C01: Cardiac physiology

C01-1

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

V. Farkasová1, L. Griecsová1, M. Murániková1, S. Čarnická1, J. Lonek1, M. Ferko1, A. Adameová2, T. Ravingerová1

1Institute for Heart Research, Slovak Academy of Sciences, Department of cardiovascular physiology and pathophysiology, Bratislava, Slovakia
2Faculty 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 arrhythmogenic effect, while in 8m SHR rats its effect was either proarrhythmic. Protective effect of RIP was accompanied with increased Akt and GSK3-β activation as well as with decreased proapoptotic signaling only in hearts from 3m and 5m animals, while in 8m rats the Akt and GSK3-β 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. Ferko1, I. Kancirová1, M. Jašová1, J. Kucharská1, O. Uličná1, O. Vančová1, M. Murániková1, T. Ravingerová1, I. Waczuškovic1

1Institute for Heart Research, Slovak Academy of Sciences, Biochemistry, Bratislava, Slovakia
2Pharmacobiochemical Laboratory, Third Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia
3Division 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 remodelling of heart mitochondrial membrane and its cardioprotective contribution to ischemic-reperfusion injury was observed.
Role of altered Ca\(^{2+}\) homeostasis during adverse cardiac remodeling after ischemia and reperfusion

A. Domínguez-Rodríguez,1 E. Díaz,1 E. Sánchez de Rojas-de-Pedro,1 I. Mayoral-González,1 A. Hmouche,1 E. Calderón-Sánchez,1 J. Avila-Medina1, A. M. Gomez,1 J. P. Benítez,1 Á. Ordoñez,1 T. Siman1
1Institute of Biomedicine of Seville, Seville, Spain
2CABIMER, Department of Stem Cells, Seville, Spain
3UMR 31180, 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 IR subjected to transient (40 minutes) ligation of left descending coronary artery. Changes in cytosolic ([Ca\(^{2+}\)]\(_c\)) and intracellular ([Ca\(^{2+}\)]\(_i\)) were studied in a cardiac myocyte isolated from remote and infarcted zone 1 week after surgery.

Results: Using echography and nuclear magnetic resonance we observed that rat undergoing IR protocols have depressed cardiac contractile capacity as soon as one week after surgery. IR treatment produces a decreased cytosolic ([Ca\(^{2+}\)]\(_c\)) and intracellular ([Ca\(^{2+}\)]\(_i\)) transients in adult cardiomyocytes, not only in the risk zone but also in the remote zone. IR 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 IR, not only in the infarcted but also in the remote area. These calcium changes may contribute to the development of adverse cardiac remodeling and further heart failure.

Fluoxetine Attenuates Remote Myocardial Ischemia Reperfusion Injury

M. O. Yaman1, I. Guner1, H. Ermaz1, O. E. Tok3, M. Pala1, M. Esrefoglu1, R. Geлизgen1, H. Uzun3, N. Yelemen1, G. Sahin1
1University of Istanbul, Cerrahpaşa Medical Faculty, Physiology, Istanbul, Turkey
2Medeniyet University, Istanbul, Turkey
3Beyzaadem University, Histology and Embryology, Istanbul, Turkey
4Bordo University, Physiology, Istanbul, Turkey
5University of Istanbul, Cerrahpaşa 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 (80-min ischemia and 120-min reperfusion); 3) Flx+IR (20 mg/kg/d, i.p. for 5 days). 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 leukocytes 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.

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

M. Zálešák1, J. Grabán3, B. Kura1, D. Pancza1, T. Ravingerová1, J. Slezák1
1Institute 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 (HpoC) against ischemia-reperfusion (IR) injury. Isolated rat hearts perfused with Krebs-Henseleit buffer (KHB) were exposed to 30-min global ischemia/120-min reperfusion. HpoC was induced by 4 cycles of 1-min perfusion with oxygen-free KHB intercepted by 1-min perfusion with normal KHB, while in H2+HpoC group, oxygen-free KHB was enriched with H2. Severity of IR injury was evaluated by measurement of infarct size (IS) within the area at risk (AR) (IS/AR, TTC staining) and recovery of function. IS was markedly reduced in HpoC group to 24.6 ± 0.9% compared with 38.7 ± 1.4% in non-conditioned controls, and even more significantly in H2+HpoC group (16.6 ± 0.8%; P<0.05 vs. both, controls and HpoC). Post-IR recovery of systolic function (LVDP) was improved in H2+HpoC 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 (HpoC: 22.1 ± 5.9 mmHg, H2+HpoC: 28.6 ± 5.6, both P<0.05 vs. 55.2 ± 6.9 mmHg in controls). Application of H2 potentiated the beneficial effect of HpoC. Grants: VEGA SR 2020115, 2020115, APVV-021115, APVV-024111, APVV-15-0376.

The Effects of Zofenopril on Cardiac Function and Pro-Oxidative Parameters in the Streptozotocin-Induced Diabetic Rat Heart

V. Živković1, P. Rtičić1, I. Srejović1, T. Nikolić1, I. Stojić1, D. Rtičić1, V. Jakovljević1
1Faculty of Medical Sciences, University of Kragujevac, Physiology, Kragujevac, Serbia
2Military Medical Academy, Belgrade, Endocrinology, Belgrade, Serbia
3Faculty of Medical Sciences, University of Kragujevac, Pharmacy, Kragujevac, Serbia
4Military 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 hyperglycaemia, 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 technic. We evaluated the influence of acute administration of zofenopril on myocardial function from rats with streptozotocin-induced diabetes mellitus (STZ-DM),
with a special emphasis on cardioselective 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.

Conclusions: 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 HYPERHOMOCYSTEINEMIC RATS

T. Nikolić1, V. Zivković2, N. Jeremić1, J. Jeremić1, I. Stojić1, I. Srejović2, D. Djurić2, V. Jakovljević1
1Faculty of Medical Sciences, University of Kragujevac, Pharmacy, Kragujevac, Serbia
2Faculty of Medical Sciences, University of Kragujevac, Physiology, Kragujevac, 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 (ICHOL), Low-density Lipoprotein (LDL), High-density lipoproteins (HDL), Triglycerides (TRI) levels, as well as pro-oxidative (superoxide anion radical, hydrogen peroxide, index of lipid peroxidation) and antioxidant markers (reduced glutathione, catalase and superoxide dismutase) in Wistar albino rats.

