Proceedings of the Czech and Slovak Physiological Societies

February 9 - 11, 2010, Prague, Czech Republic

THE EFFECT OF SILDENAPHIL AND L-ARGININ COMBINATION ON HYPOXIC PULMONARY HYPERTENSION

H. Al-Hiti[1], J. Herget[2]
IKEM[1], Department of Physiology[2], 2nd Medical School, Charles University, Prague and Center for Cardiovascular Research, Prague, Czech Republic

The interaction of free radicals and NO plays a role in etiology of hypoxic pulmonary hypertension (HPH). One possible way of treatment of HPH is the interference with NO/cGMP signal route. There is a good experimental and clinical evidence of vasodilatory effect of phosphodiesterase 5 (PDE 5) inhibition by sildenaphil. We tested whether PDE 5 inhibition and administration of L-arginin, the NO synthase substrate, has a synergistic effect on NO bioavailability and vasodilatation in HPH. Four groups of adult male Wistar rats were exposed to chronic hypoxia (10 % O_2, 3 wks) and obtained either combination of sildenaphil (25mg/kg/day by esophageal gavage, gift of Pfizer) and L-arginin (500 mg/kg/day, i.p.) or each of the substances alone. Fourth hypoxic group and normoxic controls were not treated. After sojourn in hypoxia the pulmonary and systemic blood pressures, cardiac output and plasma concentration of NO_x were measured in pentobarbital anesthesia. Heart was dissected in parts and lugs processed histologically and remodeled prealveolar vessels were assessed quantitatively. The inhibition of development of HPH was significantly most effective in rats treated with combination sildenaphil and L-arginin. Highest plasma concentration of NO_X in this group reflected highest NO bioavailability.

IMPORTANCE OF PHYSIOLOGY FOR RESEARCH IN NEUROREHABILITATION

Y. Angerová[1], M. Hralová[2], T. Gueye[1], O. Švestková[1], J. Bortelová[2], M. Lippertová – Grunerová[1]
[1]Department of Rehabilitation medicine of the First Faculty of Medicine and General Teaching Hospital Charles University, Prague, [2]Institute of Physiology of the First Faculty of Medicine, Charles University, Prague, Czech Republic

Neurorehabilitation is a multidisciplinary rehabilitation process used in patients with neurological diseases.The department of Rehabilitation Medicine of the First faculty of Medicine and General Teaching Hospital is interested especially in patients after brain damage. Brain damage is manifested in functional deficits due to both primary and secondary mechanisms. Primary mechanisms (e.g. type of traumatic event) can hardly be influenced. Secondary mechanisms are the result of biochemical and physiological events that lead to cell death. They occur within a period of hours to days even months and provide a window of opportunity for therapeutic intervention with the potencial to prevent or reduce secondary damage and to improve long term patient outcome. The physiological basis for neurorehabilitation is neuroplasticity which is responsible for functional restitution or recovery after secondary brain damage. There are several mechanisms of neuroplasticity after brain damage - vicariation, diaschisis, sprouting, long term potentiation, neuronal reorganisation, unmasking of neuronal functional pathways and others. Neurotrophicity, neuroprotection, neuroplasticity and neurogenesis are the most important endogenous basic physiological processes that act together under genetically control to generate endogenous defence activity – a continuous process facing pathophysiological processes. Neurorehabilitation could be enpowered by promising neuroprotective and neurorestorative approaches. Various substances (e.g. erythropoietin, statins, nitric oxide and others) are used for this purposes after the injury. Preclinical studies testing efficacy of those substances in animal brain damage models are essential to prepare clinical trials. The cooperation between The Department of Rehabilitation Medicine and The Institute of Physiology of The First Faculty of Medicine results in designing the rehabilitation model in three months old rats (Wistar) exposed to hyperbaric hypoxia (8000 m). After hypoxia erythropoietin was used to support rehabilitation. The results are under investigation.

MODULATION OF MYOCARDIAL CELL-TO-CELL COMMUNICA-TION IS MOST LIKELY INVOLVED IN ANTIARRHYTHMIC EFFECTS OF OMEGA-3 FA AND ATORVASTATIN

B. Bacova[1], J. Radosinska[2], L. Kolenova[2], V. Knezl[3], M. Barancik[1], P. Weismann[2], L. Okruhlicova[1], N. Tribulova[1]
[1]Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovakia, [2]Faculty of Medicine, Comenius University, Bratislava, Slovakia, [3]Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Bratislava, Slovakia

Objective: We have previously shown that hereditary hypertriglyceridemic (HTG) rats, characterized by moderate hypertension, insulin resistance and myocardial structural remodelling, are prone to malignant arrhythmias. Omega-3 fatty acids (omega-3 FA) and statins exhibit besides others effects also antiarrhythmic ability, while definite mechanisms are not yet elucidated. Our goal was to examine whether these compounds may affect susceptibility of the HTG rat heart to ventricular fibrillation (VF) and whether they affect myocardial cell-to-cell coupling protein connexin-43 (Cx43). Design and Method: Part of HTG and healthy Wistar rats was fed with omega-3 FA (Vesteralens, Norway, 30mg/100g/day/2mth). Atorvastatin (Zentiva, Slovakia, 0.5mg/100g/day/2mth) was orally applied to another part of HTG and Wistar rats. Some functional parameters and susceptibility of the heart to electrically-induced ventricular fibrillation was monitored using Langendorff-perfused isolated heart. Ventricular tissues from treated and untreated HTG and Wistar rat hearts were processed for ultrastructure examination as well as for analysis of myocardial Cx43 distribution and expression using antiCx43 MAB, immunofluorescence and immunoblotting. Results: 1/ Both, omega-3 FA and atorvastatin reduced elevated blood pressure, triglycerides and heart rate in HTG rats. 2/ VF-threshold was significantly increased due to treatment in HTG and healthy rat hearts. 3/ Abnormal localization of myocardial Cx43 was not eliminated, whereas elevated phosphorylated form of Cx43 was suppressed by the treatment in HTG rat hearts. 4/ Subcellular examination revealed an improvement of cardiomyocyte and intercellular junctions integrity. Conclusions: Results indicate that antiarrhythmic effects of omega-3 FA and atorvastatin are associated with modulation of Cx43 phosphorylation and protection of cell-to-cell junction integrity. As both compounds are ligands for PPAR, a possible modulation of Cx43 gene expression as well as the way of Cx43 phosphorylation should be examined in further studies.

PHARMACOKINETIC OF THE CHALCONE AFTER ORAL ADMINISTRATION

L. Bartošíková[1], J. Nečas[1], T. Bartošík[2], P. Fráňa[3]
[1]Department of Physiology, Faculty of Medicine and Dentistry, Palacky University Olomouc; [2]Department of Anaesthesiology and Intensive Care, [3]II[nd] Internal Clinic, St. Anne´s University Hospital Brno, Czech Republic

Trihydroxychalcone was syntetized from dihydroxyacetophenone and hydroxybenzaldehyde. The aim of the study was to determine some pharmacokinetic parameters after single oral administration of tested substance. After sample preparation the HPLC system consisted of gradient HPLC pump Knauer 64 equipped with a LCD 2084 UV detector was used. The wavelength was set on 255 nm for chalcone determination. The mobile phase consisted of KH_2PO_4:acetonitrile, 40:60 (v:v). Isocratic flow was maintained at 1.0 ml/min. Data from analysis were collected and analyzed with the CSW software. The quantification of the chalcone was achieved from areas of its peaks by comparison with calibration curves obtained using standard solution of individual chalcone in blank serum. For determination of pharmacokinetic parameters program kinetica 4.0 was used. The maximum plasma chalcone concentration was 53.0±1.2 mg/l; time to maximum plasma concentration was 50 min; $AUC_{0\rightarrow last}$ was 4912 μg/min/l; elimination half-life was 270±85 min; constant of elimination was 0.002832 min^{-1}; MRT was 409±116 min.

HORMONAL CONTROL OF INSECT LIPID METABOLISM

I. Bártů[1,2], A. Tomčala[1,2], P. Šimek[1], D. Kodrík[1,2]
[1]Institute of Entomology, Biology Centre ASCR, and [2]Faculty of Science, South Bohemian University, Branišovská 31, České Budějovice, Czech Republic

Lipids play a crucial role in insect energy metabolism. They are mobilized from the fat body as diacylglycerols (DGs) that represent main transport form of insect lipids in haemolymph (1). Most of the potential energy available from DGs is contained in fatty acid (FA) components of their molecules. The mobilization of DGs from fat body triacyglycerols (TG) is hormonally controlled by small neuropeptides from the AKH/RPCH (adipokinetic hormone/red pigment-concentrating hormone) family. It is very well known in a number of insect species that these hormones increase haemolymph lipids (= DGs) after their injection into the insect body (2). The DG molecular species and their fatty acid (FA) composition were investigated by electrospray mass spectrometry (ESI-MS) in haemolymph of Locusta migratoria after application of adipokinetic hormones Locmi-AKH-I, -AKH-II and AKH-III. (A) The analysis showed a heterogeneous distribution of individual DGs on nmol/ml level in haemolymph after the hormone application and revealed that mobilization of the DGs is molecular species-specific with the highest proportion of 34:1 DG (16:0/18:1 – molecular weight 595.0 Da) bearing palmitic acid (C16:0) and oleic acid (C18:1) residues, and forming in summary about 20 % of the total mobilized DG content. (B) Additional analysis of fat body triacylglycerols revealed that the AKHs mobilize the DGs selectively with the preference of those possessing the unsaturated C18 FAs. The fat body FAs with carbon chain longer than 18 did not participate on the mobilization. (C) A derived representation of FAs in mobilized DGs indicated a certain degree of AKH selectivity toward the DGs and hence the FAs. The Locmi-AKH-I significantly prefers mobilization of DGs containing unsaturated FAs, while Locmi-AKH-II and –III prefer mobilization of saturated ones.

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Supported by the grant No. P501/10/1215 from the Czech Science Foundation (DK).

CHANGES IN GENE EXPRESSION OF DOPAMINE RECEPTORS IN THE CENTRAL NERVOUS SYSTEM OF C-FOS KO MICE

J. Benes[1], R. Kventansky[2], J. Myslivecek[1]
[1]Inst. of Physiology, 1st Fac. Med., Charles University, Prague, Czech Republic
[2]Inst. of Exp. Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia

C-fos is considered to be one of the most important players in the intracellular signalization. We have shown previously that c-fos gene disruption affects some G protein-coupled receptors (α-adrenoceptors and muscarinic receptors), while the others (β-adrenoceptors, D_1-like dopamine receptors, D_2-like dopamine receptors) were not affected in the cortex and cerebellum. We have employed quantitative reverse-transcription pcr (RT-PCR) in order to determine the gene expression in more specific regions of CNS (frontal, parietal cortex, striatum, hippocampus and cerebellum) and to differentiate more specifically between dopamine receptor subtypes. D_1, D_2, D_3, D_4, D_5 dopamine receptor gene expression was studied in aforementioned regions. As the dopamine receptors are at least in the striatum tightly connected to cholinergic system, acetylcholinesterase gene expression was also studied. We have compared the changes of gene expression in males and females. Cortex areas (frontal and parietal) showed similar levels of expression of D_1 receptors both in males and females. D_2 receptors were found of the similar expression level in parietal cortex (both males and females) and also in frontal cortex in males. C-fos KO females showed down-regulation of D_2 in frontal cortex. Similarly, acetylcholinesterase gene expression was decreased in females (in frontal cortex only). Another picture was found in the striatum. While D_1 dopamine receptors were dereased in c-fos KO males and were not changed in females, D_2 dopamine receptors were decreased in c-fos KO females but were not changed in males. In cerebellum, no D_1 receptor expression was detected both in WT and KO animals but D_2 receptors were upregulated in KO animals. Hippocampus showed no difference between groups. In order to determine a relationship between both receptor subtypes a correlation analysis was employed. D_1 and D_2 receptor mRNA levels were found to be directly correlated both in frontal cortex and striatum. Moreover, striatal D_1 expression levels were found to directly correlate with frontal cortex D_2 expression levels. These results give evidence that c-fos affect gene expression of dopamine receptors and that these changes are sex dependent. We suggest that these changes are part of the complex adaptation of the animals to the c-fos gene disruption.

INTERACTION OF δ-OPIOID RECEPTOR WITH $G_i1\alpha$ PROTEIN REQUIRES INTACT STRUCTURE OF PLASMA MEMBRANE

J. Brejchová[1], L. Bouřová[1], P. Ostašov[1], J. Sýkora[2], P. Svoboda[1]
[1]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, [2]Institute of Physical Chemistry of Jaroslav Heyrovsky, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Cholesterol-rich membrane micro-domains were postulated to play a role in transmembrane signaling through G protein-coupled receptors. We have previously reported that delta-opioid receptors (DOR) exhibit high efficiency in fractions enriched in membrane domains when compared with fractions containing the bulk phase of plasma membranes (1). Here we investigated the effect of cholesterol depletion on the ability of DOR to activate their cognate $G_i1\alpha$ protein in HEK-293 cells expressing DOR-$G_i1\alpha$ fusion protein. In order to disrupt the membrane domains, the intact cells were treated for 60 min with the cholesterol depleting reagent β-cyclodextrin (β-CDX). Depletion of cholesterol did not alter the radioligand binding characteristics of DOR (B_{max} and K_d), but the ability of DOR to activate their cognate $G_i1\alpha$ protein was markedly impaired: EC_{50} for DADLE-stimulated [^{35}S]GTPγS binding was shifted by one order to the right. Interestingly, incubation of cells with high concentrations of cholesterol did not alter functional interaction of DOR with $G_i1\alpha$ proteins. Decrease of cholesterol content in plasma membranes was reflected in increase of membrane fluidity monitored by steady-state anisotropy of fluorescence of hydrophobic membrane probe 1,6-diphenyl-1,3,5-hexatriene (DPH). The time-resolved studies of DPH fluorescence indicated a marked change of both structural and dynamic parameters of DPH fluorescence and distinguished the two different membrane environments. One of them was effected by β-CDX more efficiently than the other. Our data indicate that the optimum signal transduction/transmission between DOR and $G_i\alpha1$ proteins requires the intact structure of membrane domains.

