH), malondialdehyde (MDA), superoxide dismutase activity (Cu,Zn-SOD), glutathione peroxidase (GSH), pro-oxidant antioxidant balance (PAB) and ferric reducing/antioxidant power (FRAP) levels were determined. Tissue leucocytes infiltration and cellular integrity were assessed histologically.

Results: IR led to a significant increase in CK and CK-MB, LOOH, PAB, MDA levels ($p<0.01$) and a decrease in FRAP, GSH, SOD levels ($p<0.01$). Flx was able to restore these parameters significantly. CK, CK-MB and MDA levels were decreased ($p<0.05$), along with LOOH and PAB levels ($p<0.01$) while FRAP, GSH, SOD levels were found increased compared to IR ($p<0.01$, $p<0.01$, $p<0.001$). Flx attenuated the disruption in cellular integrity induced by IR.

Conclusions: Our study clearly demonstrates that fluoxetine confers protection against aortic IR-induced cardiac injury, tissue leucocyte infiltration and cellular integrity.

C01-6

Benefitial effect of molecular hydrogen and hypoxic postconditioning on ischemia reperfusion injury of isolated rat hearts

M. Zálešák[1], J. Graban[1], B. Kura[1], D. Pancza[1], T. Ravingerová[1], J. Slezák[1]
[1]*Institute for heart research, SAS, Department of Cardiovascular physiology and pathophysiology, Bratislava, Slovakia*

Molecular hydrogen (H2) is considered as a selective antioxidant able to react with strong oxidants and preserve cell signaling mediated by NO and superoxide radicals. This study aimed to verify whether H2 can potentiate protective effect of hypoxic postconditioning (HpostC) against ischemia-reperfusion (I/R) injury. Isolated rat hearts perfused with Krebs-Henseleit buffer (KHB) were exposed to 30-min global ischemia/120-min reperfusion. HpostC was induced by 4 cycles of 1-min perfusion with oxygen-free KHB intercepted by 1-min perfusion with normal KHB, while in H2+HPostC group, oxygen-free KHB was enriched with H2. Severity of I/R injury was evaluated by measurement of infarct size (IS) within the area at risk (AR) (IS/AR, TTC staining) and recovery of function. IS/AR was markedly reduced in HpostC group to 24.6 ± 0.9% compared with 38.7 ± 1.4% in non-conditioned controls, and even more significantly in H2+HPostC group (16.6 ± 0.8%; $P<0.05$ vs. both, controls and HPostC). Post-I/R recovery of systolic function (LVDP) was improved in H2+HpostC group: 53 ± 11% to the levels of statistical significance vs. 23 ± 1.6% in controls. End-diastolic pressure (LVEDP) was decreased in both conditioned groups to a similar level (HpostC: 22.1 ± 5.9 mmHg, H2+HpostC: 28.6 ± 5.6, both $P<0.05$ vs. 55.2 ± 6.9 mmHg in controls). Application of H2 potentiated the beneficial effect of HpostC. Grants: VEGA SR 2/0201/15, 2/0021/15, APVV-0102-11, APVV-0241-11, APVV-15-0376.

C01-7

THE EFFECTS OF ZOFENOPRIL ON CARDIAC FUNCTION AND PRO-OXIDATIVE PARAMETERS IN THE STREPTOZOTOCIN-INDUCED DIABETIC RAT HEART

V. Zivkovic[1], P. Ristic[2], I. Srejovic[1], T. Nikolic[3], I. Stojic[3], D. Ristic[4], V. Jakovljevic[1]
[1]*Faculty of Medical Sciences, University of Kragujevac, Physiology, Kragujevac, Serbia*
[2]*Military Medical Academy, Belgrade, Endocrinology, Belgrade, Serbia*
[3]*Faculty of Medical Sciences, University of Kragujevac, Pharmacy, Kragujevac, Serbia*
[4]*Military Medical Academy, Belgrade, Ophthalmology, Belgrade, Serbia*

Questions: Renin–angiotensin–aldosterone system is one of the main modulators of chronic hyperglycaemia while hyperglycaemia-induced oxidative stress is an important factor in diabetic cardiomyopathy. The present study was designed to assess heart performance in the early stage of diabetic cardiomyopathy development after 4 weeks of hyperglycemia, in the stage known as increased tissue RAAS activity.

Methods: Investigation was carried out on 24 adult male Wistar albino rats whose hearts were perfused according to Langendorff technique. We evaluated the influence of acute administration of zofenopril on myocardial function from rats with streptozotocin-induced diabetes mellitus (STZ-DM),

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with a special emphasis on cardiodynamic and oxidative stress parameters in diabetic rat hearts. Rats were divided randomly into two groups (12 animals per group): control nondiabetic animals (C) were healthy rats perfused with 1.5 μM of zofenopril, and STZ-treated diabetic animals were diabetic animals perfused with 1.5 μM of zofenopril 4 weeks after the induction of diabetes.

Results: STZ-induced diabetic rats are characterized by a depressed cardiac performance and that these changes seems to not be mediated by via in oxidative stress. However, acute application of zofenopril failed to improve these hyperglycemia-induced changes of cardiac function.

Conslusions: Long-term follow-up intervention trials are necessary to fully demonstrate the benefit of zofenopril in this context.

Key words: zofenopril, cardiac function, diabetic rat heart

C01-8

THE LONG-TERM EFFECTS OF ATORVASTATIN ON OXIDANT/ANTIOXIDANT STATUS OF HYPEHOMOCYSTEINEMIC RATS

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

Questions
The objective of our study was to evaluate the association between atorvastatin administration 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 (superoxide 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). Atorvastatin was administrated daily for 4 weeks, 3 mg/kg i.p.

Results
After 4-wk feeding with purified diets, blood concentrations of the antioxidant GSH in blood were significantly affected, as well as CAT activity and parameters of lipid status ($p<0.05$). We found significant differences between the body weights and food intakes among all groups ($p<0.05$) and strong positive correlation between Hcy levels, prooxidative and lipid parameters, and negative correlation with antioxidant parameters in blood after administration of atorvastatin ($p<0.05$).

Conclusions
Atorvastatin could inhibit progression at any stage of oxidative stress and should therefore be proactively administered to the patient with dyslipidemia and hyperhomocysteinemia, regardless of disease severity.

Key words: HMG-CoA reductase inhibitors, homocysteine, oxidative stress

C01-9

THE EFFECTS OF CHRONIC ADMINISTRATION OF CISPLATIN ON OXIDATIVE STRESS IN ISOLATED RAT HEART

J. Jeremic[1], I. Stojic[1], T. Nikolic[1], J. Smigic[2], V. Zivkovic[2], I. Srejovic[2], T. Sabo[3], V. Jakovljevic[2]
[1]*Faculty of Medical Sciences, University of Kragujevac, Department of Pharmacy, Kragujevac, Serbia*
[2]*Faculty of Medical Sciences, University of Kragujevac, Department of Physiology, Kragujevac, Serbia*
[3]*Faculty of Chemistry, University of Belgrade, Department of General and Inorganic chemistr, Belgrade, Serbia*

Questions
Taken into consideration that molecular and cellular mechanisms involved in cardiotoxicity are still not clear, the aim of this study was to compare the production of oxidative stress parameters in the isolated rat heart between animals chronically treated with cisplatin and saline.

Methods
The hearts of male Wistar albino rats (n = 24, 12 per group, age 8 weeks, body mass 250±50 g) were excised and perfused according to the Langendorff technique at gradually increased coronary perfusion pressures (40-120 cmH2O). Over the entire CPP range, we measured levels of superoxide anion radicals, hydrogen peroxide, nitrites and index of lipid peroxidation in order to determine if oxidative stress is involved in coronary endothelium response in conditions of hypoxia (lower than 60 cm H2O) and hyperoxia (higher than 80 cm H2O).

Results
Levels of superoxide anion radicals, hydrogen peroxide, nitrites and index of lipid peroxidation were significantly altered ($p<0.05$). Higher levels of CPP increased the values of oxidative stress.

Conclusion
We can conclude that damaged endothelium of cisplatin-treated animals had weaker response to hyperoxia and also lower antioxidant capacity. This increment is more prominent in control group as a result of preserved endothelium and its more powerful response to hyperoxia.

Keywords: cisplatin, isolated rat heart, oxidative stress

C01-10

THE EFFECTS OF MODULATION OF N-METHYIL-D-ASPARTATE RECEPTORS ON OXIDATIVE STATUS IN ISOLATED RAT HEART

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

The role of N-methyl-D-aspartate receptor (NMDA-R) in cardiovascular system is not fully understood jet. The aim of the present study was to examine the effects of MK-801, as a NMDA-R blocker, alone and its combination with glycine and/or glutamate on oxidative status in isolated rat heart. The hearts of male Wistar albino rats were excised and perfused according to Langendorff technique and in samples of coronary venous effluent were spectrophotometrically determined values of biomarkers of oxidative stress – index of lipid peroxidation measured as TBARS, nitrites (NO_2^-), superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2). Only in group treated with MK-801, glutamate and glycine there was an increase in value O_2^-, while in all other groups all other measured biomarkers of oxidative stress were decreased or remained unaltered. Based on the obtained results it can be concluded that NMDA-R activation allows the entry of certain quantities of calcium and thus influence the redox balance in myocardium.

C01-11

EFFECT OF MATURATION ON RESISTANCE OF RAT HEARTS TO ISCHEMIA AND EFFECTS OF CLASSICAL AND REMOTE ISCHEMIC PRECONDITIONING. STUDY OF POTENTIAL MOLECULAR MECHANISMS

L. Griecsova[1], V. Farkasova[1], L. Lonek[1], I. Gablovsky[1], I. Bernatova[2], T. Ravingerova[1]
[1]*Institute for Heart Research SAS, Department of cardiovascular physiology and pathophysiology, Bratislava, Slovakia*
[2]*Institute of Normal and Pathological Physiology SAS, Bratislava, Slovakia*

Questions: Aging affects tolerance to ischemia/reperfusion (IR), however, its onset and cellular mechanisms behind are less known. Blunting of ischemic preconditioning (IPC) and defects in protective signaling are suggested. Although remote IPC (RIPC) protects young and aged human hearts, its age-dependency in animals is less explored.

Methods: We studied response to IR, effects of IPC and RIPC in isolated hearts of juvenile, younger and mature adult (1.5-, 3-, and 6-month-old) rats exposed to 30-min I/120-min R, and proteins of "pro-survival" pathways. IPC was induced by 1 cycle of IR, 5 min each. RIPC was evoked by pressure cuff inflation (200 mmHg)/deflation (3 cycles, 5 min each) on hind limb. We measured infarct size (IS), arrhythmias and contractile recovery (LVDP), levels of Akt, phosphorylated Akt (p-Akt), endothelial NO synthase (eNOS) and protein kinase Cε (PKCε) (WB).

Results: Maturation impaired response to lethal injury and promoted arrhythmogenesis. IPC reduced arrhythmias occurrence, IS and improved LVDP recovery in younger animals, while its effect was attenuated in mature ones. Loss of protection was associated with age-dependent decrease in p-Akt, eNOS and PKCε in the hearts of mature animals, and with a failure of IPC to upregulate these proteins. RIPC also reduced severity of arrhythmias, IS and improved LVDP recovery in younger rats. However, protection was preserved even in the mature adults coupled with upregulation of all selected proteins.

Conclusions: Maturation starts to impair the resistance of rat hearts against IR injury and causes gradual loss in IPC efficiency, while RIPC appears more effective and easily performed clinically relevant intervention.

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C01-12

EMAP II provides restoration of heart function in Langendorff ischemia-reperfusion model.

R. Fedichkina[1], Y. Goshovska[1], A. Kornelyuk[2], V. Sagach[1]
[1]*Bogomoletz Institute of Physiology, Circulation, Kyiv, Ukraine*
[2]*Institute of Molecular Biology and Genetics, Kyiv, Ukraine*

Endothelial monocyte-activating polypeptide (EMAP) II is a proinflammatory cytokine that is released from apoptotic and hypoxic cells. EMAP II negatively modulates lung neovascularization. Others data suggest that EMAP II stimulates vasodilatation via iNOS activation. However, the role of EMAP II in ischemia-reperfusion is not highlighted. The aim of our study was to examine the effect of EMAP II at heart function recovery in ischemia-reperfusion model. We used male Wistar rats aged 6 month. Recombinant human protein EMAP II in dose of 30 mkg/kg was injected in tail vein. After 30 min rats were sacrificed and hearts were perfused by Langendorff preparation. We registered contractile activity, coronary flow and oxygen consumption. Hearts were subjected to 20 min ischemia followed by 40 min of reperfusion. EMAP II prevented myocardial contracture during ischemic period and strongly supported restoration of left ventricular pressure that averaged 90% during all the reperfusion vs 30% in control rats. Notably, there was 25% increase of coronary flow right after reperfusion: we observed reaction of reactive hyperemia after perfusion renovation. As a result oxygen cost of myocardial work did not changed significantly comparing to control where it was 4 time increase

indicating non-effective oxygen utilization and ROS formation. Thus, EMAP II seems to be perspective tool for development of anti-ischemic approach against contracture and non-effective oxygen utilization by myocardium.

C01-13

Oxidative stress and deficient of nitric oxide synthesis as possible reasons of impaired Frank-Starling low in rat heart due to prolonged lighting

Y. Goshovska[1], V. Sagach[1]
[1]*Bogomoletz Institute of Physiology, Circulation , Kyiv, Ukraine*

Prolonged lighting (PL) as a result of sleep deprivation is known to decrease melatonin synthesis which contributes to the cardiovascular control. We hypothesized that PL induce disturbances of oxidative metabolism and NO production at mitochondrial level.

Wistar male rats were exposed to 24h-lighting for 1 and 3 weeks. Hearts were perfused by Langendorff preparation. We studied dependence of left ventricular pressure from volume (PV, Frank-Starling low). Activities of NO synthases as well as generation rate of reactive oxygen species in cardiac mitochondria were measured. PCR analysis for UCP3 expression was used.

PL for 1 week resulted in a pronounced impairment of heart function. The contractile activity (dP/dtmax) as well as coronary flow was decreased. Lowering of dP/dtmin indicated the impairment of diastolic function. Negative impact of PL was aggravated after 3 weeks. The coronary flow was reduced by 43%; the heart rate was slowed by 21%. 1 week of PL for did not affect the shape of PV curve. However, disturbances of heterometric regulation were significant after 3 weeks. The functional changes were accompanied with increased O2- and *OH (by 4.4- and 4-times respectively) in cardiac mitochondria. The activity of constitutive NO synthase was 3-times decreased. As a result, the level of NO2- was decreased by 34%. The 5-times increase of inducible NO synthase activity was accompanied with increase in NO3- content by 19%. Notable downregulation of cardiac UCP3 gene expression ($P<0.01$) was observed right after 1 week as well as after 3 weeks.

Deficient of NO synthesis and increased reactive oxygen species in cardiac mitochondria might underlie PL-induced heart function disturbances and decreased adaptive abilities of myocardium.

C02: Vascular physiology

C02-1

Impaired expression of voltage-gated K+ channel during early phase of diabetes in the rat mesenteric arterial smooth muscle

W. S. Park[1]
[1]*Kangwon National University School of Medicine, Department of Physiology, Chuncheon, South Korea*

This study investigated the alteration of voltage-dependent K+ (Kv) channels in mesenteric arterial smooth muscle cells from control (LETO) and diabetic (OLETF) rats during the early and chronic phases of diabetes. In the early phase of diabetes, the amplitude of mesenteric Kv currents induced by depolarizing pulses was greater in OLETF rats than in LETO rats. The contractile response of the mesenteric artery induced by the Kv inhibitor, 4-aminopyridine (4-AP), was also greater in OLETF rats. The expression levels of most Kv subtypes were increased in mesenteric arterial smooth muscle from OLETF rats compared with LETO rats. However, in the chronic phase of diabetes, the Kv current amplitude did not differ between LETO and OLETF rats. In addition, the 4-AP-induced contractile response of the mesenteric artery and the expression of Kv subtypes did not differ between the two groups. In summary, the increased Kv current amplitude and Kv channel-related contractile response

were attributable to the increase in Kv channel expression during the early phase of diabetes. The increased Kv current amplitude and Kv channel-related contractile response were reversed during the chronic phase of diabetes.

C02-2

The vasodilatory effect of repaglinide, a member of meglitinide class anti-diabetic drugs, via activation of PKG and PKA in aortic smooth muscle

M. S. Seo[1], **W. S. Park**[1]
[1]*Kangwon National University School of Medicine, Physiology, Chuncheon, South Korea*

We investigated the vasorelaxant effect of repaglinide and its related signaling pathways using phenylephrine (Phe)-induced pre-contracted aortic rings. Repaglinide induced vasorelaxation in a concentration-dependent manner. The repaglinide-induced vasorelaxation was not affected by removal of endothelium. Pre-treatment with adenylyl cyclase inhibitor or the PKA inhibitor effectively reduced repaglinide-induced vasorelaxation. Also, pretreatment with guanylyl cyclase inhibitor or the PKG inhibitor effectively inhibited repaglinide-induced vasorelaxation. However, pretreatment with voltage-dependent K+ channel inhibitor (4-AP), ATP-sensitive K+ channel inhibitor (glibenclamide), big-conductance Ca2+-activated K+) channel inhibitor (paxilline), and the inwardly rectifying K+ channel inhibitor (Ba2+) did not affect the vasorelaxant effect of repaglinide. Furthermore, pretreatment with Ca2+ inhibitor (nifedipine) and SERCA inhibitor (thapsigargin) also did not affect the vasorelaxant effect of repaglinide. From these results, we concluded that repaglinide induced vasorelaxation by activation of adenylyl cyclase/PKA and guanylyl cyclase/PKG signaling pathway independently of endothelium, K+ channels, Ca2+ channel and intracellular Ca2+ ([Ca2+]i).

C02-3

Inhibitory effect of nortriptyline, a tricyclic antidepressant, on voltage-dependent K+ channels in coronary arterial smooth muscle cells

S. E. Shin[1], **W. S. Park**[1]
[1]*kangwon National University School of Medicine, Department of physiology, ChunCheon, South Korea*

We demonstrated the effect of nortriptyline, a tricyclic antidepressant drug and serotonin reuptake inhibitor, on voltage-dependent K+ (Kv) channels in freshly isolated rabbit coronary arterial smooth muscle cells using a whole-cell patch clamp technique. Nortriptyline inhibited Kv currents in a concentration-dependent manner, with an apparent IC50 value of 2.86 ± 0.52 μM and a Hill coefficient of 0.77 ± 0.1. Although application of nortriptyline did not changethe activation curve, nortriptyline shifted the inactivation current toward a more negative potential. Application of train pulses (1 or 2 Hz) did not change the nortriptyline-induced Kv channel inhibition, suggesting that the effects of nortiprtyline were not use-dependent. Preincubation with the Kv1.5 and Kv2.1/2.2 inhibitors, DPO-1 and guangxitoxin did not affect nortriptyline inhibition of Kv channels. From these results, we concluded that nortriptyline inhibited Kv channels in a concentration-dependent and state-independent manner by changing the steady-state inactivation curves independently of serotonin reuptake.