Methods
Study 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 deficin 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: HMCoA reductase inhibitors, homocysteine, oxidative stress

C01-9
THE EFFECTS OF CHRONIC ADMINISTRATION OF CISPLATIN ON OXIDATIVE STRESS IN ISOLATED RAT HEART

J. Jeremić1, I. Stojić2, T. Nikolić1, J. Smigic3, V. Zivković1, I. Srejović2, T. Sabo1, V. Jakovljević1
1Faculty of Medical Sciences, University of Kragujevac, Department of Pharmacy, Kragujevac, Serbia
2Faculty of Medical Sciences, University of Kragujevac, Department of Physiology, Kragujevac, Serbia
3Faculty of Chemistry, University of Belgrade, Department of General and Inorganic chemistry, 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 Langendorf technique at gradually increased coronary perfusion pressures (40-120 cmH2O). Over the entire CPP range, we measured levels of superoxide anion radicals, hydrogen peroxide, nitrates 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 hypoxia (higher than 80 cm H2O).

Results
Levels of superoxide anion radicals, hydrogen peroxide, nitrates 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 hypoxia 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 hypoxia.

Key words: cisplatin, isolated rat heart, oxidative stress

C01-10
THE EFFECTS OF MODULATION OF N-METHYL-D-ASPARTATE RECEPTORS ON OXIDATIVE STATUS IN ISOLATED RAT HEART

I. Srejović1, V. Zivković1, N. Jeremić2, I. Stojić2, T. Nikolic2, D. Djurić2, V. Jakovljević1
1Faculty of Medical Sciences University of Kragujevac, Department of Physiology, Kragujevac, Serbia
2Faculty of Medical Sciences University of Kragujevac, Department of Pharmacy, Kragujevac, Serbia
3Institute 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 yet. The aim of the present study was to examine the effects of MK-801, as a NMDA-R blocker, alone and its combination with glyciné and/or glutamate on oxidative status in isolated rat heart. The hearts of male Wistar albino rats were excised and perfused according to Langendorf technique and in samples of coronary venous effluent were spectrophotometrically determined values of biomarkers of oxidative stress – index of lipid peroxidation measured as TBARS, nitrates (NO3−), superoxide anion radical (O2−) and hydrogen peroxide (H2O2). Only in group treated with MK-801, glutamate and glyciné there was an increase in value O2− 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. Grieceova1, V. Farkasova1, L. Lonek1, I. Gablovska1, I. Benmatova1, T. Ravingerova1
1Institute for Heart Research SAS, Department of cardiovascular physiology and pathophysiology, Bratislava, Slovakia
2Institute 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 l/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 Cz (PKCz) (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 PKCz 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.

P. Fedichkin1, Y. Goshovska1, A. Kornelyuk1, V. Sagach1
1Bogomolitz Institute of Physiology, Circulation, Kyiv, Ukraine
2Institute 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 mg/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. Goshovska1, V. Sagach1
1Bogomolitz 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 4.4-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. Park1
1Kangwon National University School of Medicine, Department of Physiology, Chuncheon, South Korea

This study investigated the alteration of voltage-dependent K+ (Kv) channels in mesenterial arterial smooth muscle cells from control (LETO) and diabetic (OLEFT) 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 OLEFT rats than in LETO rats. The contractile response of the mesenteric artery induced by the Kv inhibitor, 4-amipropidine (4-AP), was also greater in OLEFT rats. The expression levels of most Kv subtypes were increased in mesenterial arterial smooth muscle from OLEFT rats compared with LETO rats. However, in the chronic phase of diabetes, the Kv current amplitude did not differ between LETO and OLEFT 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. Seo1, W. S. Park1
Kangwon National University School of Medicine, Physiology, Chunchon, 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 adenyl 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 (amiloride) and 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 adenyl 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. Shin1, W. S. Park1
Kangwon National University School of Medicine, Department of physiology, Chunchon, 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 change the 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 nortriptyline were not use-dependent. Preincubation with the Kv1.5 and Kv2.1/2.2 inhibitors, DFO-1 and guanibotrocin 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. Li1, W. S. Park1
Kangwon National University School of Medicine, Department of Physiology, Chunchon, 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, 4-AP, 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. An1, W. S. Park1
Kangwon National University School of Medicine, Department of Physiology, Chunchon, 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. Li1, S. E. Shin1, M. S. Seo1, J. R. An1, W. S. Park1
Kangwon National University School of Medicine, Department of Physiology, Chunchon, 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\textsubscript{1.2} L-type Ca\textsuperscript{2+} channel form a signal complex with Orai1 and TRPC1 in vascular smooth muscle cells: Role in vascular tone regulation

J. Avila-Medina\textsuperscript{1,2,3}, E. Calderon-Sanchez\textsuperscript{2,3}, P. Callejo-García\textsuperscript{1}, J. A. Rosado\textsuperscript{1}, T. Smani\textsuperscript{1,2,3}

\textsuperscript{1}University of Seville\textsuperscript{;}Institute of Biomedicine of Seville, Medical Physiology and Biophysics, Seville, Spain
\textsuperscript{2}Institute of Biomedicine of Seville, Grupo de Fisiopatología Cardiovascular, Seville, Spain
\textsuperscript{3}CiberCV, Madrid, Spain

Rationale: Voltage-dependent Ca\textsubscript{1.2} L-type Ca\textsuperscript{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\textsuperscript{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\textsubscript{1.2} 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\textsuperscript{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\textsubscript{1.2} both in aortic and coronary VSMCs. Interestingly, Orai1 and TRPC1 enhanced their interaction with Ca\textsubscript{1.2} 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\textsubscript{1.2}-dependent LTCC and SOCC channels formed by Orai1 and TRPC1.