1. Bouřová et al.: J. Neurochem. 85: 34-49, 2003.

Supported by LC554, LC06063, GD305/08/H037 and AV0Z50110509.

METABOLIC ROLES OF EXTRACELLULAR ADENOSINE

L. Brejchová, J. Fleischmannová, L. Kučerová, M. Žurovec
Faculty of Science, University of South Bohemia and Institute of Entomology, Biological centre v.v.i., Ceske Budejovice, Czech Republic

Extracellular adenosine is a multi-potent signalling molecule implicated in many physiological and pathophysiological conditions. In order to deal with ischemia or inflammation, specific G-protein coupled receptors and their downstream cAMP and/or Ca^{2+} signalling cascades are activated by adenosine produced in the extracellular space from ATP or cAMP. The level of adenosine signalling seems to be attenuated by the action of adenosine deaminases and adenosine uptake into the intracellular space via nucleoside transporters. Adenosine has been used for decades in clinical praxis to treat tachycardia. More recently, drugs specifically targeting various adenosine receptors and transporters became promising therapeutics for pharmacological treatment of many pathological conditions including acute lung injury or various types of ischemia. Medical applications of specific adenosine derivates imply detailed understanding of extracellular adenosine metabolism and function. Despite long-lasting intensive research, complexity of adenosine signalling and metabolism has not been understood in detail. From many *in vitro* studies on both vertebrate and invertebrate cell lines, adenosine is known to reduce cell survival. The mechanism of

adenosine action seems to be complex and largely cell type specific resulting from either activation of specific adenosine receptors or adenosine uptake into cells. This study aimed to elucidate the mechanisms of adenosine toxicity in fruit fly cell lines. Adenosine was able to stop proliferation and induce cell death in cells with insufficient deaminase activity independently of adenosine receptor activation. On the other hand, adenosine toxicity was correlated with its incorporation into cellular ATP pool and could be rescued by adenosine transport blockers. Recycling of adenosine is an important nucleotide salvage pathway at low physiological adenosine levels. However, at high adenosine levels excessive adenosine phosphorylation may interfere with cellular energy homeostasis and cause growth arrest and cell death.

Supported by GA CAS CZ KJB501410801.

PROTECTIVE EFFECTS OF SIMVASTATIN AND THE LIGAND OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPAR-alpha) ON MYOCARDIAL INFARCT SIZE IN ISOLATED RAT HEART

S. Carnická[1], M. Nemčeková[1], A. Adameová[2], J. Matejikova[1], D. Pancza[1], V. Franeková[1], T. Ravingerová[1]
[1]*Institute for Heart Research, Centre of Excellence for Cardiovascular Research of the Slovak Academy of Sciences;* [2]*Department of Farmacology and Toxicology FaF UK, Bratislava, Slovakia*

Statins, specific inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase as well as fibrates, agonists of peroxisome proliferator-activated receptors alpha (PPAR-alpha) are hypolipidemic drugs used in prevention of atherosclerosis (1). However, recent findings suggest that protective effects of both groups of these farmaceuticals against ischemia/reperfusion (I/R) injury are also related to other, lipid-independent „pleiotropic effects“, such as anti-inflammatory, antioxidant and antithrombotic effects, as well as their ability to improve endothelial function (2). In addition, simvastatin (S) has been recently found to increase gene and protein expression of PPAR-alpha (3) The aim of our study was to compare the effects of S and fibrate WY 14643 (WY) on myocardial infarct size in isolated Langendorff-perfused rat hearts. For examination of pleiotropic effects of these drugs, normocholesterolemic rats were pretreated for 5 days p.o with S (10mg/kg/day) (4) or with WY (3mg/kg/day) (1). To investigate the acute effect of statins, S (10 μmol/l) (4) was added to the perfusion solution 15 min prior to global ischemia. All treated groups as well as untreated controls were subjected to 30-min global ischemia followed by 2–h reperfusion for evaluation of the infarct size (IS, expressed in % of area at risk, AR) by TTC statining. Results: 5-day treatment with S and WY significantly reduced IS to 11.5±0.4 % and 27.3±4.2 % from 33.7±4.0 % and 42.0±3.0 % in controls, respectively ($P<0.05$). In acute S-treated group, the IS was also significantly decreased (26.9±2.7 %) as compared with respective controls (41.7±1.8 %; $P<0.05$). Conclusions: administration of S and WY attenuated lethal I/R injury in the hearts of rats with normal plasma cholesterol. Moreover, chronic premedication with S decreased IS more effectively than WY and acute S treatment. The results indicate that activation of PPAR-alpha might be involved in non-lipid cardioprotective effects of both drugs, although exact molecular mechanisms of this protection require further investigation.

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Supported by grants VEGA SR 2/0173/08, 1/0620/10, APVV-LPP-0393-09.

$Ca_V1.2$ GENE SILENCING IN CULTURED RAT HIPPOCAMPAL NEURONS

A. Caro, L. Lacinova
Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia

We analyzed contribution of the $Ca_V1.2$ channel to excitability of hippocampal neurons in primary culture established from newborn rat hippocampi. Neurons used for recordings were large and had pyramidal shape and well-defined dendrite processes. We have employed whole cell configuration of the patch clamp technique. To assess the contribution of L-type calcium channels to neuronal excitability in our model we have used two experimental approaches: i) block of L-type calcium channels by 10 μM of nimodipine; and ii) selective $Ca_V1.2$ channel silencing by siRNA. Silencing decreased proportion of nimodipine-sensitive calcium current measured under the voltage clamp conditions by about 50 % suggesting that this procedure did suppress $Ca_V1.2$ channel expression. Input resistance of cultured hippocampal neurons measured under the current clamp conditions was enhanced by both nimodipine treatment and gene silencing confirming contribution of the L-type calcium channels to resting membrane properties. For analysis of the contribution of L-type calcium channel to generation of action potentials we injected appropriate holding current to clamp desired resting membrane potential (-65 to -70 mV). Injection of 300 ms long depolarizing current pulse activated firing of series of action potentials. Application of 10 mM nimodipine fully suppressed this type of excitability allowing firing of single action potential only. This effect likely involved altered conductance of not only L-type calcium channels, but also potassium and sodium channels. Silencing of the $Ca_V1.2$ channel had less dramatic effect. Frequency of action potential firing was decreased and accommodation phenomenon was less pronounced in the silenced group compared to the control cells. In conclusion, L-type calcium channels and more specific $Ca_V1.2$ channels contribute to both resting membrane properties and to generation of action potential series in cultured rat hippocampal neurons.

Supported by the Marie Curie Research Training Network CavNET MRTN-CT-2006-035367and Centre of Excellence of the Slovak Research and Development Agency “Biomembranes2008” VVCE-0064-07.

SHORT-TERM SURVIVAL OF THREE TYPES OF GRAFTS IN THE CEREBELLUM OF LURCHER AND WILD TYPE MICE

J. Cendelín[1], Z. Houdek[1], V. Kulda[2], M. Králíčková[3], V. Babuška[2], J. Hatina[4], J. Pacherník[5], F. Vožeh[1]
[1]*Department of Pathophysiology,* [2]*Department of Medical Chemistry and Biochemistry,* [3]*Department of Histology and Embryology,* [4]*Department of Biology, Faculty of Medicine in Pilsen, Charles University,* [5]*Institute of Experimental Biology, Faculty of Science, Masaryk University, Czech Republic*

There are several types of cells used for neurotransplantation. It was hypothesized that more differentiated elements survive less than undifferentiated ones. On the other hand, embryonic carcinoma stem cells are considered as dangerous due to tendency to form teratocarcinoma. The aim of this work was to compare survival of three types of grafts in the cerebellum of adult Lurcher mutant mice (+/Lc) suffering from olivocerebellar degeneration and wild type (+/+) B6CBA mice: embryonic carcinoma stem cells (line P19), neuroprogenitors derived from P19 cells and solid embryonic cerebellar grafts. The mouse P19 embryonic carcinoma stem cells were labelled with the green fluorescent protein (GFP). Neuroprogenitors were obtained by differentiation of P19 cells using retinoic acid. Cerebellar grafts were prepared from 12-13 days old GFP mouse embryos. Both naive P19 and neuroprogenitor cell suspensions were injected into the cerebellum of host mice. Solid grafts were applied towards its surface. 3 weeks later the grafts were examined histologically. Naive P19 cells survived in 32 % of +/+ and 20 % of +/Lc mice. Neuroprogenitors survived in 62 % of +/+ and 20 % of +/Lc mice. Solid grafts were found in 69 % of +/+ and 88 % of +/Lc mice. In +/+ mice solid grafts survived more often than naive P19 cells ($p<0.05$). In +/Lc, solid grafts survived more often than both naive P19 cells ($p<0.002$) and neuroprogenitors ($p<0.002$). Neuroprogenitors survived in higher percentage of +/+ than +/Lc mice ($p<0.02$), while in survival of P19 cells and solid grafts the mice did not differ. P19 cells and neuroprogenitors formed a separated tissue mass in the place of injection. Dispersion of the cells through the host cerebellum was not observed. Cell migration from the solid grafts into the host tissue was rare. The stage of differentiation of grafted cells did not influence negatively graft survival in normal cerebellum. Lurcher mutant cerebellum was more hostile to both types of grafts applied as cell suspension than to the solid tissue. In the normal cerebellum, only naive P19 cell survival differed from the solid graft.

Supported by the grants No. VZ MSM 021620816 and COST B 30/2007 OC 152 of the Ministry of Education, Youth and Sport of the Czech Republic.

EVALUATION OF THE DIASTOLIC FUNCTION IN TYPE 2 DIABETIC PATIENTS – COMPARISON WITH CONTROL HEALTHY SUBJECTS

J. Charvát, J. Chlumský, P. Šváb
Medical Department of 2nd Faculty of Medicine, Charles University, Prague, Czech Republic

The aim of study was to evaluate the diastolic function in type 2 diabetic patients with no history of cardiovascular disease in comparison with healthy subjects. Asymptomatic type 2 diabetic patients with no history of cardiovascular disease, no abnormality on ECG and ejection fraction of left ventricle at least 55 % were accepted. All the patients and control individuals were examined by transthoracal echocardiography and tissue Doppler. The type 2 diabetic patients as well as controls have been included into study only if office blood pressure was below 135/85 mm Hg however previous treatment by hypotensive agents was accepted. The diastolic function parameters E/A, E´, E/E´, E´/A´ were evaluated (E flow peak at early diastole, A flow peak during atrial systole, E´ mitral annular early diastolic velocity, A´ maximal myocardial velocity during late diastole). The results were evaluated by Mann Whitney test. In diabetic group we evaluated the correlation of E´, E/A, E´/A and E/E´ to age, gender, BMI, duration of diabetes, HbA1c, systolic and diastolic blood pressure, index of mass of left ventricle, type of treatment of diabetes and hypertension by multivariate regression analysis. Eighty two type 2 diabetic patients and 41 control subjects were examined. The age of both groups was comparable (diabetics 61±5 vs, 61±4 in controls). Twenty eight (34 %) women were in diabetic group and 14 (34 %) in control group. E/A was 0.97±0.22 in diabetic group and 1.10±0.24, NS; E´ was 7.45±1.42 in diabetic and 8.91±1.30 cm/sec in control group, p=0.001; E/E´ was 0.10±0.02 in diabetic and 0.08±0.02 in control group, p=0.001; E´/A´ was 0.69±0.14 in diabetic and 0.82±0.14 in control group, p=0.001 In diabetic group E´correlates with HbA1c (r= -0.761), duration of diabetes mellitus (r= -0.478), E/E´ correlates with age (r=0.585), duration of diabetes (r=0.314) and use of diuretics (r=0.518), E/A correlates with age only (r= =0.813) and E´/A´ correlates with age (r= -0.276) and HbA1c (r= -0.718). Conclusion: In type 2 asymptomatic diabetic patients with normal systolic function of left ventricle there are significantly deteriorated parameters of diastolic function in comparion to healthy subjects. These changes can contribute to the development of chronic left ventricle failure in type 2 diabetic patients. In diabetic group the diastolic parameters correlates mainly with diabetes compensation and duration of diabetes.

Supported by grant IGA MZ ČR NR/9520-3.