C02-4

The vasorelaxant effect of nateglinide, a member of meglitinide class of anti-diabetic drugs, via activation of voltage-gated K+ channels in aortic smooth muscle

H. Li[1], **W. S. Park**[1]
[1]*Kangwon National University School of Medicine, Department of Physiology, Chuncheon, South Korea*

We investigated the vasorelaxant effect of nateglinide using phenylephrine-induced pre-contracted aortic rings. The application of nateglinide induced vasorelaxation in a concentration-dependent manner. Pretreatment with the BKCa channel inhibitor paxilline, Kir channel inhibitor Ba2+, and KATP channel inhibitor glibenclamide, did not affect the vasorelaxant effect of nateglinide. However, pretreatment with the Kv channel inhibitor 4-AP, effectively reduced the vasorelaxant effect of nateglinide. Pretreatment with the Ca2+ inhibitor nifedipine and the SERCA inhibitor thapsigargin did not change the vasorelaxant effect of nateglinide. Additionally, the vasorelaxant effect of nateglinide was not altered in the presence of an adenylyl cyclase, a protein kinase A, a guanylyl cyclase, or a protein kinase G inhibitor. The vasorelaxant effect of nateglinide was not affected by the elimination of the endothelium. In addition, pretreatment with a nitric oxide synthase inhibitor, L-NAME, and a SKCa channel inhibitor, apamin did not change the vasorelaxant effect of nateglinide. From these results, we concluded that nateglinide induced vasorelaxation via the activation of the Kv channel independent of other K+ channels, Ca2+ channels, intracellular Ca2+ ([Ca2+]i), and the endothelium.

C02-5

The inhibitory effect of dapoxetine, a selective serotonin reuptake inhibitor on voltage-gated K+ channels in rabbit coronary arterial smooth muscle cells

J. R. An[1], **W. S. Park**[1]
[1]*Kangwon National University School of Medicine, Department of Physiology, Chuncheon, South Korea*

We investigated the inhibitory effect of dapoxetine, a selective serotonin reuptake inhibitor (SSRI), on voltage-dependent K+ (Kv) channels using native smooth muscle cells from rabbit coronary arteries. Dapoxetine inhibited Kv channel currents in a concentration-dependent manner, with an IC50 value of 2.68 ± 0.94 mM and a slope value (Hill coefficient) of 0.63 ± 0.11. Application of 10 mM dapoxetine accelerated the rate of inactivation of Kv currents. Although dapoxetine did not modify current activation kinetics, it caused a significant negative shift in the inactivation curves. Application of train step (1 or 2 Hz) progressively increased the inhibitory effect of dapoxetine on Kv channels. In addition, the recovery time constant was extended in its presence, suggesting that the longer recovery time constant from inactivation underlies a use-dependent inhibition of the channel. From these results, we conclude that dapoxetine inhibits Kv channels in a dose-, time-, use-, and state (open)-dependent manner, independent of serotonin reuptake inhibition.

C02-6

Direct inhibition of the class III anti-arrhythmic agent, amiodarone on voltage-dependent K+ channels in coronary arterial smooth muscle cells from rabbit

H. Li[1], S. E. Shin[1], M. S. Seo[1], J. R. An[1], W. S. Park[1]
[1]*Kangwon National University School of Medicine, Department of Physiology, Chuncheon, South Korea*

We examined the inhibitory effect of amiodarone, a class III anti-arrhythmic agent, on voltage-dependent K+ (Kv) currents in freshly isolated rabbit coronary arterial smooth muscle cells, using a whole-cell patch clamp technique. Amiodarone inhibited Kv currents in a concentration-dependent manner, with a half-maximal inhibitory concentration (IC50) value of 3.9 ± 1.44 μM and a Hill coefficient of 0.45 ± 0.14. Amiodarone did not have a significant effect on the steady-state activation of Kv channels, but shifted the inactivation current toward a more negative potential. Application of consecutive pulses progressively augmented the amiodarone-induced Kv channel inhibition. Another class III anti-arrhythmic agent, dofetilide, did not inhibit the Kv current or change the inhibitory effect of amiodarone on Kv channels. Therefore, these results strongly suggest that amiodarone inhibits Kv currents in a concentration- and state-dependent manner.

C02-7

$Ca_V1.2$ L-type Ca^{2+} channel form a signal complex with Orai1 and TRPC1 in vascular smooth muscle cells: Role in vascular tone regulatio

J. Avila-Medina[1,2,3], E. Calderon-Sanchez[2,3], P. Callejo-García[2], J. A. Rosado[4], **T. Smani**[1,2,3]
[1]*University of Seville/Institute of Biomedicine of Seville, Medical Physiology and Biophysic, Sevilla, Spain*
[2]*Institute of Biomedicine of Seville, Groupo de Fisiopatología Cardiovascular, Sevilla, Spain*
[3]*CiberCV, Madrid, Spain*
[4]*University of Extremadura, Physiology, Caceres, Spain*

Rationale: Voltage-dependent $Ca_V1.2$ L-type Ca^{2+} channels (LTCC) are considered the main route for calcium entry in vascular smooth muscle cells (VSMCs). However, independent studies have determined the relevant role of store-operated Ca^{2+} channels (SOCC), formed by Orai1 and TRPC1, in vascular tone regulation.

Objective: We aimed to characterize the crosstalk between Orai1- and TRPC1- dependent SOCC and $Ca_V1.2$ LTCC in VSMCs isolated from mice aorta and rat coronary artery.

Methods and results: Serotonin (5-HT) and endothelin-1 (ET-1) evoked significant vasoconstriction and intracellular Ca^{2+} increase in aorta and coronary artery isolated from mice and rat respectively. The induced vasoconstriction was sensitive to the widely used inhibitors of LTCC and SOCC. Immunofluorescence experiments using proximity ligation assay (PLA) determined that both Orai1 and TRPC1 share the same subcellular microdomains and interact with $Ca_V1.2$ both in aortic and coronary VSMCs. Interestingly, Orai1 and TRPC1 enhanced their interaction with $Ca_V1.2$ upon VSMCs with agonists or upon store depletion with thapsigargin.

Conclusions: Our data suggest that vasoactive agonists promote vessel contraction by co-activation of $Ca_V1.2$-dependent LTCC and SOCC channels formed by Orai1 and TRPC1.

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Keywords: $Ca_V1.2$; Orai1; TRPC1, Store depletion; Vascular tone regulation.

C02-8

Effects of PCSK9 inhibitor in obese Zucker (fa/fa) rats.

M. Kosutova[1], R. Rehakova[1], M. Cebova[1], Z. Matuskova[1], O. Pechanova[1]
[1]*Institute of Normal and Pathological Physiology Slovak Academy of Sciences, Bratislava, Slovakia*

Proprotein convertase subtilisin/kexin type (PCSK9) is an enzyme that binds to the LDL receptors. If PCSK9 is blocked, more LDLRs are recycled and are presented on the cell surface to remove LDL-particles from the extracellular fluid. Therefore, blocking PCSK9 can lower blood LDL-particle concentrations.
Male obese Zucker (fa/fa) rats and Zucker lean (lean) rats, aged 12 weeks were divided into three groups: Zucker (lean) - control, Zucker (fa/fa) – obese control, Zucker fa/fa – treated with inhibitor of PCSK9 (iPCSK9), n=6 in each group. Inhibitor of PCSK9 was administrated intraperitoneally three times during six weeks (10 mg/kg per one application). Blood pressure was measured by the tail-cuff-plethysmography. Lipid profile was analysed in the plasma and concentration of conjugated dienes (CD, marker of lipid peroxidation) was measured in the kidney and liver. Total nitric oxide synthase (NOS) activity was examined by measuring the rate of conversion from [3H]L-arginine to [3H]L-citrulline in the heart, aorta and kidney. Protein expression of NOS isoforms were determined by Western blot analysis in the same tissues. Administration of iPCSK9 decreased LDL-cholesterol in obese Zucker (fa/fa) rats without affecting other components of lipid profile.

Moreover, iPCSK9 was able to reduce CD concentration in the kidney and liver and increase NOS activity in the aorta, however, without affecting blood pressure yet. In conclusion, the increase of NOS activity, in addition to reducing LDL-cholesterol and lipid peroxidation, may contribute to the beneficial effects of iPCSK9 during hypercholesterolemic conditions.

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C02-9

Protective effects of nanoparticle-loaded renin inhibitor in experimental hypertension

O. Pechanova[1], M. Cebová[1], R. Rehakova[1], S. Vrankova[1], A. Barta[1]
[1]*Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Department of Neuro-cardiovascular Interactions, Bratislava, Slovakia*

Introduction: Despite beneficial effects, clinical use of renin inhibitor - aliskiren is limited by short lifetime of this drug. We aimed to determine the effects of nanoparticle-loaded aliskiren, with gradually realized drug, on blood pressure (BP), nitric oxide synthase (NOS) activity, and structural alterations developed due to hypertension.

Materials and methods: 12-week-old male SHRs were divided to the untreated group, group treated with powdered aliskiren, or nanoparticle-loaded aliskiren (25mg/kg per day), and nanoparticles only for 3 weeks by gavage. NOS activity including isoforms expressions, and collagen and elastin contents were determined in both heart and aorta. Wall thickness (WT), inner diameter (ID) and cross sectional area (CSA) were determined in the aorta.

Results: At the end of experiment, BP was lower in both powdered aliskiren and nanoparticle-loaded aliskiren groups with more pronounced effect in the second one. Moreover, nanoparticle-loaded aliskiren was able to decrease collagen content (by 11%) and CSA (by 25%) in the aorta in comparison to the powdered aliskiren group, while it had no significant effect on the similar parameters in the heart. There were no significant changes in the elastin content, WT and ID among aliskiren groups and control group. Only nanoparticle-loaded aliskiren increased the activity of NOS in the heart (7.4±0.4 pkat/g) and aorta (9.8±0.5 pkat/g) in comparison to the untreated SHR (5.1±0.3 pkat/g and 7.0±0.5 pkat/g, respectively).

In conclusion, nanoparticle-loaded aliskiren seems to be promising drug in blood vessel protection during hypertensive conditions.

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C02-10

Ranolazine improves vascular sensitivity to insulin in rabbit femoral arteries.

C. Aldasoro[1], S. Guerra Ojeda[2], A. Jorda[2], P. Marchio[2], M. Gimeno-Raga[2], M. D. Mauricio[2], S. Valles[2], M. Aldasoro[2], J. M. Vila[2]
[1]*Hospital General de Castellon, Medicina Familiar y Comunitaria, Castellon, Spain*
[2]*University of Valencia, Physiology, Valencia, Spain*

Questions: Insulin resistance impairs vascular function through an imbalance between vasoconstrictor and vasodilator pathways, and by increasing reactive oxygen species production. Ranolazine, a late Na+ current (I NaL) blocker, improves glycemic control and reduces HbA1c in type II diabetic patients. Thus, the purpose of the present study was to evaluate if three different I NaL blockers (GS967, GS6615 and ranolazine) enhance vascular sensitivity to insulin.

Methods: Rabbit femoral artery rings were mounted for isometric tension recording in organ baths. In rings pre-contracted with noradrenaline (10−6 M), cumulative concentration curves of insulin (10−13 to

10−7 M) were constructed in the absence and presence of ranolazine (10-6M), GS967 (3x10-7M) and GS6615 (3x10-7M).

Results: *Insulin induced a c*oncentration–dependent *relaxant* response in rings pre-contracted with noradrenaline (Emax = 43.5 ±8.3). Vascular relaxation to insulin was blocked by GS967 (Emax = 14.8 ± 16.9) but not by GS6615 (Emax = 50.3 ± 3.9). However, ranolazine enhanced vascular response to insulin (Emax = 64.9 ± 5.6).

Conclusions: *Ranolazine enhances vascular relaxant effects induced by insulin in rabbit femoral arteries and this effect seems to be independent of* I NaL *blockade.*

C02-11

Renal vascular Kv7.1 channels – potential targets for renoprotection

R. Schubert[1], F. Stocker[1], S. Braun[1], N. Schmidt[1]
[1]*Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany*

Question: Vascular smooth muscle Kv7 channels, mainly Kv7.4 and Kv7.5, have been shown to contribute to vasoconstriction and vasodilation. However, Kv7.1 channel function is largely unexplored. Thus, this study addressed the hypothesis that Kv7.1 channels contribute to blood flow regulation in the renal vasculature, a vascular bed with high expression of Kv7.1 channels.

Methods: Wistar rat renal segmental arteries and intact kidneys were studied using real-time qPCR, isometric vessel myography and constant-flow organ perfusion.

Results: In renal arteries, Kv7.1 channel mRNA expression was at a similar level compared to Kv7.4 and Kv7.5 channels. The Kv7.1 channel opener R-L3 reduced methoxamine (MX)-induced contraction of isolated vessels. This effect was inhibited by the pan-Kv7 channel blocker XE991 and by HMR1556, a selective Kv7.1 channel blocker. HMR1556 alone was without effect on MX-induced contraction. The Kv7.2-7.5 channel opener retigabine reduced MX-induced contractions. This effect was abolished by XE991 but was not affected by HMR1556, pointing to the absence of Kv7.1-Kv7.x heteromultimeric channels. Neither HMR1556 nor XE991 affected the anti-contractile effect of the cGMP-coupled vasodilator ANP or the cAMP-coupled vasodilator urocortin. In intact kidneys, R-L3 reduced MX-induced increases in perfusion pressure. This effect was inhibited by XE991 and HMR1556. HMR1556 alone was without effect on MX-induced increases in perfusion pressure.

Conclusion: The results show that opening of renal vascular Kv7.1 channels facilitates kidney blood flow without altering vasoconstrictor- and vasodilator-induced blood flow adaptation suggesting that these channels may serve as targets for renoprotection.

C02-12

The Effects of Nifedipine in Heart Injury Induced by Renal Ischemia Reperfusion

A. tanyeli[1], E. ERASLAN[1], E. polat[2], E. polat[3], N. kurt[2]
[1]*Atatürk University, Physiology, Erzurum, Turkey*
[2]*ataturk university, biochemistry, erzurum, Turkey*
[3]*ataturk university, histology and embryology, erzurum, Turkey*

Aim; It has been shown that acute renal injury may lead to dysfunction in far organs like the heart and liver. Ischemia Reperfusion (I/R) borne injury might occur due to the increase and activation of leucocytes, release of reactive oxygen types like hydrogen peroxide (H2O2), and intracellular calcium (Ca2+) increase. In our study, we examined the effects of nifedipine, which is a nonspecific calcium channel antagonist, in heart injury induced by Renal I/R by determining some oxidative stress markers

and the CD38 and cyclic adenosine diphosphate ribose (cADPR) levels that have roles in intracellular calcium regulation.

Methods; 24 Wistar Albino male rats weighing 240-260g were used in our study. 4 groups were formed each of which had 6 animals. The 1st Group was the Control Group (C). In the 2nd Group, the Sham (S) Group; right kidney was dissected. In the 3rd Group (I/R), 1hour ischemia 24hour reperfusion were applied to the left kidney after the right kidney was dissected. In the 4th Group (N), the same surgical procedures were applied as in the 3rd Group, and 4mg/kg nifedipine was administered intraperitoneally before the reperfusion started. The statistical analyses and the results are given as mean±SD. The differences were compared with the Tukey Post Hoc Analysis following the One-Way ANOVA test.

Results; It was observed that applying nifedipine in heart injury occurring due to renal I/R decreased the MDA, SOD, MPO and H2O2 levels in the group which received nifedipine, when compared with the I/R Group, and increased the GSH, Cat, CD38 and cADPR levels; however, these changes are not significant. In the histological examinations; the renal injury increasing with I/R, Caspase-3 expression have decreased with the application of calcium canal antagonists.

Key words: İschemia reperfusion, Nifedipine, Oxidative Stress, Calcium

This study was supported by Atatürk University SRP (Project no: 2014/146).

C03: Molecular & cellular physiology

C03-1

Iron oxide nanoparticles increase nuclear textural entropy in buccal epithelial cells

I. Pantic[1,2]
[1]*University of Belgrade, Faculty of Medicine, Institute of Medical Physiology, Belgrade, Serbia*
[2]*University of Haifa, Haifa, Israel*

Questions: Although it is known that iron oxide nanoparticles (IONPs) have certain toxic potential in cells and tissues, many issues regarding their interaction with cell nucleus remain unclear. In this study, we demonstrate that certain parameters of nuclear texture of buccal epithelial cells (BECs) change after exposure to IONPs in *in vitro* conditions.

Methods: Human BECs were kept in RPMI-1640 medium at 37^{o}C, with the addition of L-glutamine. The cells were put in special chamber/slides for tissue culture (Lab-Tek, IL, USA) and treated with magnetite, Fe3O4 nanoparticles (spherical shape, diameter 80-100 nanometers, 120 mg/L). Digital micrographs of the cell nuclei (50 nuclei of treated, and 50 of untreated, control cells) were made with Pro-DEM 200 High-Speed color CMOS Chip (Oplenic Optronics, Hangzhou, CN) mounted on optical microscope. Textural analysis was done using Grey level co-occurrence matrix algorithm. For each nucleus, average values of entropy, as well as angular second moment (ASM) and inverse difference moment (IDM), were calculated.

Results: Nuclear textural entropy of BECs significantly increased ($p<0.05$) after the treatment with iron oxide nanoparticles. Values of angular second moment, on the other hand, did not significantly change. Similarly, no significant change in average values of nuclear inverse difference moment was detected after the treatment ($p>0.05$).

Conclusions: Our study shows that iron oxide nanoparticles may, in some circumstances, increase the level of textural chaos and disorder of cell nucleus. This is the first study to demonstrate this phenomenon in buccal epithelial cells.