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Keywords: Ca\textsubscript{1.2}; Orai1; TRPC1; Store depletion; Vascular tone regulation.

C02-8
Effects of PCSK9 inhibitor in obese Zucker (fa/fa) rats.

M. Kosutova\textsuperscript{1}, R. Rehakova\textsuperscript{1}, M. Cebova\textsuperscript{1}, Z. Matuska\textsuperscript{1}, O. Pechanova\textsuperscript{1}

\textsuperscript{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 3HJ-arginine to 3HJ-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, blocking blood pressure yet. In conclusion, blocking blood pressure yet. In conclusion, blocking blood pressure yet. In conclusion, blocking blood pressure yet. In conclusion, blocking blood pressure yet. In conclusion, blocking blood pressure yet.

Supported by: APVV-14-0932, VEGA-2/0170/17, SSC grant

C02-9
Protective effects of nanoparticle-loaded renin inhibitor in experimental hypertension

O. Pechanova\textsuperscript{1}, M. Cebová\textsuperscript{1}, R. Reháková\textsuperscript{1}, S. Vranková\textsuperscript{1}, A. Barta\textsuperscript{1}

\textsuperscript{1}Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Department of Neurocardiovascular 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 SHR 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.


C02-10
Ranolazine improves vascular sensitivity to insulin in rabbit femoral arteries.

C. Aldasoro\textsuperscript{1}, S. Guerra-Ojeda\textsuperscript{2}, A. Jorda\textsuperscript{2}, P. Marchio\textsuperscript{2}, M. Gimeno-Raga\textsuperscript{2}, M. D. Mauricio\textsuperscript{2}, S. Valles\textsuperscript{2}, M. Aldasoro\textsuperscript{2}, J. M. Vila\textsuperscript{2}

\textsuperscript{1}Hospital General de Castellon, Medicina Familiar y Comunitaria, Castellon, Spain
\textsuperscript{2}University of Valencia, Physiology, Valencia, Spain

Questions: Insulin resistance impairs vascular function through an imbalance between vasconstrictor 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 concentration–dependent relaxant response in rings pre-contracted with noradrenaline (Emax = 43.5 ± 6.3). 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 1 NaL blockade.

C02-11
Renal vascular Kv7.1 channels – potential targets for renoprotection
R. Schubert1, F. Stocker1, S. Braun1, N. Schmid1
1Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

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 arterioles and intact kidneys were studied using real-time qPCR, isometric vessel myography and constant-flow organ perfusion.

Results: In renal arterioles, 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. tanyel1, E. ERASLAN2, E. polat3, E. polat3, N. Kurt2
1Atatürk University, Physiology, Erzurum, Turkey
2atalturk university, biochemistry, erzurum, Turkey
3atalturk 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 (IR) 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 nonspcific calcium channel antagonist, in heart injury induced by Renal IR 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 (IR), 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 means±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 IR decreased the MDA, SOD, MPO and H2O2 levels in the group which received nifedipine, when compared with the IR 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 IR; Caspase-3 expression have decreased with the application of calcium canal antagonists.

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

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

C03-1
Iron oxide nanoparticles increase nuclear textural entropy in buccal epithelial cells
I. Pantic1,2
1University of Belgrade, Faculty of Medicine, Institute of Medical Physiology, Belgrade, Serbia
2University of Halls, Halls, 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 vitro conditions.

Methods: Human BECs were kept in RPMI-1640 medium at 37°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 (Oplican 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 tissue damage caused by the 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. Voglova1, J. Bezakova1, R. Reis2, M. Vician2, M. Zeman1, I. Herichova1
1Faculty of Natural Sciences Comenius University in Bratislava, Department of Animal Physiology and Ethology, Bratislava, Slovakia
2University 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-21-3p than miR-21-5p 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. Forcini1, R. Dai Magro1, E. Cesana1, B. Albertini2, P. Blasi2, F. Re1, G. Sancini1
1University of Milan Bicocca, School of Medicine and Surgery, Monza, Italy
2University 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 increased its role in developing strategies to deliver drugs to the CNS. In our previous studies we administered liposomes functionalized with phosphatidyl 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 (hCMVEC/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 dynamics 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. Heim, M. Beyreith1, C. May1,2, M. Ritter1, M. Jakab1, T. Kesslich1,2, K. Plätzner1
1Paracelsus Medical University Salzburg, Institute of Physiology and Pathophysiology, Salzburg, Austria
2Salzburger Landesklinikum - SALK, Paracelsus Medical University, Department of Internal Medicine I, 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: A31 (epidermoid carcinoma) cells were treated with 3 different established chemical apoptosis inducers (actinomycin-D, TBB, RG 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 major 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. Polikarpova1, I. Levina1, L. Kulikova1, L. Morozov2, P. Rubtsov3, I. Zavarzin1, A. Guseva1, O. Smirnova1, T. Shchelkunova1
1Lomonosov Moscow State University, Faculty of Biology, Moscow, Russian Federation
2Zelinsky Institute of Organic Chemistry Russian Academy of Sciences, Moscow, Russian Federation
3Engelhardt Institute of Molecular Biology Russian Academy of Sciences, Moscow, Russian Federation

Progesterone (P4) ensures pregnancy preservation and prevents alloimmune fetal rejection. The mechanism of P4 action on immune cells is not well understood. The effects of progestins are mediated both by nuclear (nPnRs) and membrane receptors (mPRs) of the progestin and adipoQ receptor family. The mPRs and mPRa 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-hydroxyprog-4-en-20-one (I) and 19-hydroxyprog-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 mRNA 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 1 and 10 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 1 and II may be due to their different affinity for the mPR α and β subtypes, whereas P4 binds to both mPRs.

C03-6
Tolfecinude Induces Apoptosis by Increasing TNF-alpha Gene Expression in rat hepatocellular carcinoma cells

S. Akın1, M. Özkurt1, R. Uyar1, S. Kabadere1
1Eskişehir Osmangazi University, Physiology, Eskişehir, Turkey

Question: Tolfecinude 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, NkappaB and TNF-alpha on rat hepatocellular carcinoma (H4IIIE) cells.