LASER CAPTURE MICRODISSECTION FOLLOWED BY FLOW CYTOMETRY AS A NOVEL TOOL FOR INVESTIGATION OF APOPTOSIS IN THE PRIMARY ENAMEL KNOT

I. Chlastakova[1,2], E. Janeckova[1,2], J. Fleischmannova[2], J. Doubek[1], L. Dubska[1,3], E. Matalova[1,2]
[1]University of Veterinary and Pharmaceutical Sciences, [2]Institute of Animal Physiology and Genetics AS CR, v.v.i., [3]Masaryk Memorial Cancer Institute, Czech Republic

Laser capture microdissection (LCM) allows unique selection of specific cell populations within histological sections followed by contact-free catapult of the sample into a test-tube without any contamination from surrounding tissues. This modern technique has revolutionized isolation of homogenous cell populations from tissue compartments. Such approach improves data reliability also in studies on specific signalling centres such as the primary enamel knot (PEK) in developing tooth germs. PEK represents a low-number population of specific, non-dividing, tissue-bound cells, producing signalling molecules and governing transition of the tooth bud/cap into the tooth bell to finally form properly shaped tooth. After fulfilling their mission, PEK cells undergo apoptosis. Rapid progress of LCM sample analyses in nucleic acid research was facilitated by relatively easy sample manipulation. However, at proteomic level, amplification procedures are not available and therefore, the mass spectrometry serves as the major but very demanding technique. Other studies of tissue bound, exactly located cell populations of high physiological importance, thus remain limited to immunohistochemistry (IHC). Therefore, we have designed a novel approach for analysis of apoptosis in the PEK cells. PEK cells were dissected from cryopreserved frontal head sections (25 μm), catapulted and captured in an eppendorf tube. PEK cells were individualized and labelled for flow cytometry analyses (immunocytometry). TUNEL assay was performed for apoptosis evaluation, PCNA for proliferation. TUNEL positivity and apoptosis were compared with each other and correlated with IHC findings to demonstrate benefits of this combined methodical procedure.

The student work was supported by the Internal Grant Agency of the University of Veterinary and Pharmaceutical Sciences (238/2009/FVL). Thanks for primary enamel knot research support (KJB500450802) and embryonal apoptosis research support (IAA600450904) to the GAAV.

HYPERCAPNIA INHIBITS LUNG VASCULAR OXIDANT INJURY INDUCED BY CHRONIC HYPOXIA

M. Chovanec, J. Herget
Department of Physiology, 2nd Medical School, Charles University, Prague, Czech Republic

Accompanying hypercapnia partly attenuates the effect of chronic hypoxia on pulmonary vasculature. Rats exposed to hypoxia combined with hypercapnia had lower pulmonary arterial blood pressure, right heart ventricle hypertrophy, and less pronounced structural remodeling of the peripheral pulmonary arteries than rats exposed to chronic hypoxia without hypercapnia (1). According to our hypothesis, hypoxic pulmonary hypertension is triggered by hypoxia-induced radical injury to the walls of the peripheral pulmonary arteries. We assumed that hypercapnia inhibits release of both oxygen radicals and nitric oxide. We studied three groups of adult male rats exposed for 3 weeks to ventilatory hypoxia (F_{iO2} = 0.1), hypoxia + hypercapnia (F_{iC02} = 0.04 – 0.05) and normoxia (F_{i02} = 0.21). We measured the NO concentration in expired air, plasma concentration of NOx, both by chemiluminescent method and plasma concentration of nitrotyrosine (marker of peroxynitrite) by ELISA. In hypoxic rats hypercapnia resulted in the decrease of NO production and lower nitrotyrosine accumulation. We conclude that hypercapnia inhibits the development of hypoxic pulmonary hypertension by the attenuation of radical injury to the walls of peripheral pulmonary arteries.

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Supported by grants GACR 305/05/08/108 and GAUK 78007.

COMPARISON OF RENAL AND ENDOTHELIAL EFFECTS OF RAMIPRIL AND PIOGLITAZONE IN ADRIAMYCIN-INDUCED NEPHROPATHY

H. Černecká, K. Turčeková, L. Mésarošová, M. Malíková, J. Klimas, P. Křenek, J. Kyselovič, P. Ochodnický
Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia

Background and Aims. Recent studies provided evidence that drugs activating peroxisome proliferator-activating receptors – PPARγ - thiazolidinediones should have renoprotective and endothelium-protective properties (1,2). We examined whether the PPARγ agonist pioglitazone (PIO) is protective against adriamycin-induced nephropathy and compared its effects to ramipril, previously reported to be protective in this model. Methods. A single intravenous injection of adriamycin (ADRIA; 2.5mg/kg) was administered to Wistar rats, to cause renal damage. Control group received saline (CTRL). After 6 weeks, treatment with PIO or ramipril (RAM) was started (ADRIA+PIO; 12.5 mg/kg/d), (ADRIA+RAM; 1 mg/kg) or their combination for further 6 weeks. Drugs were given in drinking water. We evaluated endothelium-dependent acetylcholine-induced relaxation of norepinephrine-precontracted isolated thoracic aorta. Histological examination and RT-PCR analysis of kidney were performed and

proteinuria was assessed. Results. ADRIA reduced acetylcholine-induced relaxation, caused massive proteinuria and structural renal damage. PIO normalized endothelial function, reduced renal damage and decreased proteinuria. RAM alone did not improve relaxation, but reduced renal damage and decreased proteinuria. Combined pioglitazone and ramipril treatment normalized endothelial function, but there was no additional effect on proteinuria and renal damage. ADRIA increased renal expression of plasminogen activator inhibitor-1 (PAI-1), PIO, RAM and their combination reduced expression of PAI-1. There were no changes in expression of nephrin, tumor growth factor beta and kidney injury molecule. Conclusions. Our results show that pioglitazone reduced adriamycin-induced renal damage and improved endothelial function. Renal protective effect is comparable to ramipril. Pioglitazone may interfere with extracellular matrix deposition.

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Supported by grant No. VEGA 1/0707/09.

MENTAL COUNTING IS REFLECTED IN THE EVENT-RELATED BRAIN POTENTIALS OF THE TEMPORAL LOBE

A. Damborská[1], M. Brázdil[2], I. Rektor[2], M. Kukleta[1]
[1]Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic, [2]1st Department of Neurology, St. Anne's Faculty Hospital, Masaryk University, Brno, Czech Republic

Mental counting can be viewed as a complex of higher order brain functions that include identifying the counted object, performing the mathematical calculation, and memorizing the result. Such sequence of neural operations is believed to be reflected in the course of event-related brain potentials (ERPs). The brain structures, however, specific for counting still have not been identified. In the present study, the ERPs induced by rare (target) and frequent (non-target) stimuli of a visual oddball task were compared in order to identify differences in the post-movement period. As the target response comprised the hand movement and the mental counting of the number of stimuli presented, we supposed that the post-movement divergence could reflect the activity associated with the latter operation. Electrical activity from 165 sites in frontal and temporal lobes of 4 epileptic patients was recorded during the task by means of depth electrodes. We averaged 1800 ms long EEG periods free of epileptic activity, separately for target and non-target responses and then we compared the course of ERPs in the two responses. Post-movement non-identical ERPs were identified in structures of temporal lobe in all patients. Generators of such late latency ERPs were found in parahippocampal gyrus, superior temporal gyrus, and middle temporal gyrus suggesting involvement of these structures in different memory mechanisms engaged in closure of the target and non-target responses. In case of the target response these ERPs may represent the final phase of the mental counting process.

Supported by the grant MSM002162240.

BEHAVIORAL CONSEQUENCES OF MINIMAL CORTICAL LESIONS AND THEIR ATTENUATION BY ROS SCAVENGERS IN RATS

K. Deykun, M. Pometlová, B. Schutová, J. Mareš
Department of Normal, Pathological and Clinical Physiology, Third Faculty of Medicine, Charles University in Prague, Czech Republic

Background– The animal model of photothrombic ischemia allows its relatively accurate positioning and gradation the extension. The mechanism of lesions development includes formation of reactive oxygen species (ROS) by damaged endothelium. Persistency in ROS production further aggravates ischemic damage. The aim of the study was to determine behavioral consequences of a minimal unilateral photothrombic lesion of the sensorimotor cortex. We also tested whether ROS scavengers may influence functional outcomes of such a lesion, even though they are applied after the end of ischemia induction. Materials and methods– Adult, male rats were divided into five groups: (1) photothrombic cortical ischemia only, (2) and (3) had in addition scavenger application (tempol or melatonin), (4) was sham operated and (5) controls. Sensorimotor tests were performed 24 and 48 hours after ischemia induction, followed by the six days of learning in the Morris water maze (MWM) and a subsequent probe test. At the end of the experiment animals were sacrificed and morphological examination of the ischemic lesion was performed. Results– The group subjected to ischemia showed a significant decline in performance in sensorimotor tests and an increase on all parameters of the MWM test, comparing to control animals. Although, tempol injection improved sensorimotor function, it did not change spatial learning. Melatonin, however, significantly improved performance in all tests. Additionally, all the experimental animals had increased swimming velocity (hyperactivity) in the MWM test, compared to controls. Performance of sham operated animals did not differ from controls and hyperactivity was not observed, however, the latency in MWM tests differ neither from controls nor from experimental animals. Conclusions– In the present study we have described an animal model of minimal unilateral ischemic lesions which cause mild deficits. Moreover, our findings showed that subsequent application of ROS scavengers improve ischemia outcomes, with melatonin being more potent. Conversely, none of the scavengers affected post-ischemic hyperactivity. Green laser irradiations caused minimal thermal injury and its consequences differ from ischemic.

Supported by GAUK 45808/2008, Research goal MSM 0021620816.

FROM PHYSIOLOGY TO MEDICINE – INTEGRATION OF SCIENCE, RESEARCH, SPECIFIC EDUCATION AND PRACTICE

J. Doubek[1], E. Matalova[1], I. Fellnerova[2]
[1]Department of Physiology and Pathophysiology, University of Veterinary and Pharmaceutical Sciences Brno, [2]Faculty of Science, Palacky University, Olomouc, Czech Republic

Physiology represents a prestigious discipline awarded – together with medicine – by Nobel Prices. Physiology has been very dynamic, therefore effective orientation in recent knowledge creates the background for successful research and high quality education. The project (CZ.1.07/2.3.00/09.0219) is focused on integration of science, research, education and practical experience of students and young scientists by the way of specialized seminars, lab work and excursions guided by experienced scientists and university teachers. The project scheme is divided into two cycles, the first one will be held in Brno in 2010 and the second one in Olomouc in 2011. Active participation of students and young scientists has been scheduled in two panels (Science-Research-Education-International cooperation and Science-Research-Practice) with seven different topics: 1) Recent scientific knowledge related to Nobel Prices in Physiology or Medicine, 2) Current research in physiology, 3) Presentation of scientific results at international meetings, 4) International networks in physiology and biomedicine, 5) Physiology-pathophysiology-diagnostics, 6) Physiology-pathophysiology-human medicine, 7) Physiology-pathophysiology-veterinary medicine. The project has been executed by expert teams of the University of Veterinary and Pharmaceutical Sciences Brno and Palacky University Olomouc in cooperation with external co-workers in the Czech Republic and abroad. Related publications will be issued to each project topic. More information, passed/recent events and news are available at http://cit.vfu.cz/fyziolmed.

Supported by the European Social Fund and the national budget of the Czech Republic (CZ.1.07/2.3.00/09.0219).

EFFECT OF A DOMAIN PEPTIDE OF THE CARDIAC RYANODINE RECEPTOR ON THE STABILITY OF ARTIFICIAL LIPID MEMBRANE

A. Faltinová[1], J. Gaburjáková[1], Ľ. Urbániková[2], M. Hajduk[2], B. Tomášková[3], M. Antalík[3], A. Zahradníková[1]
[1]Institute of Molecular Physiology Genetics SAS, Bratislava, Slovakia, [2]Institute of Molecular Biology SAS, Bratislava, Slovakia, [3]Institute of Experimental Physics SAS, Košice, Slovakia

Systolic contraction of cardiac myocytes is induced by a transient elevation of cytosolic Ca^{2+} due to Ca^{2+} release from the intracellular stores through the ryanodine receptor (RyR2). RyR2 contains one

N-terminal, one central and two C-terminal domains where mutations related to the cardiac arrhythmia, CPVT, tend to be clustered (1). It is assumed that interaction between the N-terminal and the central domain plays a role in forming the "domain switch" that regulates the stability of the resting (closed) state of the RyR2 (2). The aim of our study was to test this hypothesis for the N-terminal part of the RyR2. Our experimental strategy was based on the fact that the interaction between the examined RyR2 domains can be suppressed by adding a peptide with amino acid sequence identical to a part of the "domain switch" (3). We constructed the peptide DPcpvtN2 corresponding to the N-terminal part of the RyR2 with the highest occurrence of CPVT mutations, and we examined its effect on the resting activity of the RyR2. In the concentration range of 0.5 – 2.0 μM DPcpvtN2 perforated the BLM before an effect on the RyR2 activity could be observed. Secondary structure analysis of DPcpvtN2 and mapping on the tertiary structure of the homological IP3R ligand-binding domain (4) has shown a high incidence of α-helix and ascending hydrophobicity gradient in the DPcpvtN2. These properties might explain the observed effect of DPcpvtN2 on BLM stability (5).

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Supported by grants APVV-0139-06, APVV-0441-09 and VEGA 2/0102/08 and by the European Union Contract No. LSHM-CT-2005-018833/EUGeneHeart.

DO THE MICE NEED ACETYLCHOLINESTERASE ACTIVITY IN THE BRAIN?