Keywords: Nucleus, Nanomaterial, Texture

C03-2

Gender-dependent expression of miRNA in human colorectal cancer and adjacent colonic tissues

K. Voglova[1], J. Bezakova[1], R. Reis[2], M. Vician[2], M. Zeman[1], I. Herichova[1]
[1]*Faculty of Natural Sciences Comenius University in Bratislava, Department of Animal Physiology and Ethology, Bratislava, Slovakia*
[2]*University Hospital, Comenius University Bratislava, First Surgery Department, Bratislava, Slovakia*

Key words: miR-21-5p, miR-21-3p, miR-16-5p

Questions: miRNAs are short regulatory non-coding RNA involved in post-transcriptional down-regulation of genes. Mature miRNA consists from a leading and a passenger strand. Co-existence and functionality of both miRNA strands have been reported recently. Deregulated levels of miRNAs were found in a variety of diseases including cancer. We focused on evaluation of the expression of miRNA in tumor and its comparison to the adjacent tissues and plasma levels.

Methods: The tissue and plasma samples from the patients with colorectal cancer were used. The tissue samples were taken from the tumor and proximal (min. 10cm above the tumor) and distal parts (2cm under the tumor) of resected colon. Expression of miR-21-5p, miR-21-3p and miR-16-5p was measured by Real Time PCR. miRNA expression profiling in the plasma, tumor and adjacent tissues was performed to identify changes in miRNA expression.

Results: We observed up-regulation of miR-21-5p, miR-21-3p and miR-16 in the tumor tissue compared to adjacent tissues. Tumors and adjacent tissues showed higher expression of miR-21-5p than miR-21-3p and positive correlation between them. The expression pattern exhibited gender-dependent differences in miRNA levels. miRNAs identified by profiling that showed different expression in the adjacent and cancer tissue were correlated with miRNA plasma levels.

Conclusions: Our findings indicate a gender-dependent expression of miRNA which should be considered as an important factor in generating new prognostic or diagnostic biomarkers.

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C03-3

Nanoparticles at the neurovascular unit: in vitro and in vivo studies to assess the blood-brain barrier permeability and function

G. Forcaia[1], R. Dal Magro[1], E. Cesana[1], B. Albertini[2], P. Blasi[2], F. Re[1], G. Sancini[1]
[1]*University of Milan Bicocca, School of Medicine and Surgery, Monza, Italy*
[2]*University of Perugia, Department of Pharmaceutical Sciences, Perugia, Italy*

The brain is always confronted with the dilemma of the protection from noxious substances from the blood and the delivery of vital metabolites. Endothelial cells, forming together with other cells the blood-brain barrier (BBB), are known as the “gatekeepers” of this trafficking. It is known that many common drugs cannot cross the BBB in appreciable concentrations, thus decreasing the rate of possible available treatments for many central nervous system (CNS) diseases. In the last decades, nanomedicine has increase its role in developing strategies to deliver drugs to the CNS. In our previous studies we administrated liposomes functionalized with phosphatidic acid and an ApoE-derived peptide as a potential treatment for Alzheimer’s disease (AD): their administration reduced brain beta-amyloid burden and ameliorated impaired memory in AD mice. Furthermore, we evaluated the adaptability of warm microemulsion process for ligand surface modification of solid lipid nanoparticles with ApoE to target the BBB and we investigated how the different administration routes affect their brain bioavailability.The aim of this study is to evaluate the interaction of lipid based nanoparticles (NPs) at the neurovascular unit. In light of our previous results we here assess the NPs interaction with human cerebral microvascular cells (hCMEC/D3) as in vitro BBB model and mice brain neuronal slices by means of patch clamp recordings and simultaneously calcium imaging measurements to follow calcium dinamics transients. Our studies of the NPs impact to the main neurophysiological functions should encourage further applications of NPs based drug delivery strategies for future clinical treatments of CNS diseases.

C03-4

In Vitro Cell Death Discrimination and Screening Method by Simple and Cost-Effective Viability Analysis.

K. Helm[1], M. Beyreis[1], C. Mayr[1,2], M. Ritter[1], M. Jakab[1], T. Kiesslich[1,2], K. Plätzer[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]*University of Salzburg, Department of Materials Science and Physics, Salzburg, Austria*

Questions:
There are two major different kinds of cell death: apoptosis and necrosis. Discrimination is essential for *in vitro* testing of potential drugs or signal transduction modifiers. Viability analysis performed at two different time points post treatment can provide valuable information after death induction because metabolic activity of apoptotic and necrotic cells is different. In this study this was verified by the use of specific caspase and membrane integrity tests.

Methods:
A431 (epidermoid carcinoma) cells were treated with 3 different established chemical apoptosis inducers (actinomycin-D, TBB, RO 31-8220), H2O2 and photodynamic treatment (PDT). Viability was measured 2 and 24 hours post treatment using the resazurin assay. Additionally, Caspase-Glo® 3/7 - and membrane integrity assays were conducted to verify apoptosis and necrosis and results of at least three independent experiments were plotted.

Results:
A difference curve between 2 and 24 hours of the resazurin measurements were calculated – the main features of the difference curve are: a positive difference signal indicates apoptosis while an early reduction of the viability signal indicates necrosis. This was confirmed by the results of the caspase and membrane integrity assays.

Conclusion:
Viability analysis at two different time points can provide clear and valuable information with minimal effort of time and financial resources about the concentration or dose ranges of a cytotoxic reagent where apoptotic or necrotic cell death appears.

C03-5

Progesterone and selective membrane progesterone receptor ligands as immunomodulators in human T-lymphocytes

A. Polikarpova[1], I. Levina[2], L. Kulikova[2], I. Morozov[3], P. Rubtsov[3], I. Zavarzin[2], A. Guseva[1], O. Smirnova[1], T. Shchelkunova[1]
[1]*Lomonosov Moscow State University, Faculty of Biology, Moscow, Russian Federation*
[2]*Zelinsky Institute of Organic Chemistry Russian Academy of Sciences, Moscow, Russian Federation*
[3]*Engelhardt Institute of Molecular Biology Russian Academy of Sciences , Moscow, Russian Federation*

Progesterone (P4) ensures pregnancy preservation and prevents allogeneic fetal rejection. The mechanism of P4 action on immune cells is not well understood. The effects of progestins are mediated both by nuclear (nPRs) and membrane receptors (mPRs) of the progestin and adipoQ receptor family. The mPRα and mPRβ are expressed in T-lymphocytes, whereas the nPRs expression is not detected. Among the synthesized compounds, we identified two selective ligands of mPRs that do not interact with nPRs: 19-hydroxypregn-4-en-20-one (I) and 19-hydroxypregn-3-en-20-one (II). We assessed the effects of these compounds and P4 on the levels of cytokines (IL-2, IL-10, TGF beta and TNF alpha) mRNA in Jurkat cells by means of qRT-PCR. Cells were stimulated with phorbol esters and incubated with hormones (1 to 50 μM) for 48 hours. 1-10 μM of any steroid did not significantly influence the cytokines mRNAs levels. 20 μM P4 and both selective ligands significantly reduced the TNF-alpha mRNA level (by about 30% compared to the control), 50 μM P4 reduced it even more, whereas I and II little changed their effects. The IL-2 mRNA level declined significantly after exposure to P4 and compound I at both concentrations, but not after the treatment with II. The IL-10 mRNA level significantly increased under the action of 50 μM P4 and compound II. None of the three steroids caused changes in the TGF-beta mRNA level. Therefore, progestins suppress the levels of pro-inflammatory TNF-alpha and IL-2 mRNA and augment the IL-10 mRNA level through mPRs in T-cells. The differences in effects of compounds I and II may be due to their different affinity for the mPR α and β subtypes, whereas P4 binds to both mPRs.

C03-6

Tolfenamic Acid Induces Apotosis by Increasing TNF-alpha Gene Expression in rat hepatocellular carcinoma cells

S. Akın[1], M. Özkurt[1], R. Uyar[1], S. Kabadere[1]
[1]*Eskişehir Osmangazi University, Physiology, Eskişehir, Turkey*

Question:
Tolfenamic acid (TA) is a non-steroidal anti-inflammatory drug that has shown to have apoptotic effect on many cancer cell lines. The aim of this study is to investigate the effect of TA on mRNA abundance of caspase3, IL-1beta, NfkappaB and TNF-alpha on rat hepatocellular carcinoma (H4IIE) cells.

Method:
We treated H4IIE cells with 10 and 50 μM dose of TA for 48 hours. After treatment, we collected the cells and just after total RNA were isolated using High Pure RNA Isolation Kit (Roche, Germany). cDNA was synthesized by using the reverse transcriptase cDNA synthesis kit (Roche Nano Lightcycler Roche Diagnostics, Mannheim, Germany). The abundance of caspase-3, IL-1beta, NfkappaB and TNF-alpha mRNA were analyzed using the beta-actin as a reference gene. Measurements were performed using a Roche Nano Lightcycler (Roche Diagnostics, Mannheim, Germany). Data were analyzed by relative quantification method, 2-ΔΔct calculation and statistical analyses were made by using one-way ANOVA and posthoc test TUKEY. Differences with P values <0,5 were considered significant.

Results
Caspase3, IL-1beta and NfkappaB mRNA abundance did not change significantly between the groups. However TNF-alpha mRNA abundance increased significantly in the 50 μM TA group when compared to control.

Conclusions :
The apoptotic effect of TA on cancer cell lines may be related to its transcriptive effect on TNF-alpha.

C03-7

The apoptotic effect of quercetin in human hepatoma cell line HEP3B that Nf-KB pathway suppressed by CAPE

M. Kasit[1], O. Doğanlar[1]
[1]*Trakya University, Faculty of Medicine, Medical Biology, Edirne, Turkey*

The present study was designed to investigate the antiproliferative efficiency and genetic mechanisms of the anticancer properties of caffeic acid phenethyl ester (CAPE) and Quercetin, single and combined treated to human hepatocellular carcinoma, Hep3B cell lines. After single and combined treatments with the CAPE and Quercetin, cell viability was monitored using the MTT assay, nectoptosis-apoptosis was observed following cell membrane staining by annexinV/propidium-iodide using a TALİ cytometer. The gene expression studies belong to NF-κB and mitochondrial apoptosis pathways were carried out using a qRT-PCR assay. Our results indicated that individually treated both CAPE and Quercetin dramatically reduces cell viability in dose and time depended manner and selectively induces caspase-dependent apoptosis via both CASP8 and CASP3 in Hep3B. NF-κB inhibition was observed significantly CAPE treated experimental groups. Combined application decreased death cell population and apoptosis rate in all experimental groups and IC50 dose of Quercetin 88 and 34 fold increased in CAPE IC50 and CAPE 2IC50 treated groups, respectively. The result of the present study emphasize that CAPE and Quercetin combination cause antagonistic effect in spite of individually treated CAPE and Quercetin are potential anticancer agent with sufficient antiproliferative effect and spesific apoptotic potential. (This work was supported by Research Fund of the Trakya University. Project Number: 2016/212)

C03-8

Trancriptional regulation of metabolic reactions in breast cancer cells

I. Ceslevičiene[1], I. Antanavičiūtė[1], V. Mikalayeva[1], G. Milašiūtė[1], V. A. Skeberdis[1], S. Bordel Velasco[1]
[1]*Lithuanian Uneversity of Health Sciences, Institute of Cardiology, Kaunas, Lithuania*

We found that the proliferation rate of cancer cell lines from the NCI-60 collection corellated with the expression of all the genes in the human metabolic network (Feizi and Bordel, 2013). The metabolic pathways showing highest correlation with cell proliferation resulted to be both lipid synthesis and degradation. Even if it was previously believed that these processes cannot coexist in the same cells, we hypothesized that this phenomenon could be involved in a shuttle of redox potential from the cytoplasm to the mitochondrion, in which reductive power from cytosolic NADPH is transferred to mitochondrial NADH. By comparing gene expression of cancer cell lines (in the NCI-60 collection) with 8 types of healthy stem cells. We observed that 5 enzymes involved in the degradation of valine, leucine and isoleucine were highly over-expressed. Using public data (Jain *et al.*, 2012) about uptake and secretion rates and a genome-scale human metabolic model we estimated the contribution of these 3 amino-acids to the total cellular ATP supply. We observed that this contribution is as important as the lactic fermentation.We silenced 3 genes (BCAT2, ECHS1, FASN) coding for metabolic enzymes involved in alternative supply of reductive potential for cancer cells. The silencing of these genes decreased significantly the proliferation of breast cancer cell lines (MDA-MB-231, MCF7 and BCC). We found that proliferation of cancer cells is impaired by the transcriptional supression of enzymes involved in alternative pathways to supply reductive potential to the mitochondrion. This is in agreement with our initial hypothesis and reveals new potential anti-cancer targets.

C03-9

Synthesis of New 1,1,3,3-Tetra(4′-oxy-3-substituted-chalcone)-5,5-diphenylcyclotriphosphazene Derivatives and Investigation of Their Anti-Cancer Activities

S. Tekin[1], î Tekin[2], K. Koran[3], A. O. Gorgulu[3], S. Sandal[1]
[1]*Inonu University, Physiology, Malatya, Turkey*
[2]*Inonu University, Public Health, Malatya, Turkey*
[3]*Firat University, Chemistry, Elazig, Turkey*

The compounds, so called phosphazene, contain phosphorous-nitrogen double bond. Phosphazenes are the largest class of inorganic macromolecules that cover small molecules through polymers depending on number of the repeating unit,-N=PX2-group, in their structure. In the present study, 1,1,3,3-tetra(4′-oxy-3-fluorochalcone)-5,5-diphenylcyclotriphosphazene **(1a),** 1,1,3,3-tetra(4′-oxy-3-chlorochalcone)-5,5-diphenylcyclotriphosphazene **(1b)** and 1,1,3,3-tetra(4′-oxy-3-bromochalcone)-5,5-diphenylcyclotriphosphazene **(1c)** compounds were obtained from the reactions of 1,1,3,3-tetrachloro-5,5-diphenylcyclotriphosphazene[4] with 4-hydroxy-3-fluorochalcone, 4-hydroxy-3-chlorochalcone and 4-hydroxy-3-bromochalcone respectively. The cytotoxicity effects of compounds **1a-c** against A2780 cancer cell lines at 1, 5, 25, 50 and 100 µM concentrations were determined with using MTT assay method. The anti-cancer properties of 1,1,3,3-Tetra(4′-oxy-3-substituted-chalcone)-5,5-diphenylcyclotriphosphazene derivatives were assessed *in vitro* using A2780 cell line at 1, 5, 25, 50 and 100 µM doses. All the compounds **(1a-c)** were reduced % cell-viability as dose-dependent ($p<0.05$) towards A2780 cell lines ($p<0.05$). When the structure activities of the compounds **(1a-c)** were investigated, the –Cl substituted compound (**1b)** against A2780 cell lines were observed more active than the others. In summary, cyclotriphosphazene compounds bearing phenyl and substituted chalcone compounds containing fluoro **(1a),** chloro **(1b)** and bromo **(1c)** groups at meta position were conducted to investigate the effects on A2780 cell line. The results displayed that cyclotriphosphazene derivatives bearing phenyl and substituted chalcone compounds have anticancer activity against A2780 cancer cell lines.

C03-10

Effects of N-(p-amylcinnamoyl) anthranilic acid (ACA) on various human cancer cell lines

S. Tekin[1], M. Cakir[2], A. Beytur[1], S. Sandal[1]
[1]*Inonu University, Physiology, Malatya, Turkey*
[2]*Bozok University, Physiology, Yozgat, Turkey*

Cancer is one of the most public health problem in the world. There is currently no therapy to cure of cancer, and hence studies aiming to cancer treatment are ongoing. It has been shown that N-(p-amylcinnamoyl) anthranilic acid (ACA) inhibit transient receptor potential melastatin-2 (TRPM2). TRPM2 isoforms were shown to be overexpressed in several cancers, including melanoma, breast, and lung cancer. Inhibition or RNA silencing of TRPM2 in prostate cancer cells led to decreased proliferation. This study is done to prove that TRPM2 inhibitor ACA have anticancer activity against human prostate (PC3), over (A2780) and breast cancer (MCF-7) cell lines.

We investigated of ACA in terms of antitumor properties were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on these cancer cell lines (PC-3, A2780 and MCF-7). Different concentrations (1, 5, 25, 50 and 100 µM) of ACA was treated with PC-3, A2780 and MCF-7 cell lines for 24 h. Additionally, we calculated LogIC50 concantration of ACA for PC-3, A2780 and MCF-7 cells, by using a Graphpad prism 6 programs on a computer.

ACA reduced cell viability of PC-3, A2780 and MCF-7 cells ($p <0.05$). We conclude that TRPM2 is essential for prostate, over and breast cancer cell a proliferation and may be a potential target for the treatment of these cancers. TRPM2 channels pharmacologic inhibition can potentially provide an innovative strategy to eradicate the tumors associated with many types of cancers.

C03-11

Effects of saxagliptin on human prostate and breast cancer: An in vitro study

S. Tekin[1], A. Beytur[1], M. Cakir[2], **S. Sandal**[1]
[1]*Inonu University, Physiology, Malatya, Turkey*
[2]*Bozok University, Physiology, Yozgat, Turkey*

Dipeptidyl peptidase (DPP- 4) inhibitors are class of oral antidiabetic drugs. They are used for the treatment of Type 2 Diabetes mellitus. DPP-4 is an enzyme which puts down the action of hormone, incretin. Incretins belong to the group of hypoglycaemic gastrointestinal hormones. Some studies show that DPP-4 inhibitors causes cancer and some study show that they have anticancer property. This study is done to prove that DPP-4 inhibitor (Saxagliptin) have anticancer activity against human prostate (LNCaP) and breast cancer (MCF-7) cell line. We investigated of *saxagliptin* in terms of anticancer properties were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on LNCaP and MCF-7 cells. 1, 5, 25, 50 and 100 µg of concantration of *saxagliptine* was treated with human postate and breast cancer lines for 24 h. Additionally, we calculated LogIC50 concantration of Saxagliptin for LNCaP and MCF-7 cells, by using a Graphpad prism 6 programs on a computer. We observed saxagliptin were reduced % cell-viability as dose-dependent (Expect 1 µg) on LNCaP and A2780 cell lines ($p<0.05$). This significant anticancer activity of DPP-4 inhibitor Saxagliptine could play a role as a cytotoxic agent in many tumour conditions.