Method: We treated H4IIIE 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, NkappaB 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). The abundance of caspase-3, IL-1beta, NkappaB and TNF-alpha mRNA were analyzed using the beta-actin as a reference gene. Differences with P values <0.5 were considered significant.

C03-7
The apoptotic effect of quercetin in human hepatoma cell line Hep3B that NF-KB pathway suppressed by CAPE

M. Kasi1, O. Dıoanlar1
1Trakya University, Faculty of Medicine, Medical Biology, Edirne, Turkey

Results: Caspase3, IL-1beta and NkappaB 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 transcriptionic effect on TNF-alpha.

C03-8
Transcriptional regulation of metabolic reactions in breast cancer cells

I. Cseslevicz1, I. Antanuičiūtė1, V. Mikalayeva1, G. Mišačiūtė1, V. A. Skrebédas1, S. Bordel Velasco1
1Lithuanian University of Health Sciences, Institute of Cardiology, Kaunas, Lithuania

We found that the proliferation rate of cancer cell lines from the NCI-60 collection correlated 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 shunt 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 suppression of enzymes involved in alternative supply 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-
diphenylcyclophosphazene Derivatives and Investigation of Their Anti-
Cancer Activities

S. Tekin1, A. Beytur1, M. Cakir2, S. Sandal1
1Inonu University, Physiology, Malatya, Turkey
2Bogaz University, Physiology, Yozgat, Turkey

The compounds, so called phosphazene, contain phosphorus-nitrogen double bond. Phosphazenes are the largest class of inorganic macromolecules that cover small molecules through polymers depending on the repeating unit,N=PX2-group, in their structure. In the present study, 1,1,3,3-tetra(4'-oxy-3-fluorochrome)-5,5-diphenylcyclophosphazene (1a), 1,1,3,3-tetra(4'-oxy-3-
chlorochrome)-5,5-diphenylcyclophosphazene (1b) and 1,1,3,3-tetra(4'-oxy-3-bromochrome)-5,5-
diphenylcyclophosphazene (1c) compounds were obtained from the reactions of 1,1,3,3-tetrachloro-
5,5-diphenylcyclophosphazene[4] with 4-hydroxy-3-fluorochrome, 4-hydroxy-3-chlorochrome and
4-hydroxy-3-bromochrome 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 MITT assay
method. The anti-cancer properties of 1,1,3,3-Tetra(4'-oxy-3-substituted-chalcone)-5,5-
diphenylcyclophosphazene 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, cyclotriphosphenazine 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 cyclotriphosphenazine
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. Tekin1, A. Cakir2, A. Beytur1, S. Sandal1
1Inonu University, Physiology, Malatya, Turkey
2Bogaz 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 cancer treatment newer studies are ongoing. It has been shown that N-(p-
amylcinnamoyl) anthranilic acid (ACA) inhibit transient receptor potential melastatin-2 (TRPM2).
TRPM2 isomers were shown to be overexpressed in several cancers, including melanoma, breast,
and lung cancer. Inhibition of 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 PCs, A2780 and MCF-7
cell lines for 24 h. In addition, we calculated LogIC50 for 24 h. We calculated LogIC50 for 24h. In
addition, we calculated LogIC50 for 24 h. In conclusion, calculated LogIC50 for 24 h. In conclusion, the
ACA reduced cell viability of PC-3, A2780 and MCF-7 cell (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.

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Poster Session C
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C03-11
Effects of saxaglipitin on human prostate and breast cancer: An in vitro study

S. Tekin1, A. Beytur1, M. Cakir2, S. Sandal1
1Inonu University, Physiology, Malatya, Turkey
2Bogaz 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,
increases incretin secretory levels 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 (Saxaglipitin) have anticancer activity against human
prostate (LNCaP) and breast cancer (MCF-7) cell line. We investigated of saxaglipitin in terms of
anticancer properties were evaluated by 3-(4,5-dimethylthiazol-2-y)-2,5-diphenyltetrazolium bromide
(MTT) assay on LNCaP and MCF-7 cell lines. 1, 5, 25, 50 and 100 μg of concentration of saxaglipitin was
selected with human prostate and breast cancer lines for 24 h. Additionally, we calculated LogIC50
concentration of Saxaglipitin on LNCaP and MCF-7 cells, by using a Graphpad prism 6 programs on a
computer. We observed saxaglipitin 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
Saxaglipitin 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 pathogenesis of tumor response

I. Mrakovic-Sutic1, M. Petkovic1, A. Bulog3, V. Micovic4, I. Sutic1, V. Pavisic1, I. Sutic2
1Medical Faculty, Department of Physiology and Radiology, Rijeka, Croatia
2Medical Faculty, Department of Oncology and Radiotherapy, Rijeka, Croatia
3Medical Faculty, Rijeka, Croatia
4Medical Faculty, Department of Public Health, Rijeka, Croatia
5Medical Faculty, Department of 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
with 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 (13.06.11.14 and
13.06.11.15).
C03-13
Investigation of the effects of a sulfite molecule on human neuroblastoma cells via a novel oncogene URG4/URGCP

Y. Dodurga1, M. Seçme1, C. Eroglu2, G. Gündoğdu2, C. Biray Avcı3, G. Başçı1, V. Kıpçakataç1, N. L. Satıoğlu-Tufan1, C. Biray Avcı3
1Pamukkale University Medical Faculty, Denizli, Turkey
2 Necmettin Erbakan University Medical Faculty, Konya, Turkey
3Atatürk University Medical Faculty, Erzurum, Turkey
4Ege University Medical Faculty, Izmir, Turkey
5Ankara 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 vitro conditions and elucidate underlying molecular mechanisms of sulfite and explore its therapeutic activity.