V. Farár[1], A. Hrabovská[2], J. Cendelín[3], M.P. Martres[4], E. Krejci[5], J. Myslivečekl[1]
[1]Inst. Physiol., 1st Fac. of Medicine, Charles University, Prague, Czech Republic, [2]Dpt. Pharmacol. Toxicol., Fac. Pharmacy, Comenius Univ., Bratislava, Slovakia, [3]Inst. Pathophysiology, Med. Faculty Pilsen, Charles Univ., Pilsen, Czech Republic, [4]Inserm U513, Université Pierre et Marie Curie, Paris, France, [5]INSERM U686, Biologie des Jonctions Neuromusculaires, Paris, France

Acetylcholinesterase (AChE) constitute a key element of central cholinergic system. In the mammal brain tissue AChE is present as tetramers anchored to the plasmatic membrane of neurons by anchoring transmembrane protein PRiMA (proline rich membrane anchor). Deletion of gene encoding PRiMA protein prevent attaching of enzyme to the outer cell membrane. Although PRiMA knockout mice (PRiMA KO) posses 2-3 % activity of wildtype AChE activity this activity is retained in the endoplasmic reticulum. Surprisingly these mice are viable and indistinguishable from wild type mice. It is not known which mechanisms compensate the lack of AChE enzyme activity. In the present study we focused on examination of other key players of cholinergic system in PriMA KO: density of muscarinic receptors (MR) and HACU (high affinity choline uptake). In addition to that, PRiMA KO were tested in a variety of behavioral tests (Morris water maze, open field test, Catwalk system) to reveal prospective changes that are not visible to the naked eye. Density of MR was determined by indirect quantitative autoradiography and by direct radioligand binding to the plasma membranes. HACU was measured as uptake of tritium labeled choline. Labeling of MR by two radioligands ^{3}HNMS and ^{3}HQNB showed decreased density of MR throughout the whole brain in PRiMA KO in comparison to the wilde type. The highest decrease of MR was in striatum (-58 %) while the lowest in thalamus and hypothalamus (-22 %). Kd values for ^{3}HQNB did not differ between genotypes indicating no change in affinity of receptors to ligands. However there was no change in HACU in striatum, hippocampus and cortex. Despite impaired cholinergic system we found no differences in spatial learning ability of PRiMA KO (tested in Morris water maze). There was also no difference in locomotor activity tested in open field test and no difference in gait (analysed with Catwalk system). Our results suggest that PRiMA KO are completely adapted to the lack of AChE. We suggest that the downregulation of MR is a key adaptation mechanism in PRiMA KO mice.

Supported by Grant GACR 309/09/0406 and by Grant GAUK111409.

INNOVATED TUTORIAL METHODS FOR DEMONSTRATION OF PHYSIOLOGICAL PROCESSES

I. Fellnerová, L. Hlaváček
Faculty of Scince, Palacky University in Olomouc, Department of Zoology, Olomouc, Czech Republic

Education of modern physiology is facing difficult task: To explain students comprehensibly number of complex physiological processes, which often proceeds on abstract cellular and molecular level. It requires correct interpretation and explanation the studied problems. Incompetent and flat ways of teaching decline the effect of educational process, student interest in the problems and consequently decline academic achievement. Using PowerPoint presentations belongs to current tuition ways in Universities, which are certainly very helpful. However, PowerPoint computer program is not often used intensely, as number of users is not deeply familiar with it. In our work, we endeavor to use PowerPoint graphic possibilities in maximum extent, including connections to other education and motivation activities: 1. We build up original computer animations, which descriptively show the dynamics of particular physiological processes. Animation sequences are adapted for particular groups of students focused on their fields of study. The animations allow teachers to regulate phases, rhythm and recapitulation of interpretation with a view to student's feedback. Visualization of complex physiological processes is highly effective in light of understanding and memorizing. 2. We connect interpretation with other multimedia components that evolve creativity and comprehension of contexts with related fields of knowledge. Nowadays, using informatics technologies fast expanded in this epoch is highly acknowledged by students and motivates and exudes their interest in the studies.

Supported by FRVŠ 2345/2009/F4/d and MŠMT NPV II 2E0801.

PROJECT THE HEART OF THE HEARTS – CREATIVE PHYSIOLOGICAL MODEL

I. Fellnerová[1], E. Sovová[2]
[1]Faculty of Science, Palacky University in Olomouc, Department of Zoology, Olomouc, Czech Republic, [2]1st Internal Clinic of Faculty Hospital, Olomouc, Czech Republic

Appropriate ways of promotion of physiological-medicine disciplines in the media are very important for development of personal responsibility of each person to own health. The heart of the hearts (www.srdcesrdci.upol.cz) is a unique popular-education project held by Palacky University in Olomouc in collaboration with the company sanofi-aventis, Foundation for the Heart of Haná, Olomouc region and statutory town Olomouc. The uniqueness of the project consists in original and highly attractive way how knowledge from cardio-physiology is imparted not only to students but also to public. Elaborated conception – connection of education, competitiveness, team collaboration, promotion in the media, and pleasure certainly attract interest of people of all age groups. The aim of the project leaders was to present basic mechanisms of heart contractions in healthy or defective heart. By this way, organizers wished also to demonstrate the problems of cardio-vascular illnesses, which belong to main cause of death. The key event of the project was the formation of dynamic model of human heart from hundreds of pupils and students attending elementary and middle schools in Olomouc and from students of Palacky University in Olomouc. Using inventive choreography, colorful costumes and other aids, the heart cycle was simulated when basic anatomical structures were maintained. Fibrillation of atriums, ventricles and consequent defibrillation were also demonstrated. The event, which held 25th of June in 2009 on the historical main square in Olomouc, was officially registered as constituent Czech record in the category The largest biology lesson. Simultaneously, it is registered in the Guinness World Record as attempt for constituent world record in the same category. Tens of hours of professional film (including the shots tracked from high visual point in height of 34m) will be a basis for official document describing history of this project from the beginning to the performance held in June 25th 2009 on the main square in Olomouc. Considering unusual positive feedback, the organizers of the project propose to continue in this began tradition. In the frame of the project Creative biology the organizers wish to present a series of other biology-medical processes in a future, of which understanding will

increase their universal awareness and will inspire interest of not only students in the given topic.

Supported by FRVŠ 2345/2009/F4/d and MŠMT NPV II 2E08018.

CHRONIC TREATMENT WITH HALOPERIDOL CHANGES EXPRESSION OF SIGMA-1 RECEPTOR IN RAT HEART

K. Fialová[1], S. Hudecová[2], B. Sedláková[2], O. Križanová[2], M. Nováková[1]
[1]Department of Physiology, Faculty of Medicine, Masaryk University Brno, Czech Republic, [2]Institute of Molecular Physiology and Genetics, Center of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovakia

Cardiac sigma receptors are known to affect several ionic channels and their signalling is reflected by changes in the electrophysiological properties of the heart. Numerous ligands of sigma receptors are known to cause various arrhythmias, including severe ones. The effects of prototypic sigma ligand haloperidol have been repeatedly studied in humans as well as in various animal models, mainly after acute exposure to this drug. In our previous study, certain electrophysiological changes have been observed also in isolated Langendorff hearts of chronically treated rats after subsequent acute exposure to haloperidol (QT prolongation, attenuation of arrhythmogenic effect). In this study, the effect of chronic administration of haloperidol on expression of sigma-1 receptor mRNA was explored. Ten adult male rats were given i.p. injection of haloperidol (2mg/kg, treated group) and two were given vehicle (control group) once a day, for 21 days. On day 22, the chest was opened under deep anesthesia, the heart quickly excised and dissected into four regions (cavities) – both atria and ventricles. The samples were stored at -80 °C until PCR specific for the sigma-1 receptor was carried out. Cycloidin was used as a housekeeper gene control for semi-quantitative evaluation of PCR. All PCR products were analyzed on 2 % agarose gels. Intensity of individual bands was evaluated by measuring the optical density per mm^2 and compared relative to CYCLO mRNA. Chronic treatment with haloperidol resulted in increased mRNA of sigma-1 binding sites in each heart cavity – left ventricle (9.0±1.2 vs. 4.7±0.5), left atrium (13.8±1.7 vs.6.4±0.4), right ventricle (10.2±2.2 vs. 6.3±1.1) and right atrium (7.5±0.2 vs.4.2±1.2). Prolonged exposure to antagonist is usually followed by up-regulation of particular receptor. Haloperidol is in most previous works considered antagonist on sigma receptors and our present finding supports this classification.

Supported by grant project GAČR 102/07/1473, APVV 51-0397-07 and MSM0021622402.

MODULATION EFFECT OF CAFFEINE ON THE ACTIVITY OF CARDIAC RYANODINE RECEPTOR AT PHYSIOLOGICAL CONCENTRATION OF LUMINAL Ca^{2+}

J. Gaburjakova, T. Kurucova, M. Gaburjakova
Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia

Ca^{2+} ions released from the sarcoplasmic reticulum *via* cardiac ryanodine receptor (RyR2) are the key determinant of cardiac contractility. The activity of RyR2 channel is primary controlled by Ca^{2+} entering the cytosol from the extracellular space through L-type Ca^{2+} channels. Recently, it has been shown that Ca^{2+} in the lumen of the sarcoplasmic reticulum also regulates activity as well as gating kinetics of the RyR2 channel (1). The aim of our present study was to investigate whether effects of luminal Ca^{2+} observed at high concentration (53 mM) are also physiologically relevant. The RyR2 channels were isolated from the rat heart and subsequently reconstituted into planar lipid bilayer. The cytosolic Ca^{2+} was kept at 90 nM concentration level and luminal side of the RyR2 channel complex was exposed to physiological concentration of luminal Ca^{2+} (1 mM). Under these experimental conditions we tested stimulation effect of caffeine added from the cytosolic side of the RyR2 channel. Observed results were compared with our previously published results obtained for 53 mM luminal Ca^{2+} (1). We found that 1 mM luminal Ca^{2+} was similarly effective in enhancing the RyR2 channel sensitivity to caffeine and in decelerating the channel gating kinetics. Only one significant difference was identified between 1 mM and 53 mM luminal Ca^{2+}. 1 mM luminal Ca^{2+} decreased the maximal activation reached by the RyR2 channel by two fold (P_{omax} = 0.35±0.14 for 1 mM luminal Ca^{2+} *vs.* P_{omax} = 0.76±0.15 for 53 mM luminal Ca^{2+}). Our results indicate that luminal Ca^{2+} interacts with potential Ca^{2+} binding sites localized on the luminal side of the RyR2 channel under physiological conditions and thus, the observed effects of luminal Ca^{2+} might play role in the regulation of RyR2 channel during the heart contraction.

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Supported by VEGA No. 2/0118/09.

IDENTIFICATION OF CHANGES IN FUNCTIONAL PROFILE OF THE CARDIAC RYANODINE RECEPTOR CAUSED BY THE COUPLED GATING PHENOMENON

M. Gaburjakova, J. Gaburjakova
Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic

In cardiac muscle, the intracellular trigger for contraction is a transient rise in intracellular free Ca^{2+} released from the Ca^{2+} stores through ryanodine receptor (RyR2) channels. Two or more RyR2 channels reconstituted into a bilayer lipid membrane (BLM) can open and close either independently (single gating) or simultaneously (coupled gating). Although the physiological relevance of coupled gating of RyR2 channels is largely open to debate at the present time, it has been considered as a one of termination mechanisms of Ca^{2+} release to ensure periodic contraction and relaxation of cardiac muscle (1). The objective of our work was to identify and further characterize potential changes in functional profile of the RyR2 channel caused by coupled gating phenomenon. Employing the method of reconstitution of an ion channel into a BLM we showed that coupled RyR2 channels from the rat heart were activated by cytosolic Ca^{2+} with the same efficacy and potency as was reported for the single RyR2 channel using the same experimental conditions. In contrast, all three parameters of gating kinetics were affected by the functional interaction between channels. The average open and closed times were considerably prolonged and the frequency of opening was reduced. Interestingly, Ca^{2+} activated coupled RyR2 channels did not exhibit a sudden switch from slow to fast gating kinetics at open probability of 0.5 as was reported for the single RyR2 channel when luminal Ca^{2+} was used as a charge carrier. Selected permeation properties of coupled RyR2 channels were comparable with those found for the single RyR2 channel. Ca^{2+} current amplitude - luminal Ca^{2+} relationship displayed a simple saturation and the channel selectivity for Ba^{2+} and Ca^{2+} ions was similar. Our results suggest that the major targets influenced by coupled gating are likely the gates of individual RyR2 channels recruited into a functional complex ensuring mainly the correlation of Ca^{2+} fluxes.

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Supported by VEGA 2/0118/09.