C03-12

The influence of enzyme matrix metalloproteinase-9 and innate immune cells in the pathogenesisi of tumor response

I. Mrakovcic-Sutic[1], M. Petkovic[2], A. Bulog[3,4], V. Micovic[3,4], I. Sutic[5], V. Pavisic[1], I. Sutic[3]
[1]*Medical Faculty, Department of Physiology and Immunology, Rijeka, Croatia*
[2]*Medical Faculty, deoartment of Onclogy and Radiotherapy, Rijeka, Croatia*
[3]*Medical faculty, Rijeka, Croatia*
[4]*Medical Faculty, Department of Public Health, Rijeka, Croatia*
[5]*Medical Faculty, Department od Family Medicine, Rijeka, Croatia*

Introduction: Matrix metalloproteinase-9 (MMP-9) or gelatinize B belongs to the family of enzymes that commonly called matrix metalloproteinases. Gelatinize B is synthesized in many cell types, such as: keratinocytes, monocytes, tissue macrophages, polymorphonuclear leukocytes and many types of tumor cells. The intensity of release of active enzyme is dependent on the amount of the enzymes stored in granules of these cells. Statistically significant expression of matrix metalloproteinase-9 is demonstrated in various cases of lung cancer and in inflammatory conditions, where is involved in many processes of proliferation, differentiation and migration of mast cells.

Patients and methods: we hypothesized that circulating levels of MMP-9 were abnormal in patients wih colorectal cancer and these levels were compared with those in matched controls. The method of enzyme immunoassay (ELISA) was used to determine enzyme expression of matrix metalloproteinase-9 (MMP-9).

Results: our results showed a large increase in the enzyme MMP-9 in the urine and the percentage of the cells of innate immunity (NKT cells and regulatory T cells) in peripheral blood of patients with colorectal cancer with significant correlation of these values. The increased levels of these cells, as well as, the concentration of MMP 9 correlate with the stage of tumor.

New possibilities for better monitoring the disease are very important. We verified the activity of MMPs in the urine of patients with diagnosed colorectal cancer in different stages of disease.

Acknowledgement: This work was supported by grants from University of Rijeka (13.06.1.1.14 and 13.06.1.1.15).

C03-13

Investigation of the effects of a sulfite molecule on human neuroblastoma cells via a novel oncogene URG4/URGCP

Y. Dodurga[1], M. Seçme[1], C. Eroğlu[2], G. Gündoğdu[3], C. Biray Avcı[4], G. Bağcı[1], V. Küçükatay[1], N. L. Şatıroğlu-Tufan[5], C. Biray Avcı[4]
[1]*Pamukkale University Medical Faculty, Denizli, Turkey*
[2]*Necmettin Erbakan University Medical Faculty, Konya, Turkey*
[3]*Atatürk University Medical Faculty, Erzurum, Turkey*
[4]*Ege University Medical Faculty, İzmir, Turkey*
[5]*Ankara University Medical Faculty, Ankara, Turkey*

Aim: The aim of this study is to determine the anticancer effect of sulfite on SH-SY5Y neuroblastoma cells in vitroconditions and elucidate underlying molecular mechanism of sulfite and explore its therapeutic activity.

Main methods: In this study, cytotoxic effects of sulfite in SH-SY5Y cellswere detected over time in a dose dependentmanner with the IC50 doses ranging from 0.5 to10 mM. Genotoxic effect of sulfite was shown by cometassay. IC50 doses in the SH-SY5Y cells were detected as 5 mM. Expression profiles of the target genes related toapoptosis and cell cycle control were determined by quantitative RT-PCR. Protein changes were determined bywestern blot analysis.

Key findings: URG4/URGCP, CCND1, CCND2, CDK4, CDK6, E2F4 and BCL-2 gene expression levels were significantly reduced and RB1, TP53, BAX, BID, CASP2, CASP3, CASP9 and DIABLO gene expressions were significantly increased in dose group cells. The mechanis mof this resultmay be related to sulfite dependent inhibition of cell cycle at the G1 phase by down-regulating URG4/URGCP or CCND1, CDK4, CDK6 gene expression and stimulating apoptosis via the intrinsic pathway. Sulfite suppressed invasion and colony formation in SH-SY5Y cell line using matrigel invasion chamber and colony formation assay, respectively.

Significance: It is thought that sulfite demonstrates anticarcinogenesis activity by affecting cell cycle arrest, apoptosis, invasion, and colony formation on SH-SY5Y cells. Sulfite may be an effective agent for treatment of neuroblastoma as a single agent or in combination with other agents.

C04: Endocrine, neuroendocrine and metabolism

C04-1

The Effects of Thyroid Dysfunction on Nesfatin-1 Levels in Rats

E. Atici[1], E. Menevse[2,1], A. K. Baltaci[2], **R. MOGULKOC**[2]
[1]*Baskent University,, Ankara, Turkey*
[2]*Selcuk University, Konya, Turkey*

Nesfatin-1 was recently discovered anorexigenic peptide in the brain and it has inhibitor affects on food intake as dose-dependently. The aim of this study was to determine the effects of thyroid dysfunction on nesfatin-1 levels in rat. The study was performed on the 40 male Sprague-Dawley rats. Experiment groups were designed as follows.

1.Control group (n=8): Animals in this groups were killed without any application and blood samples were collected for hormone analysis.

2.Hypothyroidism group (n=8): To induce hypothyroidism PTU was applied by intraperitoneal as 10 mg/kg/day for 3 weeks.

3. Hypothyroidism + Thyroxine group (n=8): Previously animals was made with hypothyrodism by 2 weeks PTU application and following 1 week L-thyroxine was given by intraperitoneal as 1.5 mg/kg/day.
4. Hyperthyroidism group (n=8): Rats were made with hyperthyroidism by 3 weeks L-thyroxine supplementation (0.3 mg /kg/day).
5. Hyperthyroidism +PTU group (n=8): Animals were made hyperthyroisim by L-thyroxine as groups 4, then 1 week PTU was applied to correct hyperthyrodism.

The end of supplementtaion animals were sacrifed and blood samples were collected for FT3, FT4 and nesfatin-1 analysis. FT3 ve FT4 levels were reduced significantly in hipothyroidism while increased in hyperthyroidism (P<0.001). Hypothyroidism caused to increase in nesfatin-1 (P<0.001). In experimental hyperthyroidism nesfatin-1 levels increased significantly (P<0.001). Although hypothyroidism increased nesfatin 1 level (P<0.001), hyperthyroidism increased nesfatin 1 levels much more than hypothyroidism (P<0.001).

The results of study show that experimental hypothyroidism and hyperthyroidism lead to significantly change to nesfatin-1 levels. However, changed hormone levels were normalised after correction of thyroid function.

C04-2

Experimental Hypothyroidism and Hyperthyroidism Have Similar Affects on Cardiac Irisin Levels in Rats

E. Atici[1,2], E. Menevse[1], A. K. Baltaci[1], **R. MOGULKOC**[1]
[1]*Selcuk University, Konya, Turkey*
[2]*Baskent University,, Ankara, Turkey*

Irisin is a newly discovered myokine and adipokine that increases total body energy expendature. This effect is considered to be achieved by converting the white fat tissue to brown fat tissue. The purpose of this study was to determine the effect of experimental hypothyroidism and hyperthyroidism on the levels of irisin in heart tissue in rats. The study was performed on the 40 male Sprague-Dawley rats. Experimental groups were designed as; Control, Hypothyroidism, Hypothyroidism+Thyroxine, Hyperthyroidism and Hyperthyroidism +PTU. Following 3 weeks experimental period, irisin levels were determined in heart tissues. Irisin levels in the experimental groups were respectively, 32.50 ± 6.55 ng / g tissue; 40.53 + 4.69 ng / gr tissue; 33.31 ± 6.33 ng / gr tissue; 47.52 ± 11.70 ng / gr tissue; 34.13 ± 08.07 ng / gr tissue. Hypothyroidism group values of irisin are higher than control group but lower than hyperthyroidism group. The hyperthyroidism group has the highest levels of cardiac irisin.

The results of the study show that the experimental hypo and hyperthyroidism increase the heart irisin levels but the increase in the hyperthyroidism group is much higher than hypothyroidism group.

C04-3

EFFECT OF BISPHENOL A AND DIETHYLHEXYL PHTHALATE ON PROGESTERONE SECRETION BY LUTEAL CELLS

R. Kabakci[1], A. A. Yigit[1]
[1]*Kirikkale University, Faculty of Veterinary Medicine, Department of Physiology, Kirikkale, Turkey*

Questions: This study investigates the effects of bisphenol A (BPA) and diethylhexyl phthalate (DEHP) as endocrine disrupting compounds (EDCs), on progesterone secretion by bovine luteal cells.

Methods: Luteal cells were isolated from the midluteal ovaries of healthy cows and distributed in 6 well plate wells as 3x104 cells/2 mL culture medium. Cells were incubated for 24 hours to adhere to the bottom of the plate. Then, the incubation was continued by replacing the media with different

concentrations of BPA (1, 3, 10 and 30 μM) and DEHP (1, 3, 10 and 30 μM). Media collected at hour 96 and hour 120 were stored at -20 °C until the progesterone measurement.

Results: At hour 96 of incubation, it was observed that all doses of BPA and 3 and 30 μM doses of DEHP significantly reduced ($p < 0.05$) the progesterone level as compared to the control. Also, progesterone synthesis was decreased ($p < 0.05$) in 3, 10 and 30 μM doses of BPA and in all doses of DEHP as compared to the control at hour 120 of incubation. Progesterone levels decreased ($p < 0.05$) in control and the highest dose of BPA (30 μM) and in all doses of DEHP including control depending on the length of the incubation.

Conclusions: The results of this study showed that BPA and DEHP disrupted luteal steroidogenesis by suppressing progesterone synthesis depending on the dosage and incubation time. It is thought that this effect can cause infertility problems in cows by disturbing the hormonal balance of the ovary. It should be necessary to restrict the use of these chemicals and spread in nature.

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Key words: BPA, DEHP, Luteal cell

C04-4

C-AMP DURING OESTRUS CYCLE IN RATS

V. Antevska[1]
[1]*Medical Faculty Skopje, Institute of Physiology, Skopje, Macedonia, The Former Yugoslav Republic Of*

Introduction. The mammalian pineal gland is under adrenergic control. The physiological oscillations of gonadal steroids could strongly affect the melatonin synthesis and secretion by acting on the pre- and postsynaptic levels and by modulation of the target cells replay. The aim of this study was to determine the basal levels of cAMP in the pineal gland during the various phases of oestrus cycle in normothensive (NTR), Wistar rats and spontaneously hypertensive (SHR) Okamoto and Aoki rats and to describe the histological finding of the pineal gland tissues.

Methods. Two hundred female mature rats (100NTR and 100SHR) were investigated. They were divided in 4 groups according to the phases of the oestrus cycle (diestrus, proestrus, estrus and metaestrus). The phase of oestrus cycle has been determined by microscopic analysis of the vaginal smears. The level of cAMP (RIA) in the pineal gland was the parameter of its intracellular activity. The pineal gland tissues were stained on HaEo.

Results. In SHR there is a slight shortening of the oestrus cycle. In NTR there was an increase of the cAMP level from proestrus to metaestrus, contrary to the dramatic decrease in SHR. Histological findings of pineal glands showed the presence of many changed pinealocytes with picnotic nucleuses, while the neuroepithelial cells, in the upper parts of the glands, were separated in gland-like islets. There was a normal pineal histology in NTR.

Conclusion. This study indicated significant neurohormonal differences between NTR and SHR. The changed adrenal activity in SHR correlated with histological findings in the pineal gland.

Key words: c-AMP; oestrus cycle; rats

C04-5

Effect of Zinc and Melatonin on Oxidative Stress and Serum Inhibin-B Levels in a Rat Testicular Torsion-Detorsion Model

A. Semercioz[1], **A. K. Baltaci**[2], R. Mogulkoc[2], M. C. Avunduk[3]
[1]*Bagcilar Training and Research Hospital, Urology, Istanbul, Turkey*
[2]*Selcuk University Medical School, Physiology, KONYA, Turkey*
[3]*Faculty of Meram Medicine, Necmettin Erbakan University, , Pathology, Konya, Turkey*

The present study was aimed to examine the effects of 3-week zinc and melatonin administration on testicular tissue injury caused by unilateral testicular torsion-detorsion in rats and their serum Inhibin-B levels.

The study was performed on 60 Wistar Albino type adult male rats. The animals were allocated to 6 groups. 1. Control; 2. Sham; 3. Ischemia-Reperfusion; 4. Zinc + Ischemia-Reperfusion; 5. Melatonin + Ischemia-Reperfusion; 6. Zinc + Melatonin + Ischemia-Reperfusion. Zinc and melatonin were administered before ischemia-reperfusion at doses of 5 and 3 mg/kg respectively through the intraperitoneal route for a period of 3 weeks. Blood and testicular tissue samples were collected to analyze erythrocyte and tissue GSH and plasma and tissue MDA, Inhibin-B levels.

The highest erythrocyte and testis GSH values were found in zinc, melatonin, and zinc + melatonin. Torsion-detorsion group had significantly lower erythrocyte GSH and higher MDA values. Serum inhibin-B and spermatogenetic activity levels in the torsion-detorsion group were also significantly lower than those in the other groups. However, zinc, melatonin and melatonin + zinc supplemented groups have higher inhibin-B and spermatogenetic activity.

The results of the study show that zinc, melatonin and melatonin + zinc administration partially restores the increased oxidative stress, as well as the reduced inhibin-B and spermatogenetic activity levels in testis ischemia-reperfusion in rats.

Suppressed inhibin-B levels in the testicular tissue may be a marker of oxidative stress.

C04-6

Combined Effects of Flavonoid Fisetin and Endocrine Disruptor Bisphenol A on Progesterone Production by Granulosa Cells

A. Bujnakova Mlynarcikova[1], S. Scsukova[1]
[1]*Biomedical Research Center SAS, Institute of Experimental Endocrinology, Bratislava, Slovakia*

Proper function of the ovaries is essential for maintaining female reproductive health. Currently, many industrial agents termed endocrine disruptors (EDs) are linked to the increased fertility disorders. In contrast, health protective effects of phytochemicals, e.g. flavonoids, are assumed. The possibility to address ED-involved reproductive dysfunctions by natural compounds would be desirable; yet, the data on such mutual effects are limited. We examined the ability of the flavonoid fisetin (Fis) to modulate effects of a ubiquitous ED Bisphenol A (BPA), on the function of ovarian granulosa cells (GCs). Porcine GCs were treated with different concentrations of BPA, Fis, or their combinations. Progesterone (P4) production by GCs was determined by radioimmunoanalysis, viability of GCs was assessed by MTT assay, expression of relevant genes was determined by real-time PCR. BPA inhibited P4 production by GCs at the highest concentration. Fis reduced P4 production dose-dependently, and in this manner, Fis further altered P4 production when added to BPA-treated GCs. This effect could partly result from the decreased viability of GCs via up-regulation of *CASP3*. Nevertheless, the combined action of Fis and BPA significantly down-regulated steroidogenesis-related enzymes (*STAR, CYP11A1, HSD3B*) what seems to contribute to P4 synthesis inhibition most. Our results suggest that Fis might interfere with ovarian steroidogenesis, and has no beneficial effects in terms of restoring P4 synthesis altered by BPA. Considering the constant human exposure to myriad of environmental and dietary chemicals, physiological effects of such mixtures need to be investigated. *Acknowledgements: The work was supported by the VEGA project 2/0198/15.*

C04-7

Determining the Correlation between Thyroid Hormone and Adropine Hormone in Rats which received Cold Restraint Stress

M. C. guler[1], A. tanyeli[1], E. eraslan[1], T. nacar[1], E. polat[2]
[1]*ataturk university , physiology , erzurum, Turkey*
[2]*ataturk university, biochemistry, erzurum, Turkey*

Aim: The Hypothalamic hypophyseal thyroid axis has various roles in regulation of the body temperature, protection of metabolic speed and many other physiological processes. It is already known that stress is related with neurochemical and hormonal changes including the changes in the thyroid hormone levels. In this study, we investigated the correlation between the adropine hormone whose expression is defined in central neural system and encoded by the gene that is related with energy homeostasis and the thyroid hormones in rats which received Cold Restraint Stress (CRS).

Method: 16 Wistar Albino male rats were used in this study. Two groups were formed in the study as Control and CRS Groups (n=8). No applications were made to the rats in Control Group. CRS application was made as follows: The rats were placed in a restraining chamber. The tails of the rats were fixed to the edge of the chamber. Sufficient respiration was ensured with big holes. The rats in the CRS group were subjected to CRS in groups of 4 at 4°C for 4 hours. The animals were sacrificed at the end of the study and their blood was collected. TSH, T3, T4 and adropine hormone levels in the plasma samples were determined with ELISA Method. The Spearman Correlation Analysis was used for statistical analysis.

Results: There were no correlations between the hormones in the Control group. In the groups which received CRS, no correlations were determined between the TSH and T4 hormones of the adropine hormone, and a negative correlation was detected with T3 hormone [r(8)=-0,922; p<0.01].

Conclusion: As a result of the CRS application, we showed that T3 level decreased and adropine level increased.

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C04-8

Thyroid axis functioning is associated with health status and shorter survival of brain tumor patients

A. Bunevicius[1], S. Tamasauskas[1], V. Deltuva[1], A. Tamasauskas[1]
[1]*Lithuanian University of Health Sciences, Kaunas, Lithuania*

QUESTIONS. To investigate if thyroid hormone levels are associated with health status and prognosis of brain tumor patients.

METHODS: Two-hundred and thirty brain tumor patients (70% women) before surgery were evaluated for cognitive (Mini mental State Examination; MMSE) and functional (Barthel index; BI) status, and thyroid function profile. The Low tri-iodothyronine (T3) syndrome was defined as T3 concentration below the reference range. Unfavorable hospital discharge outcomes were determined as Glasgow outcome scale score of ≤3. Follow-up continued until November, 2015.

RESULTS. Seventy-four percent of patients had Low T3 syndrome. Lower total T3 concentrations were associated with lower MMSE (p=.013) and BI (p=.023) scores independent of age, gender and histological diagnosis. Preoperative Low T3 syndrome increased risk for unfavorable discharge outcomes adjusting for age, gender and histological diagnosis (OR=2.944, 95%CI [1.314-6.597], p=.009). In all patients, lower total (p=.038) and free (p=.014) T3 concentrations were associated with greater mortality adjusting for age, gender, extent of resection, adjuvant treatment and histological diagnosis. The Low T3 syndrome was associated with greater 5-year mortality for glioma patients

(HR=2.197; 95%CI [1.160-4.163], p=.016) and with shorter survival (249 [260] vs. 352 [399] days; p=.029) of high grade glioma patients independent of age, gender, extent of resection and adjuvant treatment.

CONCLUSIONS. Reduction of T3 concentrations is common in brain tumor patients and is associated with worse health status and worse discharge outcomes.