Main methods: In this study, cytotoxic effects of sulfite in SH-SY5Y cells were detected over time in a dose dependent manner with the IC50 doses ranging from 0.5 to 10 mM. Genotoxic effect of sulfite was shown by comet assay. IC50 doses in the SH-SY5Y cells were detected as 5 mM. Expression profiles of the target genes related to apoptosis and cell cycle control were determined by quantitative RT-PCR. Protein changes were determined by western 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 mechanof this result may 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 antitumorogenesis 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

C04-2
Experimental Hypothyroidism and Hyperthyroidism Have Similar Affects on Cardiac Irisin Levels in Rats

E. Atıcı1, E. Menevşė1, A. K. Baltacı1, R. MOGULKOC1
1Selcuq University, Konya, Turkey
2Baskent University, Ankara, Turkey

Irisin is a newly discovered myokine and adipokine that increases total body energy expenditure. 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 with the 40 male Sprague-Dawley rats. Experimental groups were designed as: Control, Hypothyroidism, 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 / g tissue; 33.31 ± 6.33 ng / g tissue; 47.52 ± 11.70 ng / g tissue; 34.13 ± 80.07 ng / g 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 hyper and hyperthyroidism increase the heart irisin levels but the increase in the hyperthyroidism group is much higher than hyperthyroidism group.

C04-3
EFFECT OF BISPHENOL A AND DIETHYLBENZYL PHTHALATE ON PROGESTERONE SECRETION BY LUTEAL CELLS

R. Kabakçı1, A.A. Yigli1
1Kirkkale University, Faculty of Veterinary Medicine, Department of Physiology, Kirkkale, Turkey

Questions: This study investigates the effects of bisphenol A (BPA) and diethylbenzyl 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. Antevska1
1Medical 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 post-synaptic 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 normovolemic (NTR), Wistar rats and spontaneously hypertensive (SHR) Okamoto and Aki 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 (destrus, 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 pineocytes with picnotic nucleuses, while the neurophelial 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 Inhibit-B Levels in a Rat Testicular Torsion-Detorsion Model
A. Semercioz2, A. K. Baltaci1, R. Mogulkoc2, M. C. Avunduk1
1Bagcılar Training and Research Hospital, Urology, Istanbul, Turkey
2Sekuk University Medical School, Physiology, 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 inhibit-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, Inhibit-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 inhibit-B and spermatogonetic 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 inhibit-B and spermatogonetic 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 inhibit-B and spermatogonetic activity levels in testsis ischemia-reperfusion in rats.

Suppressed inhibit-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 Mlynarcikova1, S. Scsukova2
1Biomedical 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 Adipone Hormone in Rats which received Cold Restraint Stress

M. C. quier1, A. tanyel1, E. eristan1, T. nacer1, E. polat*
1Ataülk university, physiology, erzurum, Turkey
2Ataülk 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 hormones levels. In this study, we investigated the correlation between the adipone 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 adipone 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 adipone 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 adipone level increased.

This study was supported by Ataülk University SRP (Project No: 2015/281, 2015/39)

C04-8
Thyroid axis functioning is associated with health status and shorter survival of brain tumor patients

A. Bunievic1, S. Tamasuska1, V. Dultuva1, A. Tamasuska1
1Lithuanian University of Health Sciences, Kaunas, Lithuania

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

METHODS. Two-hundred and thirty brain tumor patients (70% women) before surgery were evaluated for cognition (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 discharge outcomes were determined as Glasgow outcome scale score of s3. 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=0.013) and BI (p=0.023) 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=0.009). In all patients, lower total (p=0.038) and free (p=0.014) 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=0.015) and with shorter survival (249 [280] vs. 352 [399] days; p=0.029) 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.

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C04-9
Pregnancy induced changes in innate immunity during autoimmune thyroid disease

I. Mračević-Sutlić1, T. Bogovic Crnic1, S. Grbac Ivanković1, V. Pavisic2, I. Sutlić2
1Medical Faculty, Department of Nuclear Medicine, Rijeka, Croatia
2Medical Faculty, Department of Physiology and Immunology, 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 ATD during human pregnancy and in the postpartum 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 cytokytic 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. Atçali1, S. Yıldız1, C. Uçer1, S. Uğraş2
1BİNGÖL University, BİNGÖL, Turkey
2İnönü University Faculty of Medicine, Physiology, Malaty, 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 unwashed hair samples were cut into small pieces by a scissors or ground by using liquid nitrogen. Afterwards, one set of samples were incubated for 16 or 36 h at 52 degrees Celsius. Another set was sonicated 30 min, 1 h or 2 h at 35 degrees Celsius. A different set was both sonicated and incubated at 52 degrees 16 h. For control comparisons, one set of samples were kept under room temperature for 90 h without using ultrasound. Following these
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 regulatory activity in bone tissue independently from exercise and may be consider.

C05-2
Cardiorespiratory fitness effect on cerebral oxygenation in chronic obstructive pulmonary patients

O. Dupuy¹, Q. Bretonneau¹, J. C. Meurice², F. Caron³,³, C. de Bisschop²
¹Univ. de Poitiers, Laboratoire MOVE EA 6314, Poitiers, France
²Service de Pneumologie, Centre Hospitalier Universitaire de Poitiers, Poitiers, France
³Centre de réadaptation du Moulin Vert, Nieul l’Esper, 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 (Vogiatzis 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 sysm, Artinis MS NL) and pulmonary gas exchanges (Ergocard, Medisoft, Dinan, B) were recorded. The NIRS optode was put on the left frontal lobe. Tissue Saturation Index, total haemoglobin, deoxyhaemoglobin and oxyhaemoglobin (TSH, THb, HbO2 respectively) were measured by a NIRS system. Correlations were performed using Pearson tests.