LACK OF CORRELATION BETWEEN β-ADRENOCEPTORS DENSITY AND INOTROPIC EFFECT IN THE RAT HEART

C. Gonzalez-Muñoz[1], J. Beneš[2], J. Hernández J., J. Mysliveček[2]
[1]Department of Pharmacology, Medical Faculty, Murcia, Spain, [2]Institute of Physiology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic

The sympathetic nervous system plays an important role in regulating cardiac function by acting on β-adrenoceptors (β-AR) and three β-AR subtypes (β_1, β_2 and β_3) have been described [1]. These β-AR subtypes are not homogeneously distributed through the heart, but whether this correlates with functional effects is not fully established yet. The aim of our study is to compare β-AR density and contractility in left atrial and right ventricular myocardium in Sprague-Dawley rats. The preparations were electrically stimulated and cumulative concentration-response curves were constructed to the β-AR agonist salbutamol in the presence of CGP 20712A (1 μM) or ICI 118551 (50 nM) to obtain a β_2- or a β_1-AR mediated effect and CL 316243 was used to explore β_3-AR. Since cyclic nucleotide phosphodiesterases (PDE) regulate inotropic

effect of β-AR agonists [2] we also studied the effects of the above agents in the absence and presence of the non selective PDE inhibitor, IBMX. These data were compared with data on receptor binding saturations with ^{3}H-CGP12177 and ^{3}H-SB206606 as specific β_1+β_2 and β_3 radioligands and competitions of ^{3}H-CGP with CGP 20712 and ICI118551 as specific β_1 and β_2 antagonists, respectively. In the presence of ICI 118551, salbutamol (1-100 μM) produces a concentration-dependent positive inotropic effect both, in ventricular an atrial tissue, which is enhanced by IBMX. In contrast, in the presence of CGP 20712A, salbutamol is devoid of inotropic effects in these tissues but IBMX reveals a contractile effect of salbutamol in ventricular but not in atrial tissue. CL 316243 (0.001-10 μM) fails to produce inotropic effect either alone or in the presence of IBMX both in atrial and ventricular myocardium. We have found similar densities of β_1, β_2-AR in right ventricles and left atria but three times higher β_3-AR density in the left atria than in the right ventricles. These results indicate that inotropic responses to sympathomimetic agents are mediated by β_1-AR. β_2-AR, only contribute to contractility in ventricular myocardium but its effect is blunted by PDEs. On the other hand, β_3-AR activation lacks contractile effect in these tissues. Thus, the presence of β_2- and the surprisingly high density of β_3-AR in rat myocardium should be related to other functions different from cardiac contractility.

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THE EFFECT OF PREBIOTIC IN EXPERIMENTAL COLON CARCINOGENESIS

E. Hijová, A. Chmelárová, A. Bomba
Institute of Experimental Medicine, Faculty of Medicine, P. J. Šafárik University, Košice, Slovak Republic

Introduction: Colorectal cancer (CRC) is a leading cause of cancer incidence worldwide. Lifestyle factors, especially dietary intake, affect the risk of CRC development. Suitable risk biomarkers are required in order to assess the effect that specific dietary components have on CRC risk. Prebiotics are generally defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of health-promoting lactobacilli and bifidobacteria. This experimental work was designed to investigate the modulatory effect of prebiotic inulin on the enzymatic activity of ß-glucuronidase, concentration of bile acids, short chain fatty acids (SCFA), total cholesterol and triacylglycerols in rats during N,N - dimethylhydrazine (DMH) induced colon cancer, also taking into consideration high intake of dietary fat as risk factor. Material and methods: Wistar albino rats were divided into control and experimental groups. In experimental group rats were treated with prebiotic (BeneoSynergy 1, ORAFTI, Tienen, Belgium) at the dose of 2 % of high - fat diet. Serum specimens were used for determination of bile acids concentration with commercial kit (Trinity Biotech, Ireland), and lipids parameters with Biolatest (Czech Republic). Enzymatic activity of ß-glucuronidase in colon contents were examined using API-ZYM kit (Biomérieux, France) and SCFA concentration were analyzed using gas chromatography Hewlett Packard (USA). Results: Treatment with prebiotic-inulin significantly ($p<0.001$) decreased enzymatic activity of ß-glucuronidase in feaces. Similar tendency was noticed in concentration of bile acids and lipid parameters. Prebiotic undergo fermentation in the colon and enhanced short chain fatty acids production especially butyric acids. Conclusions: Prebiotics may have health benefits in the prevention of colon cancer and might represent a novel therapeutic or preventive agents.

Supported by project AV 4/0028/07 and grant VEGA 1/0372/10.

CHANGES OF FUNCTIONAL ACTIVITY OF NEUTROPHILS INDUCE BY RESVERATROL IN PIGLETS

Z. Holešovská, V. Kotrbáček, O. Pěnkava, J. Doubek
University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

Our study was aimed to determine the effect of Resveratrol (R) on leukocytes in piglets, mainly the neutrophils count and their functional activity. Ten six-week old White Large piglets were administered R in alcohol (15 %) solution in a daily dose 3 mg /kg0.75 of body weight. R was given by a gastric tube for two weeks. Other ten control piglets obtained this solution without R. Both groups were housed in individual pens and fed ad libitum with a complete food mixture. In the blood samples, collected by puncture of the v. jugularis prior the experiment and after the first and the second week, number of leukocytes (WBC) were determined using the hematological analyzer CELLTACα (Nihon Kohden, Japan) and neutrophils were counted manually.The luminol-enhanced chemiluminescence (CL) of neutrophils was measured using microtitre plate Luminometer LM-01T (Immunotech, Prague, Czech Republic). The CL assay of TRAP was measured using a Luminometer 1251 (BioOrbit, Turku, Finland). Student´s t-test was used for statistical processing of all data by the program Microsoft Excel. Administration of R caused a significant decrease of WBC ($P < 0.01$) and of the neutrophils ($P < 0.01$) at the end of the first week. After two weeks no further reduction of this parameter was found. In controls a significant ($P < 0.01$) decrease of WBC was observed only at the end of the experiment and the number of neutrophils remained unchanged. In contrast to the control animals CL of R group was significantly lower at the end of the first week. However recalculation on the number of neutrophils showed that the intensity of CL was comparable in both groups. Thus inhibition of leukopoiesis caused by R administration was not accompanied by decrease of functional activity of these leukocytes. TRAP values rice in both groups with age but R significantly accelerated this development ($p < 0.01$).

FREQUENCY OF THE SPECTRAL PEAK IN THE RANGE OF FREQUENCIES BETWEEN 60 AND 120 mHz IN CROSS-SPECTRA OF BLOOD PRESSURE AND HEART RATE FLUCTUATION IN YOUNG DIABETICS

N. Honzíková[1], M. Javorka[2], I. Tonhajzerová[2], K. Javorka[2], A. Krtička[3], B. Fišer[1]
[1]Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic, [2]Department of Physiology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovak Republic, [3]Department of Electrical Engineering, University of Defence, Brno, Czech Republic

The relatively well-preserved baroreflex sensitivity (BRS) in young diabetics is accompanied by an increased time delay within the baroreflex loop and a decrease in the synchronisation level between blood pressure and heart rate fluctuations. The aim of the present study was the determination of the frequency of the spectral peak (f) in cross-spectra of systolic blood pressure and heart rate fluctuations (frequency range between 60 and 120 mHz) and BRS by the spectral method in young diabetics and matched healthy controls. We investigated 14 type-1 diabetics (age 20-25 years, diabetes duration 10-15 years) and 14 age-matched controls. Blood pressure and heart rate were recorded beat-to-beat by Finapres for 40 minutes. BRS was determined by the spectral method, f was measured in the cross-spectra of systolic blood pressure and heart rate fluctuations. Mean (±S.D.) BRS was 14.58 (±7.05) ms/mmHg in controls and 10.31 (±4.35) ms/mmHg in diabetics. The difference was statistically insignificant ($p=0.06$). Mean f was 93.70 (±3.77) mHz in controls and 88.79 (±6.74) mHz in diabetics; the difference was significant (Wilcoxon: $p<0.05$). It is concluded that the frequency of the spectral peak in the cross-spectra of systolic blood pressure and heart rate fluctuations in the range between 60 and 120 mHz is decreased in young diabetics and provides a diagnostic tool for the detection of autonomic nervous system impairment in these patients.

Supported by grants MSM 0021622402 from the Ministry of Education, Youth and Sports of the Czech Republic, by VEGA No. 1/0064/08, and by Project of the Centre of Excellence No. 262 201 200 16.

NOVEL EFFICIENT STRATEGY TO GENERATE DIVERSITY OF ANTIBODIES

A. Hrabovska[1,2], V. Bernard[1], E. Krejci[1]
[1]Centre d'Etude de la Sensori-Motricité, Université Paris Descartes, Paris, France, [2]Dpt. of pharmacology and toxicology, Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic

Background. Despite a long history, the successful generation of specific and selective antibodies is still a task fraught with considerable

uncertainty. Results. We examine a simple, fast, and highly efficient strategy to produce an antibody, which utilizes immunization of mutant mouse strains with antigens that the host strains themselves have been genetically targeted to be deficient for. To test this strategy we choose butyrylcholinesterase, an antigen that has been considered to be difficult to generate antibody against. Antigens of different origins all provided a strong immune response, while the characteristic of the resulting antibodies depended on the preparation for the antigen prior to the immunization. Conclusion. This method, introduced previously but since neglected until now due probably to the lack of specific resources, should at this time, based on our data presented here, be considered a reasonable and reliable choice for antibody production.

Work was supported by grants AFM; grant ANR Neuroscience and APVV grants (SK-FR-0031-09 a SK-CZ-0028-09).

THE COMPARISON OF THE EFFECT OF NICOTINE-ADMINISTRATION ON BIOELECTRICAL ACTIVITY OF THE BRAIN AND ON BEHAVIOR IN 12- AND 25-DAY-OLD RATS

M. Hralová, D. Marešová, V. Riljak
Department of Physiology, First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

A variety of current studies have been concentrated on the effect of short-term or long-term administration of nicotine in humans and animals. The aim of this study was to investigate the effect of nicotine in one dose in two different concentrations on bioelectrical activity of the brain and on behavior and neuromotor activity in 12- and 25-day-old rats in 25 minutes following the application. The group of younger animals which was given an injection with lower concentration of nicotine (0.75 mg/kg body weight) showed only mild alteration of the ECoG activity and almost no behavioral changes were observed. The group with higher concentration of nicotine – 1.00 mg/kg showed epileptiform discharges in about 50 %. The animals showed also slightly expressed behavioral changes. The group of 25-days-old animals which were administrated one injection of nicotine in concentration 0.75 mg/kg body weight, showed mainly a non-specific abnormity in the ECoG and the behavior and neuromotor activity were changed almost in all cases. The group of 25-days-old animals (which was given one nicotine injection in the dose 1 mg/kg) showed very expressive specific and also non-specific abnormity in the ECoG and as well pathology in behavior and neuromotor activity in the first four minutes after nicotine-application was observed. In the control groups no changes in the ECoG and behavior were observed. We conclude, that the administration of nicotine evocated the alteration in the ECoG tracing and as well as in behavior. The quantity, the quality and the length of both was in very close relation to the concentration of nicotine and to the age of animals. The time flow of bioelectrical pathology corresponds to the time of pathology in behavioral and neuromotor activity.

This study was supported by MSM 00216 208 16 and GACR 305/09/P136.

PREDICTORS OF RESTING ENERGY EXPENDITURE DURING PREGNANCY

M. Hronek[1,2], Z. Zadak[2], P. Klemera[1], J. Tosner[3]
[1]Faculty of Pharmacy, Charles University ,[2]Department of Research and Development, [3]Obstetric and Gynecological Clinic, University Hospital, Hradec Kralove, Czech Republic

The anabolic situation during pregnancy leads to a positive energy balance, with synthesis and growth of new tissue and retention of fat and protein in the mother and in the fetus. For calculation of resting energy expenditure (REE) in pregnancy the predictive equation was derived (1). The magnitude of the increase in REE during pregnancy varies considerably among women, but the factors responsible for the variation are not completely known (2). A total of 152 healthy pregnant Czech women from the Hradec Kralove region were recruited into this longitudinal prospective study. The subjects had parity ≤ 2, and were non-smokers, non-users of chronic medications and non-abusers of alcohol or drugs. These women were normoglycemic, euthyroid, and not anemic. Women were divided into two cohorts, of which Group1 (n=31) was used for determination of the association between anthropometric parameters and REE, and Group 2 (n=121) for verification that observed relationships were suitable for prediction of REE during pregnancy. Anthropometric maternal changes and REE via indirect calorimetry after 12 hours of fasting during the four periods throughout pregnancy (until the 20th week, between 21st and 29th W, the 30th and 36th W, 37th and 39th W) were determinated. Strong associations ($p<0.0001$) in Group1 between measured REE and weight, fat mass, fat-free mass (FFM), body surface area, BMI, circumference of waist and thigh were found. By method linear regression derivative equations were tested on Group2. Maternal FFM was proved as the best determinant of REE in pregnancy. Equation REE [in kcal] = 33 * FFM [in kg] corresponds closely to measured REE and maternal changes in each phase of pregnancy and can be applied for prediction of REE during gestation.

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Financial support from project MZO 00179906 of the Czech Republic.