C04-9

Pregnancy induced changes in innate immuniy during autoimmune thireoid disease

I. Mrakovcic-Sutic[1], T. Bogovic Crncic[1], S. Grbac Ivankovic[1], V. Pavisic[2], I. Sutic[3]
[1]*Medical Faculty, Department of Nuclear Medicine, Rijeka, Croatia*
[2]*Medical Faculty, Department of Physiology and Immunology, Rijeka, Croatia*
[3]*Medical Faculty, Department od Family Medicine, Rijeka, Croatia*

Aim: Autoimmune thyroid dysfunction (ATD), which comprises two main clinical entities: Graves' disease and Hashimoto thyroiditis, often affect women of reproductive age. In a healthy pregnancy predominant is Th2 over Th1 immunity, which explains the improvement of autoimmune disease during pregnancy, while after birth due to changes in Th1/Th2 ratios often leads to deterioration of ATD. NKT and Tregs seem to play an important part in mediating maternal tolerance to fetus. Although many researches have been done in the field of thyroid autoimmunity, very few studies investigated the role of innate immunity in AITD during human pregnancy and in the postpartal period

Results: We investigated the presence of ATD in pregnant and postpartum period in women with hormonal status determination, the titer of thyroid antibodies and auto antibodies and compared them with healthy pregnant women and subjects postpartum and not pregnant women. After intracellular and surface staining using flow cytometry, we analyzed the phenotype and cytolytic potential of isolated peripheral blood mononuclear cells of pregnant women and postpartum women, and not pregnant women.

Conclusion: pregnancy and the postpartum period influence the function of the thyroid gland. In the presence of thyroid autoimmunity changes are more pronounced, especially postpartum. Apart from pregnancy and postpartum period influence the course of ATD and thyroid autoimmunity affects thyroid function in pregnancy and the postpartum period.

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C04-10

Comparison of extraction methods for measurement of hair cortisol

T. ATÇALI[1], S. Yildiz[2], C. Uçar[2], S. Uğraş[2]
[1]*Bingöl Universty, BİNGÖL, Turkey*
[2]*İnönü Universty Faculty of Medicine, Physiology, Malatya, Turkey*

Introduction: Hair cortisol measurements provide an important tool for the assessment of long-term stress in humans. However, extraction methods differ between the studies. Therefore, the aim of the current study was to compare the effect of different extraction methods on hair cortisol concentration.

Materials and methods: Washed or unwashedmale hair samples were cut into small pieces by a scissor or ground by using liquid nitrogen. Afterwards, one set of samples were incubated for 16 or 36 h at 52 degrees Celcius. Another set was sonicated30 min, 1.0 h or 2 h at 35 degrees Celcius. A different set was both sonicated and incubated at 52 degreesfor 16 h. For control comparisons, one set of samples were kept under room temperature for 90 h without using ultrasound. Following these

extraction protocols, all samples were centrifuged 10000 rpm for 30 min and the supernatant was used for cortisol analyses. All supernatants were evaporated, re-suspended in phosphate buffered saline, vortexed and analyzed by a validated ELISA method.

Result: Variations were observed between the hair cortisol concentrations of different individuals. All extraction protocols resulted in cortisol concentrations that were within pg/mg range and readable with the ELISA test used.

Conclusions: Extraction methods appear to affect hair cortisol concentration. However, as all methods used resulted in levels within acceptable range, it might be recommended to use any of the extraction methods used in the current study.

C04-11

Lengths of the menstrual cycle and menstruation are positively correlated with general tiredness in long-term entrained students

S. Uğraş[1], C. Uçar[1], T. Atçalı[1], S. Yildiz[1]
[1]*İnönü Unıversty Faculty of Medicine, malatya, Turkey*

Introduction: Long-term participation in sports might affect lengths of the menstrual cycle and menstruation in young females. Therefore, the aim of the current study was to find out the correlations between parameters of stress andlengths of the menstrual cycles and menstruation in female students actively participating in sports.

Materials and methods: Female students (n=193) actively participating in sports as a part of their education were studied in the current study. Lengths of menstrual cycle and menstruation in the last three cycles were recorded together with 40-item state and trait anxiety scales. Statistical analyses were carried out by using Pearson"s correlations.

Results: Lengths of the menstrual cycle and menstruation were 29.3±0.3 and 5.6±0.1 days, respectively. Length of the menstrual cycle was positively correlated with the length of menstruation (R-sq=0.905; P<0.001). There were positive linear correlations between the scores of general tiredness and that of length of the menstruation (R-sq=0.213; P=0.003) and length of the menstruation (R-sq=0.172; P=0.017).

Conclusions: The results of the current study suggest that (1) the lengths of the menstrual cycles and menstruation were within normal range in long-term entrained female student and that (2) increased menstruation increases level of tiredness. The latter might be associated with increased iron loss by prolonged menstruation.

C05: Sports & exercise physiology

C05-1

The Effect of Resveratrol Supplementation on Element Metabolism in Bone Tissue of Rats with Acute Swimming Exercise

A. K. Baltaci[1], D. Cinarli[1], R. Mogulkoc[1], S. Patlar[2], S. B. Baltaci[1]
[1]*Selcuk University Medical School, Physiology, KONYA, Turkey*
[2]*Selcuk University, Sport Sciences, Konya, Turkey*

The aim of the present study was to investigate how affect resveratrol supplementation element metabolism in bone tissue of rats with acute swimming exercise

Animals were divided to 4 groups. 1.Control, 2.Swimming; Rats were fed by standart rat food and exposed to 30 minutes swimming exercise at the end of study. 3.Resveratrol: Animals were fed by

standard rat food plus resveratrol for 4 weeks (10 mg/kg/day) by drinking water. 4.Resveratrol + Swimming: Animals were fed by standard rat food plus resveratrol (10 mg/kg/day) by drinking water for 4 weeks and exposed to swimming exercise for 30 minutes at the end of study.

The end of 4 weeks study, bone tissue samples analyzed at the Atomic Emission (mg/L).

The findings of the study show that resveratrol supplementation increased zinc, calcium, phosphorus, magnesium and boron levels in bone tissue independently from exercise.

One of the main findings of study was that resveratrol supplementation has protective and/or regulator activity in bone tissue indepedently from exercise and may be consider.

C05-2

Cardiorespiratory fitness effect on cerebral oxygenation in chronic obstructive pulmonary patients

O. Dupuy[1], Q. Bretonneau[1], J. C. Meurice[2], F. Caron[2,3], **C. de Bisschop**[1]
[1]*Université de Poitiers, Laboratoire MOVE EA 6314, Poitiers, France*
[2]*Service de Pneumologie, Centre Hospitalier Universitaire de Poitiers, Poitiers, France*
[3]*Centre de réadaptation du Moulin Vert, Nieuil l'Espoir, France*

Introduction:
Low cerebral oxygenation is associated with cognitive decline and may lead to higher risk of neurodegenerative disease. However, positive impact of physical activity on brain health is recognized (Dupuy et al, 2015). Chronic obstructive pulmonary disease (COPD) is often associated with brain functioning deregulation and lower cerebral oxygenation than healthy during exercise (Vogziatis et al, 2014). The aim of this study was to assess the influence of cardiorespiratory fitness on cerebral oxygenation during exercise in COPD patients.

Material and Method:
Forty-one COPD patients (64.6 ± 9.8 years), classified GOLD 2-3, VEMS (%pred) 57.3 ± 14.0 were included in the study. All performed a maximal incremental test on ergocycle (10W/min). During the test, cerebral oxygenation (NIRS sytem, Artinis MS NL) and pulmonary gaz exchanges (Ergocard, Medisoft, Dinant, B) were recorded. The NIRS optode was put on the left frontal lobe. Tissue Saturation Index, total haemoglobin, deoxyhaemoglobin and oxyhaemoglobin (TSI, tHb, HHb and HbO2 respectively) were measured by a NIRS system. Correlations were performed using Pearson tests.

Results:
Mean VO2peak were 16.2 ± 4.5ml/min/Kg and power peak were 77.0 ± 19.8W. Two positive correlations were found: 1) VO2peak vs tHbpeak ($r=0.40$, $p<0.05$) and 2) VO2peak vs HbO2peak ($r=0.42$, $p<0.05$). Neither HHbpeak nor TSI were correlated with VO2peak.

Discussion Conclusion:
This study confirms the link, in COPD patients, between cerebral oxygenation and cardiorespiratory fitness. The patients who presented a higher VO2peak also had a higher cerebral oxygenation. As cerebral oxygenation is a major feature of brain functioning and health, COPD patients should be encouraged to be active.

C05-3

Effects of Acute Exhaustive Exercise on Oxidant and Antioxidant System Parameters in Rats with Streptozotocin Induced Diabetes Mellitus

A. M. Sahin[1], O. F. Sonmez[1], M. Mengi[1], M. Altan[1], M. S. Toprak[2], H. Ekmekci[2], G. Metin[1], L. Cakar[3]
[1]*Istanbul Universty Cerrahpasa Faculty of Medicine, Physiology, İstanbul, Turkey*
[2]*Istanbul Universty Cerrahpasa Faculty of Medicine, Biochemistry , İstanbul, Turkey*
[3]*Sanko Universty School of Medicine, Physiology, Gaziantep, Turkey*

Questions: Oxidative stress (OS) is responsible for both the development and complications of diabetes mellitus (DM). Acute exercises are a well known source of OS. DM patients may experience strenuous physical activity conditions in daily life. Therefore we investigated how oxidant-antioxidant system responds to acute exhaustive exercise (AEE) in an experimental DM model.

Materials and Methods: 16 Sprague-Dawley rats were randomly divided into two groups: control (n = 8) and DM group (n = 8). Streptozotocin (STZ) (65 mg/kg intraperitoneal injection) was administered to DM group rats. Three days after the administration, blood glucose levels were evaluated and rats with levels above 200 mg/dL was considered as DM. Serum was separated from blood samples immediately after AEE. 8-OH-deoxyguanosine, 3-nitrotyrosine, lipid hydroperoxide, protein carbonyl, CuZnSOD, glutathione and glutathione peroxidase assays were performed by ELISA method.

Results: 3-nitrotyrosine (p = 0.001) and protein carbonyl (p = 0.013) were significantly higher and 8-OH-deoxyguanosine was significantly lower in the DM group compared to control group (p = 0.001). There was no significant difference in lipid hydroperoxide levels between the groups. When antioxidant parameters compared, there was no significant difference in Cu-Zn-SOD but glutathione (p = 0.013) and glutathione peroxidase (p = 0.001) levels were significantly higher in the DM group.

Conclusion: Antioxidant system showed an increase in response to AEE induced OS in DM group. Although this increase may protect against DNA damage, it could not prevent protein oxidation.

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C05-4

Diving response after a one-week diet and overnight fasting

A. Di Giacomo[1], G. Ghiani[1], G. Palazzolo[1], S. Roberto[1], F. Tocco[1]
[1]*University of Cagliari, Cagliari, Italy*

Questions: We hypothesized that overnight fasting after a short dietary period could allow performing breath-hold diving with no restraint for diaphragm excursion and blood shift and without any increase of metabolism, and in turn improve the diving response. **Methods:** During two separate sessions, 8 divers carried out two trials: (A) a 30-metre depth dive, three hours after a normal breakfast and (B) a dive to the same depth, but after following a diet and fasting overnight. Each test consisted of 3 apnea phases: descent, static and ascent. An impedance cardiograph, housed in an underwater torch, provided data on trans-thoracic fluid index (TFI), stroke volume (SV), heart rate (HR) and cardiac output (CO). Mean blood pressure (MBP), arterial O2 saturation (SaO2), blood glucose (Glu) and blood lactate (BLa) were also collected. **Results:** In condition B, duration of the static phase of the dive was longer than A (37.8±7.4 vs. 27.3±8.4 s respectively, $P<0.05$). In static phases, mean Δ SV value (difference between basal and nadir values) during fasting was lower than breakfast one (-2.6±5.1 vs. 5.7±7.6 ml, $P<0.05$). Since mean Δ HR values were equally decreased in both metabolic conditions, mean Δ CO value during static after fasting was lower than the same phase after breakfast (-0.4±0.5 vs. 0.4±0.5 L·min-1 respectively, $P<0.05$). At emersion, despite the greater duration of dives during fasting, SaO2 was higher than A (92.0±2.7 vs. 89.4±2.9 % respectively, $P<0.05$) and BLa was lower in the same comparison (4.2±0.7 vs. 5.3±1.1 mmol·L-1, $P<0.05$). **Conclusions:** An adequate balance between metabolic and splancnic status may improve the diving response during a dive at a depth of 30m, in safe conditions for the athletes health.

C05-5

Relationship between regular exercise-induced cardiac hypertrophy and microRNA

M. Pala[1], M. Altan[2], O. F. Sonmez[2], M. Mengi[2], S. Dincer[3], F. Akbas[4], M. Yıldız[5], M. Kumaş[6], M. Esrefoglu[6], G. Metin[2]
[1]*Biruni University Faculty of Medicine, Physiology, Istanbul, Turkey*
[2]*Istanbul Universty Cerrahpasa Faculty of Medicine, Physiology, İstanbul, Turkey*
[3]*Istanbul Universty Istanbul Medical Faculty, Sports Medicine, İstanbul, Turkey*
[4]*Bezmialem Vakıf University Medical Faculty, Medical Biology, Istanbul, Turkey*
[5]*Istanbul Universty, Institute of Cardiology, İstanbul, Turkey*
[6]*Bezmialem Vakıf University Medical Faculty, Histology, Istanbul, Turkey*

Questions: Exercise-induced cardiac hypertrophy (CH) is a type of physiologic CH. MicroRNAs (miRNAs) are involved in cardiac development, hypertrophy and angiogenesis. We investigated the role of miRNAs in regular exercise-induced cardiac hypertrophy.

Material & Methods: Male Sprague Dawley rats were divided into Exercise-group (EG, n=9) and Control-group (CG, n=6). Swimming sessions began with 60 min/5 days/8 weeks and continued with on the 9th week 2x/day, and on the 10th week 3x/day. Dimensions of the left ventricle and myocardial wall thickness were measured by transthoracic echocardiography (TTE). miRNAs were assessed by miRNA microarray and confirmed by real time PCR. Apoptosis, necrosis, and cell proliferation were evaluated histologically.

Results: In TTE left ventricular mass, end-diastolic diameter of the left ventricle and end-systolic diameter of the left ventricle, the thickness of the posterior wall and interventricular septum thickness were found to be increased significantly in EG. Genetic analysis showed upregulation of the expression of miR-132-3p and miR-194-5p and downregulation of the expression of miR-290 in EG. In histological analysis although there was necrosis in cardiac tissue, there were no cell proliferation and apoptosis in TG.

Conclusions: We suggest that in exercise-induced CH, heart may be protected from fibrosis due to changes in the expression of the genes miR-132-3p and miR-290. Increase in expression of miR-132-3p in blood may be a predictor of fibrosis. Also an increase in the expression of miR-194-5p may be an indicator of exercise induced CH. However these findings should be validated with further research.

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C05-6

Prognostic Value of 6-Minute Walk Test in children with congenital anemia

K. AYED[1], S. YAHYAOUI[2], S. MOKADDEM[1], S. BEN JEMAA[1], I. L. HADJ KHALIFA[1], S. BEN KHAMSA JAMALEDDINE[1]
[1]*Abderrahman Mami Hospital , Department of respiratory fonctionnal explorations, Ariana, Tunisia*
[2]*Bechir Hamza Children's Hospital, Service of infantile medicine, Bab Saadoun, Tunisia*

Introduction: Anemia is the main cause of dyspnea, muscle deconditioning and than exercise intolerance. The 6-min walk Test (6MWT) is a simple and safe test that usually used to evaluate global response to submaximal exercise and which have reliable prognostic value.

Aim: The aim of this study was to evaluate the relationship between 6-Minute Walk Test (6MWT) distance, and respectively Muscle Mass (MM) and hemoglobin levels in a group of children with beta thalassemia or Sickle Cell Disease (SCD).

Methods: Our study included 24 children who regularly followed up in a pediatric consultation. This population is composed by 11 beta-thalassemia and 13 SCD patients with sex ratio equal to 0,41. We

performed for each patient a blood sampling test for hemoglobin measurement, bio-electrical impedance for MM measurement and 6MWT for distance walked measurement.

Results: The averages of age, hemoglobin level and MM were respectively 12 ± 3,4 years, 7,9 ± 0,7 g /dl and 49,5 ± 8,2 %. Contrasting with normal MM, data revealed a severe reduction of average walking distance expressed as a percentage of the theoretical value calculated according to the Troosters equation (41 ± 13,6%). The, 6MWT distance was strongly correlated with Hemoglobin levels ($p < 0.05$) but no significant correlation between MM and anemia was found.

Conclusion: This study highlights an important limitation of 6MWT distance which correlated to anemia severity and reflected poor prognosis in patients with congenital anemia. These alarming data could be seriously taken into consideration by health authorities to better management of anemia.

C05-7
Case Study of a Male Ocean Racer: body composition and nutritional intake during world solo sailing record attempt

G. Ghiani[1], S. Magnani[1], V. Pinna[1], A. Doneddu[1], G. Sainas[1], F. Tocco[1], A. Crisafulli[1]
[1]*Università Cagliari, Scienze mediche e sanità pubblica, Cagliari, Italy*

The Italian Sailor Gaetano Mura tried to beat the world record of non-stop solo globe circumnavigation in Class 40 (record held by the Chinese sailor Guo Chuan) in October 2016, without stopovers or assistance, a physically demanding challenge for which appropriate nutrition should be crucial to maintain energy balance, ensure optimum performance and to maintain optimal body composition. His daily recommended nutritional intake (NI) during the voyage, detected with sensewear armband during preparation, that had to be about 130 days, was 3000 Kcal/day with carbohydrate and protein intake goals of 335 g/day and 100g/day, respectively. Unfortunately he had to stop in Australia for a technical failure after 70 days of navigation and did not continue the challenge. Fat mass (FM) and fat-free mass (FFM) were assessed, by means of plicometry, during his preparation (4 months before the race-T0) pre- (15 days-T1) and postrace (10 days-T2), and body mass was also measured. Measurements enlightened that during the voyage the racer did not lost body mass (ΔT0-T1 2,1% Δ T0-T2 2,1%) and his body composition remained similar pre and after the race (FFM Δ T0-T11,8% Δ T0-T2 2,2%; FM Δ T0-T1 5,3% Δ T0-T2 1,8%), moreover, he reported good sensations about his nutrition on board. This intervention demonstrates that racers' nutrition strategy can be improved to facilitate meeting more optimal NI goals for performance and health. And shows that further studies can provide important information for optimizing nutritional strategies for ocean racing.