Results:
Mean VO2peak were 16.2 ± 4.3ml/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 HbO2peak nor TSH 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 Exercise on Oxidant and Antioxidant System Parameters in Rats with Streptozotocin Induced Diabetes Mellitus

A. M. Sahin1, O. F. Sommez2, M. Mengi1, M. Altan1, M. S. Toprak2, H. Ekmekci1, G. Metin1, L. Cakar2
1Istanbul University Cerrahpaşa Faculty of Medicine, Physiology, Istanbul, Turkey
2Istanbul University Cerrahpaşa Faculty of Medicine, Biochemistry , Istanbul, Turkey
Sanko University 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, Cu/ZnSOD, 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 Giacomo1, G. Ghiani1, G. Palazzolo1, S. Roberto1, F. Tocco1
1University 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 tower, 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±5.4 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 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, P<0.05). Conclusions: An adequate balance between metabolic and splanchic status may improve the diving response during a dive at a depth of 30m, in safe conditions for the athletes.

C05-5
Relationship between regular exercise-induced cardiac hypertrophy and microRNA

M. Pal1, M. Altan1, O. F. Sommez2, M. Mengi1, S. Dincer1, F. Akbas1, M. Yildiz1, M. Kumas2, M. Eseroglu1, G. Metin1
1Biruni University Faculty of Medicine, Physiology, Istanbul, Turkey
2Istanbul University Cerrahpaşa Faculty of Medicine, Physiology, Istanbul, Turkey
3Istanbul University Cerrahpaşa Faculty of Medicine, Sports Medicine, Istanbul, Turkey
4Bekzimatek Vakif University Medical Faculty, Medical Biology, Istanbul, Turkey
5Istanbul University, Institute of Cardiology, Istanbul, Turkey
6Bekzimatek Vakif University Medical Faculty, Histology, Istanbul, Turkey

Questions: Exercise-induced cardiac hypertrophy (CH) is a type of physiological CH. MicroRNAs (miRNAs) are involved in cardiac development, hyper trophy 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=8). Swimming sessions began with 60 min/5 days/8 weeks and continued with on the 5th week 2x/day, and on the 10th week 3x/day. Dimensions of the left ventricle and myocardial wall thickness were measured by trans thoracic 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. AYED1, S. YAHAYOUI2, S. MOKADDEM2, S. BEN JEMAA1, I. L. HADJ KHALIFA1, S. BEN KHAMSIA JAMAL EDDINE2
1Mohammed V Mamoun Hospital, Department of respiratory functional explorations, Ariana, Tunisia
2Bechir 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. Ghiani1, S. Magnani1, V. Pinna1, A. Donedda1, G. Sainas1, F. Tocci1, A. Crisafulli1
1Università 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 Quan Hua) 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 sensors-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 stop 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-T11.6 %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. Barak1, K. Caljurić2, R. Holland3, S. Thom4, P. Jovanov3, T. Mijačić4, Z. Đuđić4
1Faculty of Medicine of Novi Sad, Department of Physiology, Novi Sad, Serbia
2University of Split School of Medicine, Split, Croatia
3University of British Columbia, Okanagan Campus, Kelowna, Canada
4University of Maryland, School of Medicine, Baltimore, Maryland, Baltimore, United States
5Institute 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 intervention 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 measures ANOVA for the two studies (time – pre/30/60/90min, trial – placebo/ViC). Velocity in the middle cerebral artery (MCAv) increased 30 minutes post-dive from 60 ± 8.99 cm/s to 63.14±10.01 cm/s and in the posterior cerebral artery (PCA) 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 Expended Measurements

Z. ALTINIKAYA1, U. DAL1, N. OZEL2
1Mersin University, Faculty of Medicine, Department of Physiology, Mersin, Turkey
2Mersin 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 mismatched 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 recorded, 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 and 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 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. Nicholson1, H. Palfrey2, C. Chruč3, D. Baker2, S. Jones1
1University of Birmingham, Institute of Inflammation and Ageing, Birmingham, United Kingdom
2Medimmune, Cardiovascular and Metabolic Disease (CVMID), 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 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. Tayfur1, K. Gökçe3, S. Yılmaz2, O. Barutçu3, E. O. Oğuz1, N. Sü1, S. A. Vardar1

1Trakya University Medical Faculty, Physiology, Edirne, Turkey
2Trakya University Medical Faculty, Physical Therapy, Edirne, Turkey
3Trakya University Biostatistics, Edirne, Turkey

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

Sprague-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/visceral 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, 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 trygliceride (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. Kandir1, G. Tekin1, C. Er2, S. Karakurt2

1Çukurova University, Ceyhan Faculty of Veterinary Medicine, Physiology, Adana, Turkey
2Selçuk University, Faculty of Science, Biochemistry, Konya, Turkey
3Pebibir 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 metalloprotease with thrombospondin motifs (ADAMTS) protease family play an important role in many physiological and pathophysiological 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 Setle 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 centrifuged at 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.

Acknowledgment: This study was supported by Çukurova University Scientific Research Projects (BAP), Project No: 9288

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

O. F. Kalkan1, Y. E. Sürmeneli1, O. Aktaş1, B. P. Yucel2, A. Ayar3

1Kademintz Technical University, Physiology, Trabzon, Turkey
2Karaman Technical University, Physiology, Trabzon, Turkey
3Karaman 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 Sprague-Dawley rats were kept under standart conditions with free access to water food. Animals were habituated to both treadmill exercise and pain threshold measurement set up before being divided into control (normoglycemic) and diabetic groups. Diabtes (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, diabetes + 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 response 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 statistically 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 diabetes-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. Unal1, A. K. Baltaci2, R. Mogulkoc1, M. C. Avunduk2
1Selcuk 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 metallothionein 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 metallothionein 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
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 <50mmHg.

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 reflex 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. GUNDOGDU1, Y. DODURGA2, L. ELMAS2, S. YILMAZ TASCIO3, E. S. KARAOGLAN3
1Ataturk University, Physiology, Erzurum, Turkey
2Pamukkale University, Medical Biology, Denizli, Turkey
3Ataturk 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 Phyloostachys pubescens, Patricia, 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 pathways.

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 Tr-Restagent protocol. Effects of ISO on apoptosis related gene were determined by using RT-PCR. The analyses of findings were made by using ΔACT method and quantitated with a computer program. The comparison of groups was done with "VolcanoPlot" analysis, from "RT" Profiles13 PCR Array Data Analysis", which assessed statistically using the Student t-test.