EFFECT OF METHAMPHETAMINE EXPOSURE AND CROSS-FOSTERING ON BEHAVIOR OF ADULT MALE RATS

L. Hrubá, B. Schutová, R. Rokyta, R. Šlamberová
Department of Normal, Pathological and Clinical Physiology, Third Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

Our previous study demonstrated that postnatal development of rat pups and their cognitive functions in adulthood are affected by both, exposure to methamphetamine (MA) and cross-fostering. Further, we showed that MA administered during gestation and lactation periods impairs maternal behavior. The aim of the present study was to investigate the impact of MA exposure and cross-fostering on behavior and anxiety in adult male rats. Mothers were daily exposed to injection of MA (5 mg/kg) or saline (S): prior to impregnation and throughout gestation and lactation periods. On postnatal day 1, pups were cross-fostered so that each mother received some of her own and some of the pups of mother with the opposite treatment. Based on the prenatal and postnatal treatments 4 experimental groups (S/S, S/MA, MA/S, MA/MA) were tested in Open field (OF) and Elevated plus maze (EPM). Locomotion, exploration, comforting behavior and anxiety were evaluated in OF, while anxiety and exploratory behavior were assessed in EPM. Our results showed that adult male rats postnatally exposed to MA via breast milk (S/MA and MA/MA) had decreased locomotion and exploratory behavior in OF compared to rats postnatally exposed to saline (S/S and MA/S). Further, S/MA and MA/MA showed increased anxiety in OF as well as in EPM. Thus, the present study demonstrates that cross-fostering may affect overall psychomotor activity and anxiety in adulthood. Postnatal exposure to MA via breast milk and maternal care of mother exposed to MA decrease locomotion, exploration but incresed anxiety to novel environment. On the other hand, decreased psychomotor activity and increased anxiety are not demonstrated in rats prenatally exposed to MA and fostered by control saline dams.

Supported by: GACR 305/09/0126, GACR P303/10/0580 and MSM 0021620816.

ANALYSIS OF REPETITIVE ELEMENTARY CALCIUM RELEASE EVENTS IN RAT CARDIAC MYOCYTES

R. Janíček, A. Zahradníková jr., E. Poláková, J. Pavelková, I. Zahradník, A. Zahradníková
Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia

The calcium release in cardiac myocytes during excitation-contraction coupling is the result of elementary calcium release events activated by the calcium current. These release events, observed as calcium spikes using confocal microscopy, are not fully understood. We have studied the activation of calcium spikes by the calcium current in isolated rat ventricular myocytes. Calcium currents were activated by 80 – 100 ms voltage pulses from -50 to 0 mV by means of the whole cell patch

clamp method. The evoked local calcium release events/calcium spikes were recorded by confocal microscopy using the calcium indicator Fluo-3 (60 μM) in the presence of EGTA (1 mM) to limit Ca^{2+} diffusion (1), and analyzed by fitting with a theoretical function (2). In the set of 33 recordings made in 18 myocytes, a large majority of the 398 observed calcium release sites responded to stimulation by a single calcium spike. In 16 % of observations the release sites responded by two subsequent (twin) spikes. The amplitude, latency, time to peak and duration of the single spikes were compared to those of the first and the second of the twin spikes. Both the first and the second of the twin spikes had lower amplitude than the single spikes. On average, the second spikes had a shorter time to peak and a shorter duration than the first and the single spikes,. The latency and the time to peak of the single and the first spikes were not significantly different. The mean interval between the first and the second spike was 30 ms. The incidence of twin spikes was higher when both the density and the inactivation rate of calcium current were reduced.

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Supported by grants APVV-0139-06 and VEGA 2/0102/08 and by the European Union Contract No. LSHM-CT-2005-018833/EUGeneHeart.

THE PROGESTERONE PROFILE IN BLOOD AND MILK SERUM OF EWES AFTER GESTAGEN AND OVSYNCH TREATMENT

J. Kaľatová, D. Sopková, S. Ondrašovičová
Institute of Physiology, University of Veterinary Medicine, Košice, Slovak Republic

Aim of our work was to observe the progesterone (P_4) concentrations in blood (BS) and milk serum (MS) in ewes Improved Wallachian after different treatment. Group 1 (n=10) was subsequently induced to oestrus with 40 mg FGA (intravaginal sponges) for 12 days. After withdrawal of sponges 500 IU eCG was applied to them. Group 2 (n=5) was treated according to OvSynch Protocol (0.5 ml GnRH per head; after five days 0.5 ml $PGF_{2\alpha}$ per head; after 48 hours 0.5 ml GnRH per head) (1). Forty-eight hours after ending of treatment ram was introduced. The milk and blood taking were realized before, during and after treatment. The P_4 concentration was determined in blood and milk serum by RIA. In group 1 and 2 before treatment P_4 flowed on the basal level. The influence of synthetic gestagen (FGA) became evident to increase P_4 but intermediate decrease in MS and BS. We registered the graduated increase of P_4 in dependence of ram introducing. The analysis of group 2 showed the analogous profile of P_4 in MS and BS as group 1. In the same time as group 1 ram was introduced and we observed mild increase of P_4 in BS and MS. The changes of progesterone concentration in blood and milk serum were significant ($P<0.001$). The changes of P_4 in blood and milk serum was probably induced after withdrawal of sponges and eCG injection, which increasing of blood 17β-oestradiol concentration during periovulation period in primarily anoestrous ewes, where these are probably responsible for oestrus and ovulation synchronization (2). From our results we can conclude that however treatment according to OvSynch presents less markedly changes of P_4 concentration than treatment with FGA and eCG, this method of oestrus induction appear with positive oestrus–synchronization effect for ewes breeding in Slovakia.

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This work was supported by Project AV 4/0113/06.

THE CYSTEINES IN THE EXTERNAL LOOP OF THE FIRST DOMAIN OF THE CA,3.1 CHANNEL ARE ESSENTIAL FOR CHANNEL FUNCTION

M. Karmazinova[1], S. Beyl[2], N. Klugbauer[3], L. Lacinova[1]
[1]Institute of Molecular Physiology and Genetics, SAS, Bratislava, Slovakia, [2]Institut für Pharmakologie und Toxikologie, Universität Wien, Wien, Österreich, [3]Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Albert-Ludwigs Universität, Freiburg, Germany

All voltage-gated calcium channels are composed of four homologous domains, each containing six transmembrane segments S1-S6 and a pore loop (P) between segments S5 and S6. Four voltage-sensing domains each composed of the transmembrane segments S1 - S4 are placed peripheral to a central pore domain formed by four sets of S5-P-S6 (1). LVA calcium channels contain a unique large external domain located between the IS5 region and the pore loop (P), which is not found in HVA calcium channels. Six cysteines located within this region are conserved among all three CaV3 channels. The aim of our study was to analyze role of these six cysteines in external IS5-P linker of the Cav3.1 channel. Each of cysteines was exchanged for an alanine. Resulting mutants (C241A, C271A, C282A, C323A, C298A and C313A) were transfected into HEK 293 cells. Whole-cell patch-clamp was used for current analysis. The bath solution contained (in mM): NaCl 155, HEPES 10, CaCl2 2, MgCl2 1, CsCl 5; ph 7.4 with NaOH. The pipette solution contained: (in mM): CsCl 130, Na-ATP 5, EGTA 10, HEPES 10, TEA-Cl 10, MgCl2 5; ph 7.4 with CsOH. Four of channel mutants (C241A, C271A, C282A and C323A) did not form functional channels. Two remaining channels C298A and C313A were capable of carrying inward calcium current. Both mutations shifted channel activation into the depolarizing direction and significantly reduced the voltage sensitivity of channel activation, inactivation and deactivation. The redox agents dithiothreitol (DTT) and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) inhibited the current amplitude through the wild type channel. Both mutations C298A and C313A reversed the effect of DTT and shifted the voltage dependence of current activation in the hyperpolarizing direction. Furthermore, both mutations reduced the current inhibition by DTNB. We conclude that all six cysteines are essential for proper channel function, but their contribution is of a different nature.

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This work was supported by the Slovak Grant Agency VEGA, project No. 2/7001/7 and VVCE-0064-07.

THE EFFECT OF SELECTIVE INHIBITION OF 11 BETA HYDROXYSTEROID DEHYDROGENASE TYPE 1 ON METABOLISM OF THE PRAGUE HEREDITARY HYPERTRIGLYCERIDEMIC RAT

P. Klusoňová, P. Ergang, I. Mikšík, J. Kuneš, J. Zicha, J. Pácha
Institute of Physiology, Academy of Sciences of the Czech Republic

An increasing number of people in advanced countries suffers from a cluster of metabolic disorders that are collectively termed metabolic syndrome and that include hyperinsulinemia, hypertension, glucose intolerance, hypertriglyceridemia and obesity. Glucocorticoids strongly influence the metabolism of nutrients. Local level of active glucocorticoids in cells is markedly influenced by 11beta-hydroxysteroid dehydrogenase 1 (11HSD1), which metabolizes inactive form of glucocorticoids into active form. Inhibition of 11HSD1 has been shown to be a promising target for treatment of metabolic syndrome in a number of rodent models. We decided to study the effect of selective inhibition of 11HSD1 on metabolism of female Prague hereditary hypertriglyceridemic rat (HHTg), a nonobese model of metabolic syndrome, which displays increased 11HSD1 activity in liver and adipose tissue. As a selective inhibitor we used a 2-adamantyl triazole also described as Compoud 544. Animals were treated at a dose of 10 mg/kg/day for two weeks. We measured serum levels of corticosterone (CS), insulin (INS), triglycerides (TG), nonesterified fatty acids (NEFA) and cholesterol. We also measured systolic and diastolic blood pressure. We then examined an effect of chronic 11HSD1 inhibition on expression of 11HSD1, glucocorticoid receptor (GR) and hexoso-6-phosphate dehydrogenase (H6PDH), an enzyme that cooperates with 11HSD1. Long term inhibition of 11HSD1 resulted in increased expression of GR in liver but had no effect on expression of 11HSD1 and H6PDH. In contrast, 11HSD1 and H6PDH mRNA was increased in ovarial adipose tissue but no changes in GR expression were observed. No changes in mRNA expression were found in subcutaneous adipose tissue. Chronic inhibition of 11HSD1 significantly decreased serum levels of corticosterone, TG and increased serum levels of HDL cholesterol, however it had no effect on serum levels of NEFA and insulin and blood pressure. Our data suggest that action of 11HSD1 is connected to impaired lipid metabolism in HHTg rats. Inhibition of 11HSD1 improved hypertriglyceridemia in HHTg rats

thus Compound 544 should be considered for further research of metabolic syndrome treatment.

Supported by The Grant Agency AS CR: KJB500110703 Compound 544 was kindly provided by Merck.

ACUTE ANTIARRHYTHMIC/DEFIBRILLATING EFFECTS OF ATORVASTATIN AND OMEGA-3 FATTY ACIDS DEMONSTRATED IN RATS SUFFERING FROM HYPERTRIGLYCERIDEMIA

V. Knezl, B. Bacova[1], J. Radosinska[1], S. Karas[2], N. Tribulova[1]
Institute of Experimental Pharmacology and Toxicology Slovak Academy of Sciences, Bratislava, [1]Institute for Heart Research, Slovak Academy of Sciences, Bratislava, [2]Institute of Measurements, Slovak Academy of Sciences, Bratislava, Slovakia

Background and purpose: It has been reported that atorvastatin and omega-3 fatty acids exhibit antiarrhythmic effect in clinic but underlying mechanisms are not elucidated yet. We have previously shown that prolonged treatment of hypertriglyceridemic rats (HTG) with these compounds reduced the incidence of malignant arrhythmias and our findings suggest that myocardial intercellular connexin channels, which ensure electrical coupling and synchronisation, are implicated. To elucidate further how atorvastatin and omega-3 FA may modulate intercellular coupling, this study was aimed to examine whether these compounds exert acute antiarrhythmic effects. Design and Methods: Experiments were conducted on male and female five-month-old HTG rats known to be much prone to ventricular fibrillation (VF) than healthy rats. The heart was excised from anesthetized rats and perfused via aorta with oxygenated Kreb-Henseleit solution at constant flow (10-14 ml/min, female-male). Upon equilibration, VF inducibility was tested using electrical stimulation. Repetitive stimulation was performed during 5 min period unless sustained (>2 min) VF occurred earlier. The hearts were perfused with Atorvastatin (Zentiva), eicosapentanoic acid (EPA) or docosahexanoic acid (DHA) (1.5, 7, 15 µmol) during 10 min prior el. stimulation. Results: Sustained VF was induced in all HTG rat hearts without treatment. In contrast, the hearts subjected to atorvastatin, EPA and DHA were less susceptible and incidence of sustained VF was reduced to 33 %, 71.4 % and 80 % in male and to 60 %, 75 % and 60 % in female rats. Atorvastatin suppressed VF inducibility in male rats already in concentration 1.5 µmol while EPA and DHA were efficient at higher 7 and 15 µmol. Strikingly, bolus of either EPA or DHA (150 µmol) administered directly to fibrillating heart defibrillated it. Conclusions: Atorvastatin, EPA and DHA exhibit clear antifibrillating and defibrillating efficacy when acutely applied. This fact suggests that these compounds can likely affect directly connexin channels function in addition to their chronic effects on cell membrane lipid compositions (fluidity) that can affect protein channels conformation and function.