C05-8
VITAMIN C SUPPLEMENTATION MITIGATES DIVING-INDUCED CHANGES IN CEREBRAL CIRCULATION

O. Barak[1], K. Caljkusic[2], R. Hoiland[3], S. Thom[4], P. Jovanov[5], T. Mijacika[2], Z. Dujic[2]
[1]*Faculty of Medicine University of Novi Sad, Department of Physiology, Novi Sad, Serbia*
[2]*University of Split School of Medicine, Split, Croatia*
[3]*University of British Columbia, Okanagan Campus, Kelowna, Canada*
[4]*University of Maryland, School of Medicine, Baltimore, Maryland, Baltimore, United States*
[5]*Institute of Food Technology in Novi Sad, Novi Sad, Serbia*

SCUBA related decrements may be associated with impairment in the cerebral circulation and we investigated if it could be prevented by oral antioxidant supplementation. Fourteen divers performed a single SCUBA dive and participated in a follow-up study involving 60% oxygen breathing at ambient pressure. Prior to both studies, participants ingested ascorbic acid (2g) and two weeks later placebo daily for six days. After two weeks of study interruption subjects switched groups and received the opposite pre-treatment. Transcranial Doppler ultrasound was used to measure cerebral blood velocities (CBV) for 10 minutes pre-dive and through 90 minutes post-dive. CBV measures were analyzed by two-way repeated measure ANOVA for the two studies (time – pre/30/60/90min, trial – placebo/VitC). Velocity in the middle cerebral artery (MCAv) increased 30 minutes post-dive from 60.08±8.99 cm/s to 63.14±10.01 cm/s and in the posterior cerebral artery PCAv from 40.05±6.14 cm/s to 43.94±5.79 cm/s, respectively ($p<0.05$). Thirty minutes post-dive MCAv and PCAv were significantly higher in the placebo trial compared to the Vitamin C trial ($p<0.05$). There were no main effects of time or trial in the oxygen breathing study. Transient elevations of CBV were present only 30 minutes post-dive and were mitigated by vitamin C; but hyperoxia as a diving related stress factor showed no independent influence on CBV and did not explain diving related changes in the cerebral vasculature.

C05-9
The Investigation of the Effects of Mask and Mouthpiece Types with Different Dead Space Volumes on the Energy Expenditure Measurements

Z. ALTINKAYA[1], U. DAL[1], N. OZEL[2]
[1]*Mersin University, Faculty of Medicine, Department of Physiology, Mersin, Turkey*
[2]*Mersin University, Faculty of Medicine, Department of Biostatistics and Medical Informatic, Mersin, Turkey*

The indirect calorimetry is widely used technique for evaluating the energy expenditure (EE) of the subjects. During respiration, gases can be collected by using different types of devices. We aimed to investigate the effect of the miscalculation of dead space volume (DSV) of two masks and mouthpiece (actual, 25% less and 25% more of DSV) during resting and walking EE measurements, and also to compare comfortableness of these apparatuses.

There was no significant agreement among the masks and mouthpiece in terms of resting EE data (ICC= 0.65). Although ICC for the actual, 25% less and 25% more of the DSV of 1st mask was moderate, the resting EE data for the three DSVs of 2nd mask were not significantly agreed (ICC=0.68). There was an excellent agreement among the resting EE measurements of the three DSVs of the mouthpiece (ICC=0.91). Among two masks and mouthpiece used for walking EE measurement, ICC was moderate. Although ICC for 1st mask was good, ICC for 2nd mask was excellent for all the three DSVs during walking. There was a moderate agreement among the measurements with mouthpiece (ICC=0.81).

We suggested that same apparatus should be used for whole study for the resting EE measurement. Although the 25% error in DSV for the 1st mask and mouthpiece may not have a significant effect on the resting EE data, the DSV of the 2nd mask needs to be correctly entered to the program, which was the most comfortable one. The 25% error in DSV for the both masks and mouthpiece also had no significant effect on the walking EE. In addition, three apparatuses can be used instead of each other in the walking EE measurement.

C05-10
The Contraction-Induced Hypertrophic Response of Myostatin Suppression Is Intrinsically Impaired in Myotubes from Obese Individuals.

T. Nicholson[1], H. Palfrey[1], C. Chruch[2], D. Baker[2], S. Jones[1]
[1]*University of Birmingham, Institute of Inflammation and Ageing, Birmingham, United Kingdom*
[2]*Medimmune, Cardiovascular and Metabolic Disease (CVMD), Cambridge, United Kingdom*

Introduction
Loss of skeletal muscle mass and function with age is a key contributor to frailty and the incidence of chronic disease. Importantly, such loss of muscle mass and quality is associated with increased adiposity. However, the intrinsic molecular mechanisms that underpin the relationship between adiposity and loss of muscle mass are poorly understood. This study aimed to characterise the hypertrophic response of primary human myotubes from lean and obese individuals in response to muscle contraction *in vitro*.

Methods
Skeletal muscle (gluteus maximus) was obtained from lean and obese patients undergoing elective total hip replacement surgery (NRES 14ES1044). Myostatin mRNA expression in skeletal muscle, cultured myotubes and myotubes subject to electrical pulse stimulation (EPS) was quantified by qRT-PCR. EPS was performed using an Ion Optix C-Pace EP for 24 h (1Hz, 2ms and 11V). All data are presented as mean ± SEM. Data was analysed by paired and unpaired t-tests as appropriate.

Results
Myostatin expression was significantly greater in skeletal muscle of obese (n=6), compared to lean subjects (n=6) (p=<0.01). Myostatin expression was also significantly greater in myotubes cultured from obese subjects (n=5), compared to lean (n=4) (p=<0.01). EPS for 24h reduced myostatin expression (2-fold) in myotubes from lean subjects (n=4) (p=<0.01). No effect of EPS on myostatin expression was observed in myotubes cultured from obese subjects (n=5).

Discussion
These data suggest that skeletal muscle myotubes from obese individuals are intrinsically altered, resulting in an impaired hypertrophic response to exercise stimulated downregulation of myostatin.

C05-11
The Effects of Voluntary Physical Activity in Female Rats Fed with Fructose Rich Diet

P. Tayfur[1], K. Gokce[2], S. Yilmaz[2], O. Barutcu[2], E. O. Ozgur[2], N. Sut[3], S. A. Vardar[1]
[1]*Trakya University Medical Faculty, Physiology, Edirne, Turkey*
[2]*Trakya University Medical Faculty, Edirne, Turkey*
[3]*Trakya University Medical Faculty, Biostatistics, Edirne, Turkey*

The aim of this study was to investigate the effects of voluntary physical activity on body weight, blood pressure, serum lipid and glucose levels in rats that were fed with fructose rich diet during six weeks.

Spraque-Dawley female rats were separated as control (C; n=7), voluntary physical activity (A; n=7), fructose (F; n=7) and fructose active group (FA; n=7). Fructose groups were fed 20% fructose in drinking water for six weeks. The rats were kept in cages with running wheel during six weeks. Lee Index (body weight$^{1/3}$/naso-anal length) was used in order to determine obesity. Blood pressure was measured with the tail-cuff method at the last day of feeding period. Serum triglyceride, total cholesterol, HDL, LDL and glucose levels were determined by using enzymatic method, insulin level measured by using the ELISA method. Two-way ANOVA and Student's t-Test were used for statistical comparisons.

Fructose intake increased systolic blood pressure (p=0.001), diastolic blood pressure (p=0.002), liver weight (p=0.035), glucose (p=0.041), insulin (p=0.001), cholesterol (p=0.001) and trygliseride (p=0.001) levels. Physical activity decreased heart rate and Lee index (respectively p=0.016; p=0.018). No significant interaction was observed between fructose intake and voluntary physical activity in groups. There was no significant difference of daily walking distance between FA and A groups.

Our findings considered that voluntary physical activity decreases obesity and heart rate but may not be effective on increased blood pressure, blood glucose and lipid levels in female rats fed with high fructose diet. This study has been supported by TUBAP (2016/84).

Key Words: voluntary physical activity, fructose rich diet, exercise

C05-12
Effects of Exercise on ADAMTS-4 and ADAMTS-5 Levels in Sport Horses

S. KANDIR[1], G. TEKIN[2], C. ER[3], S. KARAKURT[2]
[1]*Cukurova University, Ceyhan Faculty of Veterinary Medicine, Physiology, Adana, Turkey*
[2]*Selcuk University, Faculty of Science, Biochemistry, Konya, Turkey*
[3]*Petibör Veterinary Clinic, Internal Medicine, Istanbul, Turkey*

The wellness and early diagnosis of the diseases in the locomotor system of sport horses are important. A disintegrin-like and metalloproteinase with thrombospondin motifs (ADAMTS) proteinase family play an important role in many physiological and physiopathological processes. In this study, we aimed to determine the changes of ADAMTS-4 and ADAMTS-5 levels on sport horses before and after exercise. The Oldenburg and Selle Français horse-breed types which are healthy, 6-15 years old, around 650-750 kg, and distinct genders were used (n=10). Following the physical examinations, the horses were subjected to 50 minutes regular exercise program. Blood samples were collected into anticoagulant-free tubes which were centrifugated as earliest as possible for 10 minutes at 3000 rpm in order to determine ADAMTS-4 and ADAMTS-5 levels before and after exercise. Horse specific ELISA kits (Sunred Bio, China) were used and results were evaluated by GraphPad Prism 5.0 software. Interestingly, although no differences were observed with at the level of ADAMTS-4 (p=0.39), ADAMTS-5 level significantly increased 1.2 fold (p=0.0032). In conclusion, ADAMTS-4 and ADAMTS-5, known as the potential therapeutic targets and responsible for the enzymatic cleavage of the major component of the cartilage tissue aggrecan proteoglycan and contribution to the restructuring of cartilage, play an important role in the early diagnosis and treatment of articular cartilage injuries and diseases observed in humans and various animals. In this terms, the increase in the serum ADAMTS-5 levels may be one of the potential biomarkers of these disorders and it is necessary to investigate more extensively to clarify its action with clinical evidence.

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C05-13
Eight-weeks of treadmill exercise ameliorates neuropathic pain in diabetic rats

O. F. Kalkan[1], Y. E. SURMENELI[1], O. AKTAS[1], B. P. YUCEL[1], **A. AYAR**[1]
[1]*Karadeniz Technical University, Physiology, Trabzon, Turkey*

The aim of this study was to investigate effects of exercise on diabetes-induced neuropathic pain and possible role of endogenous irisin.

Adult male Spraque-Dawley rats were kept under standart conditions with free accces to water food. Animals were habituated to both treadmil exercise and pain threshold measurement set up before being divided into control (normoglycemic) and diabetic groups. Diabates (serum glucose >=300 mg/dL) was induced by i.p. injection of streptozotocin. *Diabetes was confirmed* by glucose measurement from blood of fasting animals collected from the *tail* vein, 48 hours after STZ injection.

Animals in the diabetes group was further divided into diabetes only, diabates + low intensity exercise, diabetes + high intensity exercise groups. The low intensity exercise protocol was 30 min/day by running at 0.5 km/h for 5 days/week and animals on high intensity exercise group performed 60 min/day by running at 1 km/h for 5 days/week, for 8 weeks.

Pain threshold, paw withdrawal in response to *radiant heat,* measurements were performed at baseline and at 4, 6 and 8th weeks after STZ by heat-induced plantar test. Data are compared using Dunnet test.

At the beginning of the experiment, the pain threshold values were not statisticaly different among the groups. After induction of diabetes, the pain threshold values were significantly increased. Exercise,

both low and high-intensity exercise, attenuated the diabetes-induced increase in pain threshold, only being significant at 8th weeks of exercise.

Results from this study indicates that chronic exercise provides beneficial effect on diabates-induced neuropathic pain.

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C07: Gastrointestinal physiology

C07-1

Effect of Pinealectomy and Melatonin Supplementation on Metallothionein, Zinc Transport Protein Levels in the Small Intestine Sections of the Rat

O. Unal[1], **A. K. Baltaci**[1], R. Mogulkoc[1], M. C. Avunduk[2]
[1]*Selcuk University Medical School, Physiology, KONYA, Turkey*
[2]*Necmettin Erbakan University, Pathology, Konya, Turkey*

The objective of the present study is to explore the relationship between levels of metallothionein, zinc transport protein levels, which comprise a basic mechanism in the absorption of zinc, in the parts of the small intestines, of rats whose pineal glands were removed, which were supplemented with melatonin after pinealectomy, and which were supplemented with melatonin without touching the pineal gland.

The study was carried out at the Wistar type adult male rats.

Group 1, Control, Group 2, Pinealectomy, Group 3, Pinealectomy + Melatonin, Group 4, Melatonin.

The percentages of ZnT2, ZIP2, ZIP4 and metallotionin were determined using the immunohistochemical method.

The results of the study indicate that reduced levels of ZnT2, ZIP-2, ZIP-4, and metallothionein, especially in the duodenum after pinealectomy are almost restored to control values after melatonin supplementation.

C07-2

Comparative study between esophageal hypomotility and inefficient esophagus about 420 cases

W. kacem[1]
[1]*University of medicine of Tunis, Physiology, Tunis, Tunisia*

Questions: Esophageal hypomotility is defined by an average pressure of contractions following a deglutition at the esophagus <50mmHg. It is fairly frequent pathology (31%) with a new character described these last years as an inefficient esophagus defined by an average pressure at the esophagus <30mmHg.

Aims:
* To study epidemiological and manometric data of a population of patients with moderate esophageal hypomotility and a population of patients with inefficient esophageal motility.

* To compare the collected data

Methods: A retrospective study of all esophageal manometers collected by the digestive functional exploration unit of the Gastro-enterology department during five years.

Results: We examined 420 patients: 223 patients with moderate esophageal hypomotility and 197 patients with inefficient esophageal motility. Comparing the two hypomotility groups, we found the following: our population is quite young whatever the intensity of the esophageal hypomotility. The two groups include a majority of females. The main esophageal manometric indicators (dysphagia, scleroderma and a reflux pre-intervention medical checkup) are quite similar. However, indicators distribution is different across groups. The two groups had a hypotonia at the lower sphincter of the esophagus, with a slightly higher frequency observed for the moderate hypomotility group. A statistically significant difference between the two groups (p=0, 02) is found at the motor disorder as highlighted by the manometer.

Conclusions: In this study, we found evidence pointing to some differences between moderate esophageal hypomotility and inefficient esophageal motility. Mainly, we found a higher frequency for achalasia and contamination of the scleroderma of the esophagus in the serious hypomotility group while the reflex and the diffused spasms disease are often associated with moderate hypomotility.

C07-3

Investigation of anticancer mechanism of isoorientin isolated from eremurus spectabilis leaves in HT-29 human colorectal adenocarcinoma cells.

G. GUNDOGDU[1], Y. DODURGA[2], L. ELMAS[2], S. YILMAZ TAŞCI[1], E. S. KARAOGLAN[3]
[1]*Ataturk University, Physiology, Erzurum, Turkey*
[2]*Pamukkale University, Medical Biology, Denizli, Turkey*
[3]*Ataturk University, Department of Pharmaceutical Botany, Faculty of Pharmacy, Erzurum, Turkey*

Question: Isoorientin is a flavonoid compound that can be extracted from plant species some of them are Phyllostachys pubescens, Patrinia, and Drosophyllum lusitanicum. The main aim of this study is to investigate the potential anti-proliferative effects of isoorientin in HT-29 human colorectal adenocarcinoma cell line in vitro, specifically on cell viability, apoptosis, and cell cycle patways.

Method: The cytotoxic effect of ISO isolated from E. Spectabilis was measured by XTT method in HT-29 cell lines. Total RNA was isolated with Tri-Reagent protocol. Effects of ISO on apoptosis related gene were determined by using RT-PCR. The analyses of findings were made by using $\Delta\Delta$CT method and quantitated with a computer programme. The comparison of groups was done with "VolcanoPlot" analysis, from "RT^2 . Profiles^{TM} PCR Array Data Analysis", which assessed statistically using the Student t-test.

Result: In our study, IC_{50} (inhibitory concentration where 50% of the cells die) of ISO was detected as 125 µM at the 48th hours in HT-29 cells by XTT assay. Real-time PCR analysis in HT-29 cells showed that CCND1, CDK6, casp-3, casp-8, Bax, Bcl-2, CHEK1, CHEK2 and ERCC1 expressions were reduced in ISO treated group of cells compared with the control group of cells. P53, p21, caspase-9 and ATR expressions were increased in ISO treated group of cells compared with the control group of cells ($p<0.05$).

Conclusion:. The effects of isoorientin were given in this study. ISO effected cell proliferation of colorectal cancer cells via cell cycle patways. It also altered apoptosis gene expression. These results demonstrated that ISO can be therapeutic agent for colorectal cancer treatment, however, further studies are needed to clarify the mechanism ofactions of ISO.

C07-4

Association between chromatin fractal lacunarity and nuclear envelope circularity in mice hepatocytes

J. Paunovic[1], D. Vucevic[1], T. Radosavljevic[1], **I. Pantic**[2,3]
[1]*University of Belgrade, Faculty of medicine, Institute of pathological physiology, Belgrade, Serbia*
[2]*University of Belgrade, Faculty of Medicine, Institute of Medical Physiology, Belgrade, Serbia*
[3]*University of Haifa, Haifa, Israel*

Questions: Relationship between chromatin structural properties and nuclear shape remains poorly understood. In our study, we tested the existence and strength of correlation between nuclear envelope circularity as the main parameter of nuclear shape, and chromatin fractal lacunarity in mice hepatocytes.

Methods: A total of 100 nuclear structures from 10 healthy male mice were evaluated using National Institutes of Health (Bethesda, MD) software and its subprogram / mathematical algorithm for fractal analysis. Chromatin was stained using DNA/RNA - specific toluidine blue method. Circularity of nuclear envelope was calculated based on nuclear area and perimeter. Chromatin fractal lacunarity was determined using box-counting algorithm.

Results: There was a statistically highly significant negative correlation ($p<0.01$) between the chromatin fractal lacunarity and nuclear envelope circularity. Circularity decreased as the lacunarity increased and vice versa. No such correlation was evident between nuclear perimeter and lacunarity, nor between nuclear area and lacunarity.

Conclusions: The results are in accordance to previously published research indicating that fractal organization of chromatin architecture is related to nuclear shape. The study presents a basis for further research in the field of cell physiology, molecular biology and biophysics.