Result: In our study, IC50 (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, Bid-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 affected cell proliferation of colorectal cancer cells via cell cycle pathways. 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. Paunović1, 2, D. Vucelić1, T. Radosavljević1, J. Pantić1, 2
1University of Belgrade, Faculty of Medicine, Institute of pathological physiology, Belgrade, Serbia
2University of Belgrade, Faculty of Medicine, Institute of Medical Physiology, Belgrade, Serbia

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. Grassalkovich1, M. Maléh1, T. Madácsy1, P. Pallagi1, V. Venglovecz1, Z. Rakonczay Jr.1, P. Hegyi1
1University of Szeged, 1st Department of Medicine, Szeged, Hungary
2University of Szeged, Department of Pharmacology and Phamacotherapy, Szeged, Hungary
3University 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-770 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. Gundogdu1, Y. Dodurga2, M. Celis1, M. Secme1, B. Cicek1
1Atatürk University, Physiology, Erzurum, Turkey
2Pamukkale University, Department of Medical Biology, Faculty of Medicine, Denizli, 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 genotoxic 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 cytotoxicity 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, further studies are needed to clarify the mechanisms of cytotoxic and genotoxic action of daidzein.

C07-7
Mechanism of glutamate secretion on the pancreatic juice by acinar cells

D. Gluch1, S. Camargo1
1University of Zurich, Physiology, Zurich, Switzerland

The pancreas efficiently absorbs amino acids for the synthesis of enzymes, but also secretes 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 is 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 LRRC8A-ER, and as previously showed the connexin 26 (Cx26). TMEM16A expression was unchanged, but the VRAC isomor LRRC8A and Cx26 increased and LRRC8B 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. Molnár1, L. Alsandí1, J. Fanczal1, T. Madácsy1, P. Hegyi1, J. Mallet1
1University of Szeged, First Department of Internal Medicine, Szeged, Hungary

Intonation: Pancreatic ductal fluid and HCO₃⁻ 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 NH₄Cl in standard HEPES or CO₂/ HCO₃⁻ buffered solution resulted in rapid intracellular alkalinization, which was followed by a recovery phase. Removal of NH₄Cl 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 10mM CFTR,172, a selective inhibitor of cystic fibrosis transmembrane conductance regulator decreased the regeneration from alkali load. Basolateral administration of 20mM amiloride and 20mM H₂DIDS decreased the intracellular pH suggesting the activity of Na⁺/H⁺ exchanger and Na⁺/HCO₃⁻ 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 can 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. Öztürk1, B. Aksoy1, O. Çetin1, H. I. Karatay2, B. Görey3, Z. N. Özdemir Kumral1, D. Özyeşil4, S. Aratçan Tamer4, H. Zorlu4, F. Arıçöğlu5, B. Ç. Yeşen1, I. Imeryu1
1University of Mersin, Department of Physiology, Mersin, Turkey
2University of Mersin, Department of Pharmacology, Mersin, Turkey
3University of Mersin, Department of Neurophysiology, Mersin, Turkey
4University of Mersin, Department of Pathology, Mersin, Turkey
5University of Mersin, Department of Cardiology, Mersin, Turkey

Questions: 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 paired with Chow (fat 2-7%) (n=18) or HFD (% 45 fat) (n=20) for 5 weeks until decapitation. In both 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 charcoals 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 <0.001 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 HCO₃⁻ SECRETION AND CFTR ACTIVITY ARE INHIBITED BY CIGARETTE SMOKE EXTRACT IN GUINEA PIG PANCREATIC DUCTAL CELLS

D. Tálas1, P. Palla7i1, V. Venglevszky2, E. Gál3, H. Tóth1, A. Schnir4, J. Malé3, D. Czupor5, Z. Rakonczay Jr.1, 6, P. Hegyi1
1University of Szeged, First Department of Medicine, Szeged, Hungary
2University of Szeged, Department of Pharmacology and Pharmacotherapy, Szeged, Hungary
3University of Szeged, Department of Pharmacognosy, Szeged, Hungary
4University of Szeged, Department of Pathophysiology, Szeged, Hungary
5University of Pécs, Institute for Translational Medicine & First Department of Medicine, Pécs, Hungary
6MTA-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

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

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 UNKP.

C14: Ion channels

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

L. Lacínová1, K. Ondácová1, L. Lapínová1, D. Jurkovicová2
1Center of Biosciences, Institute of Molecular Physiology and Genetics, Bratislava, Slovakia
2Biomedical 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 (CCG) 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.

CCGs 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 CCGs 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 CCGs 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. Jakab1, M. Kozák2, M. Beyreis1, H. Dobias1, M. Gaisberger1, M. Ritter1, H. Kerschbaum1
1Paracelsus Medical University, Institute of Physiology and Pathophysiology, Salzburg, Austria
2University of Salzburg, Department of Cellular Biology, Division of Molecular and Cellular Neurobiology, Salzburg, Austria

![Image](https://via.placeholder.com/150)

Questions: In microglial cells formation of engulfment pseudopodia and particle uptake is associated with activation of a swelling-dependent Cl- current (IClswell) and blockers of IClswell inhibit phagocytosis. Likewise, an increase in extracellular glycine stimulates phagocytosis, causes cell swelling and depolarizes the cell membrane potential (Vmem) due to glycine uptake via Na+/neutral amino acid transporters (SNATs). Here we investigated, cell swelling under glycine induces IClswell, IClswell activation affects Vmem 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 IClswell. Glycine uptake via SNATs induced a rapid, stable depolarization, which was enhanced by additional hypotonic stimulation of IClswell, but also under isotonic conditions upon long-term (>20 min) exposure to glycine. Cell migration was stimulated by glycine (0.6-5 mM). The IClswell inhibitor DCPIB (10 μM) completely counteracted both hypotonic- 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. Michael1, M. Jakab1, T. S. Steininger2, M. Beyreis1, M. Ritter1, H. H. Kerschbaum1
1Paracelsus Medical University, Institute of Physiology and Pathophysiology, Salzburg, Austria
2University of Salzburg, Department of Cellular Biology, Division of Molecular and Cellular Neurobiology, 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 (IClswell) 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 IClswell 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 (IClswell), which was reduced by addition of NA (1 nM or 1 μM). Similarly, IClswell was suppressed by the β2/AR agonist isoproterenol, the Epac-specific analog 8-pCPT2’-O-Me-cAMP and the PKA inhibitor H89. NA in the pM and nM range suppressed phagocytosis and the α2/AR antagonist yohimbine enhanced the suppressive effect of NA. Conclusions: We show that AR stimulation suppresses IClswell in microglial cells, probably via altered CAMP levels. Given the role of IClswell 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.
C14-4
Cloxyquin is a selective and state-dependent activator of TWIK-related spinal cord K⁺ channel (TRESK)