INFLUENCE OF VEGETARIAN DIET ON THE CONCENTRATION OF LIPOPROTEIN (a) - Lp(a), APOLIPOPROTEIN B_{100} – apo B_{100} AND LIPIDS

J. Koprovičová, D. Petrášová
Institute of Experimental Medicine, Faculty of Medicine, Šafarik University, Košice, Slovak Republic

Increased serum concentration of Lp(a) is an important independent risk factor of premature atherosclerosis and myocardial infarction (1). Apo B_{100} is a significant marker of early atherosclerosis (2). The combination of elevated serum concentration of Lp(a) and apo B_{100} is considered a severe atherogenic risk (3). Unhealthy diet correlates with etiopathogenesis of atherosclerosis. The goal of this study was to determine the concentration of Lp(a), apo B_{100} and lipids (total cholesterol – TCH, triacylglycerols – TG, LDL-cholesterol – LDL-CH, non HDL-cholesterol – non HDL-CH and HDL- cholesterol – HDL-CH) in blood serum in 35 Vegetarians (V) with BMI: 23±2 kg/m^2 and age: 37±5 years. 27 healthy men with conventional diet, BMI: 24±2 kg/m^2 and age: 35±7 years were examined in the control group (C). Lp(a) was determined using the immunoturbidimetric method and apo B_{100} by electroimmunoassay. TCH and TG were determined using the biochemical tests of Pliva- Lachema company (Czech Republic). We found significantly decreased serum concentration of apo B_{100} ($p<0.001$), TCH ($p < 0.001$), LDL-CH ($p<0.001$) and non HDL-CH ($p<0.001$) in the group V. Concentration of Lp(a) was also significantly decreased ($p<0.05$) and concentration of HDL-CH was significantly elevated ($p<0.05$). in group V. The very favourable status of lipids including Lp(a) an apo B_{100} is influenced by vegetarian diet rich in vitamins, fibers, bioflavonoids and unsaturated fatty acids.

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ACUTE AND CHRONIC INHIBITION OF RHO/RHO – KINASES INDUCES VASODILATATION IN ONE WEEK HYPOXIC PULMONARY HYPERTENSION

K. Koubský, J. Herget
Department of Physiology, 2nd Medical School, Charles University, Prague and Center for Cardiovascular Research, Prague, Czech Republic

Rho/Rho – kinases regulate the sensitivity of vascular smooth muscle cells to intracellular calcium. They increase the phosphorylation of myosin light chains mainly by inhibition of myosin phosphatase activity. We tested whether increase in calcium sensitivity by Rho/Rho – kinase mechanism participates on the increase in pulmonary vascular tonus in chronic hypoxia. In the first experiment (A) we studied the effect of acute administration of Rho/Rho kinases inhibitor Fasudil (10 µM) in chronically hypoxic and normoxic rats, in the second study (B) the result of chronic Fasudil treatment in hypoxic rats (30 mg/kg b.w. daily) was observed. Chronic hypoxia was induced by 7 days exposure in isobaric hypoxic chamber (F_{iO2} = 0.1). Relevant control groups were in air. The vascular resistance was assessed by measurement of perfusion pressure – perfusion flow relationship (P/Q) in saline perfused isolated ventilated lungs. The acute administration of Fasudil (A) to hypoxic rats resulted in significant decrease of intercept of P/Q line with pressure axis. Chronic treatment of hypoxic rats by Fasudil lowered the slope of P/Q line with no change in intercept with pressure axis. No effect of Fasudil was observed in normoxic rats. Conclusion: Acute inhibition of Rho/Rho – kinases in chronic hypoxia decreases the critical closing pressure of pulmonary vessels (vascular smooth muscle tonus). Chronic treatment during hypoxia decreases the pressure increment induced by the increase in perfusion flow.

GATING OF THE NEURONAL $CA_V3.3$ CHANNEL IS DETERMINED BY GATING BRAKE IN I-II LOOP

L. Lacinova[1], M. Karmazinova[1], E. Perez-Reyes[2]
[1]Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia, [2]Department of Pharmacology, University of Virginia, Charlottesville, Virginia 22908, USA

Low-voltage activated Ca_V3 Ca^{2+} channels are characterized by activation threshold about -60 mV, which is lower than the activation threshold of other voltage-dependent calcium channels (VDCC). Kinetics of their activation at membrane voltages just above activation threshold is much slower that the activation kinetics of other VDCC. It was demonstrated (1, 2) that intracellular loop connecting repeats I and II of all three Ca_V3 channels contains a so-called “gating brake”. Disruption of this brake led to channels that activated at even more hyperpolarized potentials with significantly accelerated kinetics. Principal α1 subunit of all VDCC consists of four homological domains, each containing six transmembrane segments S1-S6. Conformational change of the channel leading to opening of conductive pore is preceded by the movement of S4 segments, which represent putative voltage-sensing part of the channel. Movement of these positively charged segments can be detected as a so-called gating current or charge movement. We have compared gating currents measured from the wild type $Ca_V3.3$ channel and a mutated ID12 channel, in which putative gating brake was removed. Voltage dependence of the charge movement was shifted by about 20 mV towards more hyperpolarized potentials in ID12 channel. Further, kinetics of the gating current was significantly accelerated and value of maximal charge moved normalized in respect to maximal inward current was doubled. We

concluded that the putative gating brake in I-II loop hinders not only opening of the conducting pore but also the activating movement of voltage sensing S4 segments.

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Supported by grants VEGA 2/7001/7 and VVCE-0064-07.

IMPARED CALCIUM SIGNALLING IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF PATIENTS WITH CHRONIC KIDNEY DISEASE

I. Lajdová, V. Spustová, A. Okša, D. Chorvát, Jr.[1]
Department of Clinical and Experimental Pharmacotherapy, Slovak Medical University, Bratislava, Slovak Republic; [1] Department of Biophotonic, International Laser Centre, Bratislava, Slovak Republic

Elevations of free cytosolic calcium concentration ($[Ca^{2+}]_i$) have been found in association with cellular dysfunctions in variety conditions. Frequently, chronic renal failure has been referred to as a state of cell calcium toxicity. The aim of the present study was to determine $[Ca^{2+}]_i$, concentration of intracellular calcium reserves, the calcium entry through CRAC channels and to investigate the participation of purinergic $P2X_7$ receptors in intracellular calcium homeostasis regulation in early stages of chronic kidney disease (CKD). The study involved 22 healthy volunteers and 22 CKD patients with stage 2-3 patients. The peripheral blood mononuclear cells (PBMCs) were separated by Ficoll gradient centrifugation and $[Ca^{2+}]_i$ was determined by using Fluo-3 AM fluorimetry. Intracellular calcium reserves were emptied using thapsigargin (Tg), a specific inhibitor of endoplasmic reticulum Ca^{2+}-ATPase. 2-Aminoethyl-diphenyl borate (2APB) was used to examine the capacitative calcium entry. KN-62, a specific inhibitor of purinergic $P2X_7$ receptors, and 2',3'-*O*-(4-benzoyl) benzoyl ATP (BzATP), a specific agonist of these receptors, were used to examine function of cation channel $P2X_7$ receptors. All experiments were carried out at 37 °C. Results are expressed as mean±S.E.M. In PBMCs of CKD patients the $[Ca^{2+}]_i$, calcium concentration Tg-sensitive stores and capacitative calcium entry were significantly increased when compared with healthy subjects. The specific agonist of purinergic receptors (BzATP) caused a sustained increase in $[Ca^{2+}]_i$ in both groups, but the effect was significantly decreased in CKD patients. The application of specific inhibitor of purinergic $P2X_7$ receptors (KN-62) decreased $[Ca^{2+}]_i$ in CKD patients from 126±1.7 to 114±3.9 nmol/l ($P < 0.01$), but had not the effect on $[Ca^{2+}]_i$ in healthy volunteers. The effect of KN-62 was attenuated in BzATP acitvated PBMCs of CKD patients when compared with healthy volunteers. Presented results demonstrate that the calcium signalling pathway is defect in PBMCs of CKD patients and purinergic $P2X_7$ receptors are involved in altered intracellular calcium signalling.

Supported by grants No. APVT-21-033002 and VG SZU 19-90-07.

DIFFERENT RESPONSE TO MYOCARDIAL ISCHEMIA/REPERFUSION INJURY IN ISOLATED HEART OF ADULT MALE AND FEMALE RATS

V. Ledvényiová, D. Pancza, D. Stakheev[1], T. Ravingerová
Institute for Heart Research, Centre of Excellence for Cardiovascular Research of the Slovak Academy of Sciences, Bratislava, Slovak Republic, [1]Institute of Physiology, Academy of Sciences of the Czech Republic, Centre for Cardiovascular Research, Prague, Czech Republic

Gender-related differences is one of the major problems underlying differences in cardiovascular morbidity and mortality in human population, where men are generally at higher risk than age-matched premenopausal women (1). Studies have shown that compared to males of the same age, premenopausal women have a lower incidence of left ventricular hypertrophy, coronary artery disease, and cardiac remodeling following myocardial infarction (2). Gender specifity represents one of the important factors that may determine differences in myocardial susceptibility to ischemia/reperfusion (I/R) injury, however, the experimental data on the mechanisms of cardioprotection are not always consistent. The present study was designed to investigate potential differences in the response to acute myocardial I/R injury in adult Wistar rats of both sex. Isolated Langendorff-perfused hearts of female rats and of age-matched males were subjected to 30-min occlusion of the LAD coronary artery for the measurement of ischemia-induced ventricular arrhythmias (3) followed by 2-h reperfusion for the evaluation of lethal injury (myocardial infarction determined by tetrazolium double staining and computerized planimetry; 4). Results: Female hearts exhibited lower susceptibility to I/R injury that was documented by a 29 % decrease of the infarct size (IS; expressed as % of area at risk size) to 21.8±2.4 % in comparison with 30.7±0.4 % in the male hearts ($P<0.05$). In addition, the total number of premature ventricular complexes (PVC) in female hearts (81±15) was significantly smaller than in males (402±86; $P<0.05$). Likewise, the total duration of ventricular tachycardia (VT) and the number of its episodes were significantly reduced in females (3.2±1.7 s and 1.7±0.8 vs. 15.2±5 s and 11.3±3.2, respectively, in males; $P<0.05$). Conclusions: The results suggest that the adult female rats are more resistant to acute myocardial infarction and severe ventricular arrhythmias than male rats of the same age. Molecular mechanisms of enhanced ischemic tolerance in female animals may involve effect of sex hormones or activation of different cell pathways and still require further investigation.

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Supported by grants VEGA SR 2/0173/08, APVV-SK-CZ-0049-07 and IAAX01110901.

THE INFLUENCE OF SELENIUM AND ZINC ON THE INNATE IMMUNE SYSTEM IN KIDS

S. Malá[1], F. Kovářů[1], L'. Mišurová[2], L. Pavlata[2], R. Dvořák[2], M. Čížˇ[3]
[1]Institute of Physiology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, [2]Ruminant Clinic, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, [3]Department of Free Radical Pathophysiology, Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic

Selenium and zinc can influence several components of innate immunity. There is little information that can suggest which form of trace elements (inorganic or organic) is better for ruminants. The experiment was conducted to determine the effect of adequate level of selenium (Se) and zinc (Zn) supplementation on innate immune response of goats. Selenium is needed for proper function of neutrophils, macrophages, NK cells and other immune mechanisms. Zinc is necessary for the normal function of the innate immune response such as phagocytosis of neutrophils and generation of reactive oxygen species (ROS). Eighteen kids after weaning (69 days of life) were divided into three groups: (1) control (no supplemental selenium), (2) inorganic form (sodium selenite), and (3) organic form (lactate protein selenium complex). Content of selenium is 1 mg in 1kg concentrate. Twenty four kids after weaning (69 days) were divided into four groups: (1) control (no supplemental zinc), (2) inorganic form (zinc oxide), (3) organic form (zinc lactate) and (4) organic form (zinc proteinate). Content of zinc was 100 mg in 1kg concentrate. The blood samples were investigated at the age of 120 days of kids in both the experiments. The observed parameters were the count of white blood cells, leukocyte differential count, phagocytic activity and phagocytic index. The production of ROS by goat's blood neutrophils was detected by luminol-enhanced chemiluminescence (CL). CL was performed to determine peak CL, integral CL and peak-time after stimulation with various agents. Calcium ionophore A23187 (Cal-I), opsonised zymosan (OZP) and phorbol 12-myristate 13-acetate (PMA) were used. A significant increase in the value of peak-time ($p \leq 0.05$) was shown in the inorganic-Se-treated group (2) when the activator OZP was used. Phagocytic activity was 60.17±9.15 % in the control group and phagocytic index was 21.72±0.41. Phagocytic activity was lower in the group treated by organic Se (54.5±4.32 %) in contrast to the control group. Our data indicate that inorganic form of Se can be beneficial for

neutrophil function of kids. A significant ROS increase reflected in peak CL was found in the lactate-Zn-treated group (3) when Ca-I was used as activator ($p \leq 0.05$). The other parameters in the experiment with supplementation of Zn did not exhibit significant changes. We can assume that both the forms of Zn are suitable for enhancement of innate immune system in kids.

This work was supported by Ministry of Education, Youth and Sports of the Czech Republic (project no. 6215712403) and by Grant Agency of the Academy of Sciences of the Czech Republic (grant no. IAA 6011680801).