Keywords: Chromatin; Lacunarity; Shape; Nucleus

C07-5

VX-809 restores the alcohol-induced expression defect of cystic fibrosis transmembrane conductance regulator in Capan-1 cells

A. Grassalkovich[1], J. Maléth[1], T. Madácsy[1], P. Pallagi[1], V. Venglovecz[2], Z. Rakonczay Jr.[3], P. Hegyi[3]
[1]*University of Szeged, 1st Department of Medicine, Szeged, Hungary*
[2]*University of Szeged, Department of Pharmacology and Pharmacotherapy, Szeged, Hungary*
[3]*University of Pécs, Institute for Translational Medicine and 1st Department of Medicine, Szeged, Hungary*

Introduction: Heavy alcohol intake is one of the most common causes of acute pancreatitis (AP). Our group previously showed that ethanol and fatty acids cause severe functional defect and impaired expression of the cystic fibrosis transmembrane conductance regulator (CFTR), which increases the severity of acute ethanol-induced pancreatitis. New compounds, (such as ivacaftor-VX-707 and lumacaftor-VX-809), are available that correct the impaired CFTR function and expression in cystic fibrosis patients with specific mutations, which might be utilized in the treatment of alcohol-induced AP.

Aims: Our aim was to test the effect of VX-809 treatment on the CFTR expression during ethanol exposure.

Materials & methods: CFTR expression was evaluated by immunofluorescent staining in Capan-1 cells and isolated guinea pig pancreatic ducts. Images were captured by confocal microscopy.

Results: Exposure of Capan-1 cells and guinea pig pancreatic ductal cells to 100mM ethanol for 12 hours significantly decreased the plasma membrane expression of CFTR. In parallel the cytoplasmic CFTR expression was increased. 10μM VX-809 alone had no effect on the CFTR expression. Whereas the application of 10μM VX-809 in pretreatment (treatment started 6 h prior to ethanol exposure), or post-treatment (treatment started 6 h after to ethanol exposure) significantly improved the plasma membrane expression of CFTR in Capan-1 cells.

Conclusion: These preliminary findings suggest that VX-809 might be able to restore the CFTR expression defect caused by alcohol. Further extended in vitro and in vivo studies need to clarify the effect of VX-809 on alcohol-induced pancreatic injury.

C07-6

THE CYTOTOXIC AND GENOTOXIC EFFECTS OF DAIDZEIN IN MIA PACA-2 HUMAN PANCREATIC CARCINOMA CELLS

G. Gundogdu[1], Y. Dodurga [2], M. Cetin[3], M. Secme [2], B. Cicek[1]
[1]*Atatürk University, Physiology, Erzurum, Turkey*
[2]*Pamukkale University, Department of Medical Biology, Faculty of Medicine, Denizli, Turkey*
[3]*Atatürk University, Department of Pharmaceutical Technology, Faculty of Pharmacy, Erzurum, Turkey*

Question: Pancreatic cancer is one of the most fatal malign diseases, with a worse survival prognosis, rapid growth and metastatic distribution. Daidzein, a flavonoid compound extracted from soybeans, has anticancer activity. The results of the *genotoxicity tests* play a significant role in the assessment of heritable and carcinogenic risks. The main object of the study was to investigate cytotoxic and genototoxic effects of daidzein in MIA PaCa-2 human pancreatic carcinoma cells.

Method: The cytotoxic effect of daidzein in MIA PaCa-2 cell line was measured by XTT method according to time and dose dependent manner within the range of 25-1000 μM. In addition, its genotoxic effects were also investigated with Comet Assay. Data were analyzed by using student t-test in SPSS 20.

Result: In this study, the IC50 (inhibitory concentration where 50% of the cells die) of daidzein was found as 200 μM in Mia Paca-2 cells at the 48th hour by XTT assay. Comet assay analysis in Mia Paca-2 cells showed that Head Length and Head Intensity were reduced in the experimental cell groups treated with daidzein compared with the control group. Tail Length, Tail Intensity, Tail moment and Tail migration were increased in the cell groups treated with daidzein compared with the control group ($p<0.01$).

Conclusion: This study displayed that daidzein has cytotoxic and genotoxic effects in MIA PaCa-2 human pancreatic carcinoma cells. These results suggest that daidzein may be used as a therapeutic agent for the treatment of pancreatic carcinoma alone or in combination with other drugs. However, furthers studies are needed to clarify the mechanism/s of cytotoxic and genotoxic action of daidzein.

C07-7

Mechanism of glutamate secretion on the pancreatic juice by acinar cells

D. Gluch[1], **S. Camargo**[1]
[1]*University of Zurich, Physiology, Zurich, Switzerland*

The pancreas efficiently absorbs amino acids for the synthesis of enzymes, but also secrets free amino acids in the pancreatic juice (PJ). From the 20 proteinogenic amino acids analyzed, glutamate (Glu) is the most concentrated. Under protein restriction, the PJ enzymes are decreased, but free Glu secretion is maintained. The aim of this study is to investigate the mechanism of Glu concentration in acinar cells and its mechanism of secretion.

Using mouse pancreata we analyzed the expression of possible carriers for Glu secretion. Freshly isolated acini were used for measuring Glu secretion in the presence of enzyme and channel inhibitors.

Our results showed that acinar cells accumulated Glu mainly via the metabolism of glutamine (Gln). The inhibition of the enzyme glutaminase (DON) reduced Glu accumulation in the cells and its secretion. The efflux mechanism of Glu in secretory cells in unknown, but recently several anion channels were showed to be able to efflux Glu and we analyzed their expression in pancreas. We observed that acinar cells express the calcium activated chloride channel ANO1/TMEM16, all the subunits forming the volume regulated anion channel LRR8CA-E/VRAC, and as previously showed the connexin 26 (CX26). TMEM16A expression was unchanged, but the VRAC isoform LRR8CA and CX26 increased and LRR8CB expression decreased in the pancreas of mice under protein restriction, suggesting that they may be involved in Glu secretion and or cell volume regulation. We are currently testing the effect of anion channel inhibitors in acinar Glu secretion.

Our results suggested that Glu is mainly synthesized from Gln in acinar cells. Our ongoing experiments will clarify the role of anion channels in the secretory mechanism of Glu by acinar cells.

C07-8

Investigation of the pancreatic ductal ion secretion in pancreatic ductal organoid cultures

R. Molnár[1], L. Alsardih[1], J. Fanczal[1], T. Madácsy[1], P. Hegyi[1], J. Maléth[1]
[1]*University of Szeged, First Department of Internal Medicine, Szeged, Hungary*

Intoruction: Pancreatic ductal fluid and HCO_3^- secretion are crucially important in the physiology and pathophysiology of the exocrine pancreas. However the study of human pancreatic secretory processes is great challenge due to the limited access to human pancreatic ductal cells. The recently developed three-dimensional pancreatic organoid cultures (OC) may help to overcome this limitation. However the ion secretory processes in pancreatic OC is not known.

Aims: Our aim was to characterize the ion transport processes in mouse pancreatic OCs.

Materials and Methods: Mouse pancreatic ductal fragments were isolated by enzymatic digestion. The isolated ducts were grown in Matrigel on 37°C for a week in OC media. Changes of the intracellular pH was measured to characterize the ion transporter activities of the epithelial cells in OC.

Results: Basolateral administration of 20mM NH_4Cl in standard HEPES or CO_2/ HCO_3^- buffered solution resulted in rapid intracellular alkalization, which was followed by a recovery phase. Removal of NH_4Cl induced rapid acidification followed by regeneration to the resting pH levels. The regeneration phase was inhibited by the removal of extracellular Na^+. The administration of 10μM $CFTR_{inh}172$, a selective inhibitor of cystic fibrosis transmembrane conductance regulator decreased the regeneration from alkali load. Basolateral administration of 20mM amiloride and 20mM H_2DIDS decreased the intracellular pH suggesting the activity of Na^+/H^+ exchanger and Na^+/HCO_3^- cotransporter on the basolateral membrane.

Conclusion: The ion transport activities in mouse OC are similar to those observed in freshly isolated primary tissue. This suggest that OC will be suitable to study human ductal epithelial ion transport.

C07-9

Role Of Vagal Afferents On High Fat Diet Induced Alterations in Rat Behaviour And Gut Motility

Y. Öztürk[1], B. Akgün[1], O. Çetin[1], H. î Karataş[1], B. Güney[1], Z. N. Özdemir Kumral[2], D. Özbeyli[2], S. Arabacı Tamer[2], H. Zortul[3], F. Arıcıoğlu[3], B. Ç Yeğen[2], N. İmeryüz[4,2]
[1]*Marmara University, Medicine, Istanbul, Turkey*
[2]*Marmara University, Physiology, Istanbul, Turkey*
[3]*Marmara University, Pharmacy, İstanbul, Turkey*
[4]*Marmara University, Gastroenterology, Istanbul, Turkey*

Questions: If is there any role of vagal afferent nerves on high fat diet (HFD) induced alterations in cognitive functions and gut motility?

Method: Ten-week old male Sprague-Dawley rats (n=38) were treated with either perivagal 1% capsaicin (n=19) or vehicle (10% Tween 80 in oil) (n=19). After 3 weeks of recovery, rats were pair-fed with chow (fat 2-7%) (n=18) or HFD (% 45 fat) (n=20) for 5 weeks until decapitation. In between 4th and 5th weeks of HFD rats were subjected to elevated plus maze and open field tests (anxiety and depression-like behaviour); novel object recognition, passive avoidance tests (memory). Food and water intake were measured after 16 hrs. of food and 24 hrs. of water deprivation. Weight of faeces for 16 hrs. and transit time with charcoal were measured. Data was expressed as mean standard deviation, comparison between groups were done with two- way ANOVA.

Results: VAD increased body weight significantly ($p < 0,05$) during feeding period irrespective of fat content of the diet. Fat content and VAD had no effect on 1 hr food intake after food deprivation. HFD decreased water intake ($p<0,0075$), VAD blunted this effect ($p<0,05$). Both HFD and VAD decreased faeces weight significantly ($p < 0,0001$ and $p<0,05$ respectively) but there was not any change in intestinal transit. HFD impaired short -term memory ($p<0,02$), whereas VAD compromised spatial learning ($p<0,04$). HFD rats were more anxious in OFT ($p < 0,01$) and in EPM ($p<0,03$).

Conclusion: HFD induced alterations in memory and anxiety were not affected by VAD but VAD blunted effect of HFD on water intake and faeces weight, suggesting that their operating mechanisms are different. VAD by itself impaired spatial memory that requires further investigation.

C07-10

FLUID AND HCO_3^- SECRETION AND CFTR ACTIVITY ARE INHIBITED BY CIGARETTE SMOKE EXTRACT IN GUINEA PIG PANCREATIC DUCTAL CELLS

D. Tálas[1], P. Pallagi[1], V. Venglovecz[2], E. Gál[1,2], K. Tóth[1], A. Schnúr[1], J. Maléth[1], D. Csupor[3], Z. Rakonczay Jr.[1,4], P. Hegyi[1,5,6]
[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 Pharmacognosy, Szeged, Hungary*
[4]*University of Szeged, Department of Pathophysiology, Szeged, Hungary*
[5]*University of Pécs, Institute for Translational Medicine & First Department of Medicine, Pécs, Hungary*
[6]*MTA-SZTE, Translational Gastroenterology Research Group, Szeged, Hungary*

Background: Smoking represents an independent risk factor for the development of chronic pancreatitis (CP). It is well documented that secretion of pancreatic ductal alkaline fluid (which is regulated mostly by the anion exchanger and CFTR) is diminished in CP. Aim: In this study we would like to understand whether smoking has any effects on pancreatic ductal fluid and HCO3- secretion.

Materials & methods: Guinea pigs were exposed to cigarette smoke four times a day for 30 min for 6 weeks. The CFTR expression was analysed by immunohistochemistry. Pancreatic ducts were isolated from guinea pig pancreas. Cigarette smoke extract (CSE) was prepared by smoking of 15 cigarettes into 10 ml distilled water by a smoking machine. Intracellular Ca2+ concentration and pH were evaluated by microfluorometry. Fluid secretion was measured by video microscopy. CFTR currents were detected by whole cell configuration of patch clamp technique.

Results: Cigarette smoking significantly diminished the expression of CFTR and the fluid and HCO3- secretion in guinea pig pancreas. CSE dose dependently decreased fluid and HCO3- secretion in guinea pig pancreatic ducts via inhibition of anion exchanger, Na+/H+ exchanger and Na+/HCO3- cotransporter and also forskolin-stimulated Cl- current of CFTR Cl- channel. CSE incubation altered

the pattern of carbachol–induced Ca2+ signal in pancreatic ducts suggesting that some of the inhibitory effects may be regulated by calcium signalling.

Conclusion: Cigarette smoking and CSE inhibits pancreatic ductal fluid and HCO3- secretion and the activity of the CFTR which may play role in the smoke-induced pancreatic damage. This study was supported by OTKA, MTA, SZTA and ÚNKP.

C14: Ion channels

C14-1
Different modulation of the excitability of hippocampal and cerebellar neurons by a fibrotic scar model

L. Lacinova[1], K. Ondacova[1], L. Lapinova[1], D. Jurkovicova[2]
[1]*Center of Biosciences, Institute of Molecular Physiology and Genetics, Bratislava, Slovakia*
[2]*Biomedical Research Center, Cancer Research Institute, Bratislava, Slovakia*

Multiple functional and morphological changes accompany a traumatic brain injury. Little is known about alteration of single neuron excitability at, or close to, the site of injury. We have used a fibrotic scar model developed by Kimura-Kuroda and coauthors to compare changes in excitability of rat hippocampal neurons (HN) and/or cerebellar granule cells (CGC) under the conditions mimicking those in injured brain.

When HN from newborn rats were cultured on a fibrotic scar model, they started to fire action potential series already at the Day 3 *in vitro* (DIV3). Control HN fired at the DIV3 single action potential only. Further, the density of voltage activated sodium, and potassium, but not calcium currents was significantly increased. Chondroitin sulfate proteoglycans played substantial role in these effects, as they were fully reversed by Chondroitinase ABC.

CGCs from 6 day old rats generated single action potential only when they were cultured for 3 days either on a fibrotic scar or in a control conditions. In a sharp contrast to HN, both sodium and potassium currents were significantly inhibited in CGCs cultured on a fibrotic scar at the DIV3. In line with our observation on HN, calcium currents were not altered. Again, observed effects were fully reversed by Chondroitinase ABC.

In conclusion, environment modeling the conditions of traumatic brain injury may have strikingly different effects on neurons in different parts of the brain. The hippocampal excitability was significantly enhanced and such enhancement may facilitate rise of epilepsy, which usually follows after brain injury. In contrast, excitability of CGCs was attenuated under these conditions.

C14-2
Glycine Uptake *via* Sodium/Neutral Amino Acid Transporters Activates a Swelling-Dependent Anion Conductance in Microglial Cells

M. Jakab[1], M. Kittl[1,2], M. Beyreis[1], H. Dobias[1,3], M. Gaisberger[1,3], M. Ritter[1,4], H. Kerschbaum[2]
[1]*Paracelsus Medical University, Institute of Physiology and Pathophysiology, Salzburg, Austria*
[2]*University of Salzburg, Department of Cellular Biology, Division of Molecular and Cellular Neurobiology, Salzburg, Austria*
[3]*Paracelsus Medical University, Gastein Research Institute, Salzburg, Austria*
[4]*Ludwig Boltzmann Cluster for Arthritis and Rehabilitation, Department for Radon Therapy Research, Salzburg, Austria*

Questions: In microglial cells formation of engulfment pseudopodia and particle uptake is associated with activation of a swelling-dependent Cl^- current (ICl_{swell}) and blockers of ICl_{swell} inhibit phagocytosis. Likewise, an increase in extracellular glycine stimulates phagocytosis, causes cell swelling and depolarizes the cell membrane potential (V_{mem}) due to glycine uptake *via* Na^+/neutral amino acid transporters (SNATs). Here we investigated if cell swelling under glycine induces ICl_{swell}, ICl_{swell} activation affects V_{mem} and glycine influences cell migration. **Methods:** Flow cytometric mean cell volume (MCV) measurements, whole-cell patch clamp and trans-well migration assays were used on murine BV-2 cells. **Results:** Glycine (5 mM) caused an increase in MCV under isotonic conditions by ~8% within 15 min. This was paralleled by the activation of a Cl^- conductance with biophysical and pharmacological characteristics of ICl_{swell}. Glycine uptake *via* SNATs induced a rapid, stable depolarization, which was enhanced by additional hypotonic stimulation of ICl_{swell}, but also under isotonic conditions upon long-term (>20 min) exposure to glycine. Cell migration was stimulated by glycine (0.6–5 mM). The ICl_{swell} inhibitor DCPIB (10 μM) completely counteracted both hypotonicity- and glycine-induced depolarizations, inhibited glycine-stimulated migration and augmented glycine-induced cell swelling. **Conclusions:** The findings indicate an interplay between cell volume regulatory processes and glycine-stimulated phagocytosis/migration in microglial cells – a mechanism which might be particularly relevant in case of brain trauma or ischemia, where high interstitial glycine concentrations occur due to cell damage.

C14-3
Noradrenaline Suppresses a Cl^- Current as well as Phagocytosis in Murine Microglia

K. Michael[1,2], M. Jakab[3], T. S. Steininger[2], M. Beyreis[1], M. Ritter[1,4], H. H. Kerschbaum[2]
[1]*Paracelsus Medical University, Institute of Physiology and Pathophysiology, Salzburg, Austria*
[2]*University of Salzburg, Department of Cellular Biology, Division of Molecular and Cellular Neurobiology, Salzburg, Austria*
[3]*Paracelsus Medical University Salzburg, Institute of Physiology and Pathophysiology, Salzburg, Austria*
[4]*Ludwig Boltzmann Cluster for Arthritis and Rehabilitation, Department for Radon Therapy Research, Salzburg, Austria*

Questions: In the central nervous system (CNS), neurodegenerative diseases are associated with a decrease in noradrenaline (NA). As microglial cells, macrophage-derived immune cells of the CNS, express adrenergic receptors (AR), their response to catecholamines is of interest. We found that in microglia a cell volume-regulatory Cl^- current (ICl_{swell}) is involved in volume-related functions like migration and phagocytosis. Since NA has been shown to suppress phagocytosis in microglial cells and fMLP-induced migration in neutrophils, we investigated if NA affects ICl_{swell} in microglial cells. **Methods:** Whole-cell Cl^- currents were recorded in murine BV-2 microglial cells using perforated patch clamp leaving the cytosolic milieu intact. Phagocytosis was quantified by exposing BV-2 cells or primary murine microglia to polystyrene microspheres for 15 min and counting the number of cells containing at least one microsphere using scanning electron microscopy. **Results:** Hypotonic cell swelling induced an outwardly rectifying Cl^- current (ICl_{swell}), which was reduced by addition of NA (1 nM or 1 μM). Similarly, ICl_{swell} was suppressed by the β-AR agonist isoproterenol, the Epac-specific analog 8-pCPT-2'-O-Me-cAMP and the PKA inhibitor H89. NA in the pM and nM range suppressed phagocytosis and the α_2-AR antagonist yohimbine enhanced the suppressing effect of NA. **Conclusions:** We show that AR stimulation suppresses ICl_{swell} in microglial cells, probably *via* altered cAMP levels. Given the role of ICl_{swell} in cell volume regulation and cell volume-related processes like formation of lamellipodia/engulfment pseudopodia and cell migration, its inhibition might underlie the observed suppression of phagocytosis upon AR stimulation.