M. Lengyel¹, A. Dobolyi¹, G. Czíják¹, P. Enyedi¹
¹Semmelweis University, Physiology, Budapest, Hungary

Questions:
Cloxyquin (5-chloroquinolin-8-ol) has been previously identified as an activator of TRESK background K⁺ channel (Kᵦ18.1, TWIK-related spinal cord K⁺ channel). We have examined the specificity of the drug by testing several Kᵦ 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, EC₅₀=26.4 μM) and human TRESK (3.9±0.3-fold, EC₅₀=43.9 μM). TRESK was potently activated by cloxyquin in the resting state. The activation was not mediated by cysolic [Ca²⁺] (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 Kᵦ channel family. In a subpopulation of isolated DRG neurons cloxyquin application activated the background K⁺ current.

Conclusions:
Cloxyquin activates TRESK by a Ca²⁺-calcineurin-independent mechanism. The drug is specific for TRESK within the Kᵦ 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. Cophignon¹, S. Naïk¹, N. Milosavljević², M. Poët², L. Counillon¹
¹LP2M-CNRS-UMR7310, Nice, France
²The 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 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 linitus.

As cisplatin had been shown to modify membrane properties in different cell systems, we first had investigated its effects on mechanosensitive channels and found 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 promoter 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 modulated by each of its two targets. Moreover, we observed a reversion of these effects by using drug activity 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.

C15-1
EVALUATION OF ESTRADIOL LEVEL AND SERUM LIPIDS IN WHITE WISTAR RATS OF FEMALE GENDER DURING THEIR GENERATIVE LIFE

S. Petrovska¹, B. Dejanova¹, S. Mancevska¹, J. Pluncvecigilovskıa¹
¹Faculty 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.

Material 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 eight months. Estradiol level was determined with the standardized method and 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. Chaves¹², C. Deer¹, A. Fransen², Y. Mashimo³, R. Machida³, B. Musset²
¹IMM Nürnberg, Institut für Physiologie, Nürnberg, Germany
²Forschungszentrum Jülich, ICS-4, Jülich, Germany
³Universität zu Köln, Zoologisches Institut, Köln, Germany
⁴University of Tsukuba, Sugadaira Montane Research Center, Ueda, Japan

A new H⁺1 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 NpH₁ following the common nomenclature used for proton channels. Interestingly, NpH₁ is gene is different to its human homolog (33 % of identity) than to other species studied. NpH₁ was successfully expressed in human cells presenting proton currents higher than 400 pA, suitable for electrophysiological studies. The detailed electrophysiological characterization has proved that NpH₁ is highly proton selective, and shows other hallmarks of H₁ as voltage-dependent gating and pH-dependent gating. Curiously, NpH₁ has demonstrated to have an enhanced pH-dependence of gating when comparing with the human one (H₁). This pH-dependent gating is a unique characteristic for H₁ which allows their main physiological role, cell’s pH regulation. However, how the channel sense and adjusts its gating
C15-3
The determination of interaction between naringin and different chemotherapy agents in neuroblastoma and astrocyte cell lines

N. P. Turker¹, Z. B. Doganlar²
¹Trakya University, Technology Research and Application Center (TUTAGEM), Edime, Turkey
²Trakya University, Medical Biology, Edime, 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 with antioxidant, apoptotic, antiinflammatory properties. In this study, we aimed that the determination of single and combined 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: TUBAP-2016-231), Edime/Turkey

C15-4
Critical analysis of dietary habits in people with type 2 diabetes

K. INCHIRAH¹
¹Faculty of sciences of Bizerta, tunisia, biology, bizerte, Tunisia

Diabetes is a formidable disease for the complications it causes (infection, 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 change. 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 norm diet A hyper-carbohydrate, normo-protein and normo-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.

C16-1
Association of TNFAIP3 and TRAF1 polymorphisms with susceptibility to systemic lupus erythematosus and rheumatoid arthritis in Egyptian Population

A. ISMAIL²
²Faculty of Medicine, Physiology Department, Sinar, 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 (rs2330926) and tumor necrosis factor (TNF)-receptor associated factor 1 (TRAF1) 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 rs2330926 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: 0.87; 95% CI: 0.43 -1.08; 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. Türkoglu¹, M. N. Öğün¹, Ü Karabörk¹, H. S. Oraillar², S. Yıldız¹
¹Abant Izzet Baysal University, Boğaziçi, 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, dysmyelinating disease, multiple sclerosis, anti-phospholipid antibody ischemic stroke, were demoted 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 manufacturer’s recommendations ≥ 1/100 dilution of serum titre are considered positive. Three of the female cases had ischemic cerebrovascular disease. Only 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 dysmyelinating 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, 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. Hnilcová¹, A. I. Daponte¹, P. Vitová², A. Dal Grande¹, F. Schmitt¹, Y. Senoo¹, P. Hnilica³, D. Ostárníkova¹
¹Comenius University in Bratislava, Institute of Physiology, Bratislava, Slovakia
²Faculty of Medicine, Comenius University, Department of Simulations and Virtual Medical Education, Bratislava, Slovakia
³SI 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. Geiger¹
¹Medical 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 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.