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C14-4

Cloxyquin is a selective and state-dependent activator of TWIK-related spinal cord K^+ channel (TRESK)

M. Lengyel[1], A. Dobolyi[1], G. Czirják[1], P. Enyedi[1]
[1]*Semmelweis University, Physiology, Budapest, Hungary*

Questions:
Cloxyquin (5-chloroquinolin-8-ol) has been previously identified as an activator of TRESK background K^+ channel (K_{2P}18.1, TWIK-related spinal cord K^+ channel). We have examined the specificity of the drug by testing several K_{2P} channels. We also investigated the mechanism of cloxyquin-mediated TRESK activation, with emphasis on the differences between the physiologically relevant regulatory states of the channel.

Methods:
Potassium currents were measured by two-electrode voltage clamp in *Xenopus* oocytes and by whole-cell patch clamp in mouse dorsal root ganglion (DRG) neurons.

Results:
Cloxyquin (100 μM) activated both mouse (4.4±0.3-fold, EC_{50}=26.4 μM) and human TRESK (3.9±0.3-fold, EC_{50}=43.9 μM). TRESK was potently activated by cloxyquin in the resting state. The activation was not mediated by cytosolic [Ca^{2+}] (it was maintained in EGTA-injected oocytes) or activation of calcineurin (verified using calcineurin inhibitors and mutant channels with abolished calcineurin binding). The compound did not influence mouse TRESK and only slightly affected the human channel after activation via calcium signal evoked by the stimulation of Gq-coupled receptors. Constitutively active mutants could not be further stimulated by cloxyquin. The drug selectively targeted TRESK in the K_{2P} channel family. In a subpopulation of isolated DRG neurons cloxyquin application activated the background K^+ current.

Conclusions:
Cloxyquin activates TRESK by a Ca^{2+}/calcineurin-independent mechanism. The drug is specific for TRESK within the K_{2P} channel family and useful for studying TRESK currents in native cells. Cloxyquin may be a useful parent compound for the development of selective TRESK modulators.

C14-5

Ion channels in anticancer drugs painful side effects

A. Cophignon[1], S. Naik[1], N. Milosavljevic[2], M. Poët[1], L. Counillon[1]
[1]*LP2M/CNRS-UMR7370, Nice, France*
[2]*The University of Manchester, Manchester, United Kingdom*

Platin-based drugs and taxane are used in the treatment of breast, ovary, testes, kidney, or head and neck solid tumors. Platin-based drugs cause cell death through the formation of DNA adducts while taxane class of drugs are mitotic inhibitors. Interestingly in patients, these anticancer drugs with two completely different mechanisms of action exhibit a range of similar side effects that occur shortly after treatment and can last for years. Those include modification of touch perception, allodynia, cold hypersensitivity, imbalance, and tinnitus.

As cisplatin had been shown to modify membrane properties in different cell systems, we first had investigated its effects on mechanosensitive channels and found some interesting candidates for its action (Milosavljevic N. Cancer research – 2010). In second part, we investigated if platinum-drugs and taxanes can modulate gene expression of several channels involved in touch and pain perception. We also studied the expression of transcription factors modulate by xenobiotics and carrying a site to bind the promotor of our identified targets.

Strikingly, both platinum-based drugs and taxanes at doses used in chemotherapy, reveal a common profile of modified gene expression for two ion channels among those tested, which is correlated with

a modification of their protein activity. Interestingly, we identified a transcription factor specifically modulates as its two targets. Moreover, we observed a reversion of these effects by using drug acting on this transcription factor in parallel of the anticancer drugs treatment. Taken together, we hope that these results will provide us with new clues on possible common denominators to previously-unrelated side effects of these drugs.

C15: Other

C15-1

EVALUATION OF ESTRADIOL LEVEL AND SERUM LIPIDS IN WHITE WISTAR RATS OF FEMALE GENDER DURING THEIR GENERATIVE LIFE

S. Petrovska[1], B. Dejanova[1], S. Mancevska[1], J. Pluncevic-Gligorovska[1]
[1]*Faculty of Medicine, Department of physiology, Skopje, Macedonia, The Former Yugoslav Republic Of*

Objectives. Clinical and experimental data underscore the cardioprotective effects of female sex hormones, particularly estrogens. 50% of the antiatherogenic effects of estrogens are attributable to effects on lipoprotein metabolism. The values of estradiol and serum lipids were examined in white Wistar rats of female gender during their generative life.

Matherial and methods. The study included a total of 40 white Wistar rats of female gender divided into two groups according to their age (sex maturity): control group of 22 mature rats, with regular estrus cycle and experimental group of 18 rats in the period of reproductive involution at the age of eighteen months. Estadiol level was determined with standardized tests based on the radioimmunological method and serum lipid concentration was determined with the method of fractionation sedimentation according to the specific weight.

Results. The investigation has shown that there was a significant reduction of the estradiol level in experimental group (12.4± 3.8 pg / ml) in comparison to control group (23.9± 1.5 pg / ml), ($p< 0.05$) and a significant increase of the level of LDL-CH in experimental group (2.6± 1.3) in comparison to control group of female rats (1.1 ± 0.6) ($p< 0.05$). Nevertheless, there were no significant differences in the level of HDL-CH, total cholesterol, and triglycerides in two groups.

Conclusions. We can conclude that there is a severe impairment of lipid profile (increase of LDL cholesterol), during the involution period of female white Wistar rats, in comparison with the reproductive period of life.

C15-2

Discovery of a new voltage-gated proton channel

G. Chaves[1,2], C. Derst[3], A. Franzen[2], Y. Mashimo[4], R. Machida[4], B. Musset[1]
[1]*PMU Nürnberg, Institut für Physiologie, Nürnberg, Germany*
[2]*Forschungszentrum Jülich, ICS-4, Jülich, Germany*
[3]*Universität zu Köln, Zoologisches Institut, Köln, Germany*
[4]*University of Tsukuba, Sugadaira Montane Research Center, Ueda, Japan*

A new H_V1 gene was discovered in *Nicoletia phytophila*, an insect species from the Zygentoma order, one of the first terrestrial animals on Earth. We have called the protein NpH_V1 following the common nomenclature used for proton channels. Interestingly, NpH_V1 is genetically closer to its human homolog (33 % of identity) than to other species studied. NpH_V1 was successfully expressed in human cells presenting proton currents higher than 400 pA, suitable for electrophysiological studies. The detailed electrophysiological characterization has proved that NpH_V1 is highly proton selective, and shows other hallmarks of H_V1 as voltage-dependent gating and pH-dependent gating. Curiously, NpH_V1 has demonstrated to have an enhanced pH-dependence of gating when comparing with the human one (hH_V1). This pH-dependent gating is a unique characteristic for H_V1 which allows their main physiological role, cell´s pH regulation. However, how the channel sense and adjusts its gating

depending on the pH across the cell membrane is still unknown. Here, further studies focused in differences on the amino acids sequence between NpH_V1 and hH_V1 could elucidate the intrinsic mechanism. Finally, substitutions of Asp66 by non acidic amino acids have shown anion permeation meanwhile those done with acidic amino acids showed conserved proton selectivity. Therefore, we probed that Asp66 (Asp112 for humans) is the selectivity filter for NpH_V1, confirming it as the general selectivity mechanism for all known H_V1.

Conclusions

- This work is the first description of a proton channel in insects.
- NpH_V1 presents the same biophysical characteristics for all known proton channels.
- NpH_V1 shows an enhanced pH-dependent gating in comparison with the human protein.
- Asp66 is the selectivity filter for NpH_V1 and confirms the general selectivity mechanism for all the proton channels.

C15-3

The determination of interaction between naringin and different chemotherapy agents in neuroblastoma and astrocyte cell lines

N. P. Turker[1], **Z. B. Doganlar**[2]
[1]*Trakya University, Technology Research and Application Center (TUTAGEM), Edirne, Turkey*
[2]*Trakya University, Medicinal Biology, Edirne, Turkey*

Neuroblastoma is a cancer type seen in children under five years old. Chemotherapy (doxorubicin, cisplatin and etoposide) use for the treatment in addition to surgery, radiation and stem cell transplantation. Because of the side effects of chemotherapeutic agents, some plant-derived components are used for protecting healthy cells. Naringin is a citrus flavonoid have antioxidant, apoptotic, antiinflamatuar properties. In this study, we aimed that the determination of single and combine effects of naringin and chemotherapy agents (doxorubicin and cisplatin) in neuroblastoma N1E-115 (ATCC® CRL-2263™) and astrocyte C8-D1A [Astrocyte type I clone] (ATCC® CRL-2541™) cell lines. With this aim, the effects of the combinations following exposure to the sequentially and simultaneously on apoptosis analyzed by image-based cytometer and gene expressions of apoptosis pathway. According to results of the study, naringin induced intrinsic apoptosis pathway as evidenced by the induction of p53, Bax, Cyt-c and caspase-3 in neuroblastoma cells. In addition, pre- or post treatment of naringin with chemotherapy agent caused different apoptotic effects. In conclusion, naringin treatment before cisplatin and after doxorubicin caused more apoptosis in neuroblastoma cells. Furthermore pretreatment of naringin showed protective effect against cisplatin toxicity in astrocyte cell lines. This study was supported by Trakya University Research Project Foundation (Project Number: TÜBAP-2016-231), Edirne/Turkey

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C15-4

Critical analysis of dietary habits in people with type 2 diabetes

K. INCHIRAH[1]
[1]*faculty of sciences of Bizerta, tunisia, biology, bizerte, Tunisia*

Diabetes is a formidable disease for the complications it causes (infarction, renal insufficiency, blindness, ...). Thus, it is better to diagnose this disease early to learn more about its different forms, its screening and its treatment. Thus, in this work, we proposed to evaluate and criticize the quantitative and qualitative aspects of the spontaneous feeding of a group of patients with type 2 diabetes, and to highlight the different diet gaps for a good catch in charge. The objectives of this study:
To highlight the different dietary habits of a group of patients with type 2 diabetes, Assess the quantitative and qualitative aspects of the spontaneous feeding of this group, Criticize the main regime differences.

This was a prospective study involving 70 patients with type 2 diabetes, who were recruited from the outpatient department of the National Institute of Nutrition and Food Technology in Tunis over a period of one month. We were interested in the various anthropometric parameters, as well as a food survey and a questionnaire on personal data of the patients.

From our results, it emerges: A caloric normo diet A hyper-carbohydrate, normo-protein and normi-lipid ration. Atherothrombogenic diet (AGPI / AGMI)> 1

These results show that there is an imbalance in the dietary intake of diabetics studied which contributes to diabetes imbalance especially with excessive carbohydrate intake. Our results can be explained by high consumption of cereals and moderate consumption of other foods.
The situation is alarming for both young people and adults. However, they continue to neglect preventive measures by adapting unbalanced eating behaviors. Critical analysis of dietary habits in people with type 2 diabetes.

C16: Neuro-immunology

C16-1

Association of TNFAIP3 and TRAF1 polymorphisms with susceptibility to systemic lupus erythematosus and rheumatoid arthritis in Egyptian Population.

A. ISMEIL[1]
[1]*Faculty of Medicine, Physiology Department, Sinnar, Sudan*

Background:
Recent genome-wide association studies demonstrated association of single nucleotide polymorphisms (SNP) in the TNFAIP3 and TRAF1 with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in European populations.

Aim of study:
To determine whether the Tumor necrosis factor, alpha-induced protein 3 (TNFAIP3) polymorphism (rs2230926) and tumor necrosis factor (TNF)-receptor associated factor 1 (TRAF 1) polymorphism (rs10818488) confer susceptibility to systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in Egyptian population.

Materials and Methods:
This was a case-control study in which 90 individual with SLE and 105 individual with RA and 75 healthy controls were included. Genotyping was performed using TaqMan genotyping assay for two single nucleotide polymorphisms (SNPs) that showed the best evidence of association in the previous Caucasian studies.

Results:
We detected significant differences in allele frequency of rs2230926 G allele with SLE (OR: 3.13, CI: 1.37-7.12; P=0.006) and RA (OR: 2.9; CI: 1.31-6.65; P =0.008). A allele of TRAF1 was significantly increased in RA compared to control (50% versus 40.6%). Carriers of the A allele were significantly more likely to develop RA (OR: 1.45; 95% CI: 0.95-2.22; P=0.008), while TRAF 1 polymorphism did not exhibited any statistical significant difference in the frequencies of genotypes or alleles in SLE and controls (OR: 0.67; 95% CI: 0.43 -1.06; P=0.03).

Conclusion:
These results indicated that TNFAIP3 is a susceptibility gene to SLE and RA in the Egyptian population. Also Association of TRAF1 locus with RA susceptibility was detected in the Egyptian population, while no significant association was observed for SLE.

Keyword: TNFAIP3; TRAF1; polymorphisms; systemic lupus erythematosus; rheumatoid arthritis; Egyptian.

C16-2

Antibodies against vimentin -An early biomarker of ischemia?

S. A. Türkoglu[1], M. N. Öğün[1], Ü Karabörk[1], **H. S. Orallar**[1], S. Yıldız[1]
[1]*Abant Izzet Baysal University, Bolu, Turkey*

Although anti-vimentin (cytoskeletal protein) autoantibodies (AVA) are not associated with a specific autoimmune disease, they are also a patency that can be seen in diseases such as Rheumatoid Arthritis, anti-phospholipid syndrome, SLE and some infections. The clinical provision is not yet fully understood. In this study, we aimed to investigate the clinical features of AVA positive patients who were followed up in our clinic. The patients who came up with different diagnostic diseases such as vasculitis, dysmyelinizing disease, multiple sclerosis, anti-phospholipid antibody syndrome, ischemic stroke were demended from Neurology Department to be tested anti-nuclear antibodies (ANA). These tests were conducted Department of Medical microbiology and Immunology laboratory at the desired and Indirect Fluorescent antibody test 10 cases were studied retrospectively. According to the manufacturers recommendations ≥ 1/100 dilution of serum titres are considered positive.Three of the female cases had ischemic cerebrovascular disease.One of these patients, had an APS, one had actinic keratosis and the other had FMF. A 53 year old patient had coronary artery disease. A 68-year-old patient had CAD and additionally hashimoto thyroiditis. A 33 year old patient was diagnosed with MS. A 3 year old dysmyelinizing patient and her investigations were still continuing. Two patients aged 34 and 39 had RA diagnosis. A 32-year-old male patient was diagnosed with MS and vasculitis.AVA positivity in patients with ischemic processes is at the forefront, this in addition to autoimmune patients and additional diseases in character. In patients with rheumatic disease in particular autoimmune character AVA is positive in terms of the early biomarker of ischemia caused by more extensive studies are needed.

C18: Teaching & e-learning

C18-1

Near-Peer Teaching Program in Medical Physiology at Comenius University

S. Hnilicova[1], A. I. Daponte[1], P. Vitovič[2], A. Dal Grande[1], F. Schmitt[1], Y. Senoo[1], P. Hnilica[3], D. Ostatnikova[1]
[1]*Comenius University in Bratislava, Institute of Physiology, Bratislava, Slovakia*
[2]*Faculty of Medicine, Comenius University, Department of Simulations and Virtual Medical Education, Bratislava, Slovakia*
[3]*SI Medical, Bratislava, Slovakia*

Traditional curriculum in Human Physiology at Faculty of Medicine, Comenius University in Bratislava involves lectures for 300 students and direct teaching in small group labs, all taught by Faculty. Recently, near-peer(NP) teaching pilot program has been added as a novel method of teaching in English program.

The aim of our study was to analyze if adding NP teaching model would increase understanding and motivation among our students. Students who finished Physiology curriculum were selected as NP teachers based on academic performance, leadership skills, motivation, and willingness to teach. Preclinical students participated in 6 structured, three hour-long tutorials for each module. 2 sessions were held on each topic by 2-3 tutors, using different modes of teaching (manikin simulators, OSCE, PBL, hands-on experiments, power-point presentations, NP teachers also provided self-made online videos and handouts).

Total of 17 NP teachers (N=17, 9 female and 8 male) participated in the study. 100% of them considered teaching beneficial for their knowledge, teaching skills, and would consider to do it again, if asked.

35 (92,1%) anonymous self-reported detailed Likert-style questionnaires were collected from students (n=35, 20 female and 15 male). 90% of them reported that NP program increased their knowledge and

improved final test results. 85% of them mentioned that they would like to participate again, if asked. For 85% of them, program enhanced their inner motivation towards studying Physiology.

NP program was found to be beneficial for both students and NP teachers, as valuable addition to Physiology traditional classes. In the future, we plan on expanding tutorials to give equal opportunities for all students.

C18-2

Team-Based Learning in Medical Physiology

M. Geiger[1]
[1]*Medical University Vienna, Department of Vascular Biology and Thrombosis Research, Vienna, Austria*

Team-based learning (TBL) is a teaching concept which allows performing small group teaching a in a lecture hall setting. We introduced TBL for Physiology teaching for 740 students/semester. TBL consists of a preparatory phase, where the students acquire all knowledge necessary for the actual TBL. In our setting the preparatory phase (4 weeks) includes plenary lectures, seminars, practical courses, and self-studying of various physiological contents. For the actual TBL (4 x 2 hours distributed over 1 week) students are randomly assigned to a team consisting of 5-7 persons; we teach 10 teams per lecture hall. At the beginning of each of these 4 courses students have to individually complete a readiness assurance test consisting of 5 to 8 multiple choice questions presented as PowerPoint slides. Students write down their answers, and immediately after this individual test, they take the same test as a team. After a discussion within the team, the teams have to decide for one answer and display their answer per audience response system. Teachers and students see the answers of the different teams, and teams have to defend their answers against those of other teams. Teachers facilitate the discussion between teams, ask questions to explore the topic, and give explanations if necessary. The most important task for teachers is to prepare multiple choice questions that connect many different fields of Physiology and that stimulate discussion. Teachers have to be open for surprising questions and answers from the students, and should not be afraid of noise in the class room. In TBL students are motivated to reflect what they have learned in the preparatory phase and make the experience that they usually perform better as a team than as an individual.