CASPASES IN MOUSE LIMB DIGITALIZATION

E. Matalova[1,2], L. Dubska[2,4], M. Buchtova[1,2], I. Chlastakova[1,2], J. Doubek[2], K. Klepárník[3], I. Misek[1,2]
[1]Institute of Animal Physiology and Genetics AS CR, v.v.i., Brno, [2]University of Veterinary and Pharmaceutical Sciences Brno, [3]Institute of Analytical Chemistry AS CR, v.v.i., Brno, [4]Masaryk Memorial Cancer Institute, Brno, Czech Republic

The best known example of embryonal apoptosis is the digit separation during limb development. Limbs start to form as undifferentiated buds consisting of an outer layer of ectoderm and an inner core of mesenchymal cells. Future digits form within these protrusions as cartilaginous condensations separated by mesenchymal cells that are destined to be eliminated by apoptosis. Caspases, a large protein family of cysteine aspartate proteases, are the key proteins involved in major apoptotic pathways. At least 15 mammalian caspases being involved in inflammation or/and apoptosis have been reported so far. Initiator caspases (caspase-8, -9, and -10) are activated during the initiation steps of apoptosis via death adaptor molecules proximity and oligomerization induced proteolytic processing. Once activated, the initiator caspases cleave other members of the caspase family called effector caspases. Subsequently, effector caspases (caspase-3, -6, -7) cause degradation of several cellular polypeptides that are essential for cell survival (lamin, PARP) resulting in DNA fragmentation, cytoskeleton break-up, and cell death.Caspase-3 was under study using explants culture *ex vivo* approach, pharmacological inhibitions (Z-DEVD-FMK inhibitor, 200μM), histochemistry (anti-caspase-3 immunohistochemistry, TUNEL assay) and flow cytometry (anti-caspase-3 cytochemistry, TUNEL assay). Mouse front limbs at E12.0 and E13.5 were used in control groups (DMEM only, DMEM+1 % DMSO) as well as in experimental (general caspase inhibition, caspase-3 inhibition) and cultured for 72 h. Inhibition of caspase-3 blocked apoptosis in the interdigital webbing only temporally showing redundancy of caspase-3 in normal limb development. Other caspases in interdigital apoptotic network and applications of highly sensitive antibody-bound nanoparticles are under study.

Research of cell death pathways is supported by the Grant Agency of the Czech Academy of Sciences (IAA600450904), nanotechnologies in functional diagnostics of cell populations by GA ČR (203/08/1680). The MMCI lab is supported by the Czech Ministry of Health (MZ0MOU2005).

c-MYB IN MOUSE ODONTOGENESIS

E. Matalova[1,2], E. Janeckova[1,2], M. Buchtova[1,2], V. Lungova[2], I. Misek[1,2], J. Doubek[2], J. Smarda[3]
[1]Institute of Animal Physiology and Genetics AS CR, v.v.i., Brno, [2]University of Veterinary and Pharmaceutical Sciences Brno, [3]Faculty of Science, Masaryk University Brno, Czech Republic

The c-*myb* gene encodes a transcription factor involved in control of cell proliferation, differentiation, and survival and is generally present in undifferentiated cells. It is required for fetal hematopoiesis, however, its potential roles in other embryonal tissues are not well understood also due to incomplete knowledge of c-Myb expression patterns. As controlled proliferation, differentiation and elimination of particular cell populations are considered to determine the final tooth shape, size and position in the jaw, c-Myb expression was investigated in mouse molar tooth germs. The major aims of this study were to evaluate c-Myb distribution in prenatal development of the first mouse molar, and to correlate c-Myb expression with proliferation and apoptosis during formation of tooth-bone complex. Frontal sections of mouse heads, starting at the embryonic day (ED) 12.5 up to birth (P0) were used in this study. Immunohistochemical detection of c-Myb (Abcam) and PCNA (proliferating cell nuclear antigen, Santa Cruz) was applied to evaluate proliferation, TUNEL assay (Chemicon) to detect apoptotic cells. c-Myb protein at ED12.5 was found in both, epithelial and mesenchymal parts of the tooth germ. At ED15, the expression was prevalent in the epithelial part of the tooth germ and at ED was located particularly in the forming odontoblast and ameloblast layers. With gradual mineralization of the tooth germ, c-Myb expression was detected particularly in the mesenchyme (future tooth pulp). c-Myb expression did not show any exact pattern, however, at all stages correlated positively with proliferation and negatively with apoptosis.

Supported by the Grant Agency of the Czech Republic 524/08/J032 and GA AV IAA600450904.

INTERACTIVE CARDIOVASCULAR PHYSIOLOGY AND PATHOPHYSIOLOGY FOR PRACTICAL COURSES

E. Matalova, P. Scheer, J. Doubek
Department of Physiology and Pathophysiology, University of Veterinary and Pharmaceutical Sciences, Brno

Interactive materials represent a priority approach in recent education due to easy updating, attractiveness and broad applications of computers and networking. The Department of Physiology and Pathophysiology UVPS Brno has been gradually modernizing student laboratories for pre-gradual teaching in veterinary medicine, and veterinary hygiene and ecology. One of the important topics in education represents the physiology of cardiovascular system, particularly non-invasive methods for investigation. Therefore, an electronic stethoscope and a modern electrocardiograph were purchased to be applied in practical training of students. The electronic stethoscope is connected with an analyser allowing for processing of sound waves and evaluation using computer techniques. All data can be monitored, saved and also simultaneously commented using data-projection during the practical courses. The electrocardiograph with radio transmission serves as a mains-independent, wireless device for ECG records, heart rate measurement with optical and acoustical signals, medical diagnosis support and ECG documentation. Also in the case of this ECG device, the monitored data are simultaneously transmitted on the screen via data-projection and can be immediately discussed. Operation manual and protocols to corresponding practical courses have been summarized in an electronic form and accompanied by background knowledge on cardiovascular system with attractive animation schemes, figures and videos.

Supported by the Ministry of Education, Youth and Sports, Czech Republic (FRVS 1837/F3/d).

EXPRESSION OF TYROSINE HYDROXYLASE AND NEUROPEPTIDE Y IN THE HEART OF RATS WITH CHRONIC RENAL FAILURE

E. Mistrová, M. Chottová Dvořáková, J. Kuncová, J. Švíglerová, J. Slavíková
Department of Physiology, Faculty of Medicine, Charles University, Plzeň, Czech Republic

Tyrosine hydroxylase (TH) is the rate-limiting enzyme of the synthesis of catecholamines (CA) in the catecholaminergic neurons. Neuropeptide Y (NPY) is a cotransmitter of CA that is produced by cleavage from a large precursor preproNPY. In the heart, TH is localized in the sympathetic nerve fibers and in the intrinsic cardiac adrenergic (ICA) cells. While TH of sympathetic origin is synthesized in neuronal cell bodies located in the sympathetic ganglia, the mRNA of TH in the heart tissue is produced by ICA cells. PreproNPY mRNA is expressed in the cell bodies of intrinsic neurons, and in the endothelial cells. Chronic renal failure (CRF) is associated with cardiovascular diseases, which can be explained in part by abnormalities in autonomic regulation (1). Here we investigated the impact of CRF on expression and tissue distribution of TH and NPY. CRF was induced in adult rats by surgical 5/6 nephrectomy. At 10 weeks after operation, rats were sacrificed by

decapitation, heart atria and ventricles were dissected, and separately analyzed by real-time PCR and immunohistochemistry. Relative expression of TH and preproNPY mRNAs was expressed as a ratio of target gene C_T value to housekeeping gene – beta-actin. The results were considered significantly different when $p<0.05$. The basal expression of TH mRNA was about 30 times higher in the atria than in the ventricles. CRF caused an increase of the TH mRNA expression in the left atrium, however, there were no significant changes in the right atrium and in the ventricles. The relative expression of preproNPY mRNA in both atria and ventricles did not significantly differ from controls. Indirect immunofluorescence showed no change in distribution and density of immunoreactivity for TH and NPY in CRF rats compared with controls.

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Supported by the Research Project MSM 0021620819, Replacement of and support to some vital organs.

MITOCHONDRIAL FUNCTION IN HEART, KIDNEY AND LIVER OF SPONTANEOUSLY HYPERTENSIVE RATS: EFFECT OF CAPTOPRIL

J. Mujkošová[1], M. Ferko[1], O. Uličná[2], I. Waczulíková[3], A. Mišák[3], A. Ziegelhöffer[1]
[1]Institute for Heart Research, Slovak Academy of Sciences, [2]Pharmacobiochemical laboratory, 3rd Medical Clinic, Faculty of Medicine, Comenius University, [3]Division of Biomedical Physics, Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovak Republic

Hypertension (HYP)-induced functional abnormalities on subcellular level, were described in many organs and tissues. Nevertheless, there are still only scarce data available about HYP-induced alterations in functional properties of the mitochondria (MIT). Present paper deals with HYP-induced alterations in heart, kidney and liver MIT in the same individual, when the disease is treated with the ACE inhibitor captopril. Twenty week old male, spontaneously hypertensive (SH) rats were treated for 4 weeks with captopril (80 $mg.kg^{-1}$ per os, daily). Systolic blood pressure (SBP) was monitored at the beginning, the 14th and 28th day of captopril treatment. Heart to body weight ratio (HW/BW) was estimated at termination of the experiment. MIT from heart, kidneys and liver were isolated by differential centrifugation. Estimations: membrane fluidity (MF) and the parameters of oxidative energy production (OEP): O_2 consumption (QO_2) with and without exogenous ADP (states S3 and S4), the respiratory control index (RCI), the rate of oxidative phosphorylation (OPR) and the ADP:O ratio with succinate (SUC) and glutamate/malate (GLUT/MAL) as substrates (Clark oxygen electrode). Captopril prevented ($p<0.05$ or more) the increase in SBP in untreated SH rats between the 16th and 20th week of age but, its effect on decrease in HW as well as HW/BW in SH rats did not reach statistical significance. MIT from SH hearts and liver exhibited elevated QO_2 in S3 and S4, RCI and OPR values (mostly $p<0.05$) with both SUC and GLUT/MAL as substrates. An exception represented the decrease in S4 QO_2 (GLUT/MAL) in liver ($p>0.05$), but this remained without any effect on RCI and OPR values. Results point to an enhancement in OEP as a compensation for a HYP- induced increase in energy demands of tissues. Due to elimination of increase in SBP by captopril, the values of QO_2, RCI and OPR in heart and liver MIT remained below those in non-treated SH rats ($p<0.05 – 0.01$). HYP did not exerted any influence ($p>0.05$) on ADP:O ratio in heart and liver MIT but captopril decreased this parameter in liver of SH rats significantly ($p<0.05$). In contrast to heart and liver MIT, kidney MIT from SH rats exhibited considerable perturbations in parameters of QO_2 S3 and S4 which led to significant ($p<0.05$- 0.01) depression of RCI and OPR values estimated with SUC. With GLUT/MAL we also registered a decrease in these variables which, however, did not reach statistical significance ($p>0.05$). Captopril failed in preventing the decrease in the above parameters of OEP. Oppositely, it supported their further depression, particularly in QO_2 S3 and S4 with each substrate and in OPR with SUC ($p<0.05$). In kidney MIT HYP induced a depression of the ADP:O ratio with all substrates. Application of captopril failed to remove this disturbance in coupling the oxidation with phosphorylation ($p>0.05$). Liver and heart MIT from SH rats showed a moderate increase in MF. This trend was supported by captopril. Oppositely, kidney MIT of SH rats exhibited a moderate decreased MF which was, however, slightly corrected by captopril.

Support: VEGA 2/7126/27; 1/0755/09; 1/0620/10; 2/0173/08 and 1/0142/09.

PHARMACOKINETIC OF THE MORIN AFTER ORAL ADMINISTRATION

J. Nečas[1], L. Bartošíková[1], T. Bartošík[2], P. Fráňa[3]
[1]Department of Physiology, Faculty of Medicine and Dentistry, Palacky University Olomouc, [2]Department of Anaesthesiology and Intensive Care, [3]IInd Internal Clinic, St. Anne´s University Hospital Brno, Czech Republic

Morin belongs to the flavonol group. Basic skeleton is substituted by – OH groups in positions 3, 5, 7, 3', 5'. The aim of the study was to determine some pharmacokinetic parameters after single oral administration of tested substance. After sample preparation the HPLC system consisted of gradient HPLC pump Knauer 64 equipped with a LCD 2084 UV detector was used. The wavelength was set on 255 nm for morin determination. The mobile phase consisted of KH_2PO_4:acetonitrile, 65:35 (v:v). Isocratic flow was maintained at 1.0 ml/min. Data from analysis were collected and analyzed with the CSW software. The quantification of the morin was achieved from areas of its peaks by comparison with calibration curves obtained using standard solution of individual morin in blank serum. For determination of pharmacokinetic parameters program Kinetica 4.0 was used. The maximum plasma morin concentration was 229.0±2.9 μg/l; time to maximum plasma concentration was 40 min; $AUC_{0\rightarrow last}$ was 10914 μg/min/l; elimination half-life was 148±16 min; constant of elimination was 0.004737 min^{-1}; MRT was 190±20 min.

ULTRASTRUCTURAL AND FUNCTIONAL MYOCARDIAL CHANGES INDUCED BY 3WEEKS LASTING ADDITION OF LOW DOSES OF ISOPROTERENOL TO RATS

Z. Nichtová[1], E. Kráľová[2], M. Novotová[1], T. Stankovičová[2],
[1]Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, [2]Comenius University, Faculty of Pharmacy, Department of Pharmacology and Toxicology, Bratislava, Slovakia

Repeated activation of sympathoadrenal system induces the development of myocardial hypertrophy (1). Prolonged mechanical cardiac overwork exhausted compensatory mechanisms issued in the myocardial failure and increased incidence of death. Myocardial remodeling in rats and high mortality (80-90 %) were induced by repeated injections of isoproterenol (5 mg/kg s.c.) for 21 days (Iso5/21, n=5). ECG were recorded from awaked or anesthetized (thiopental 45 mg/kg) animals as well as from the isolated spontaneously beating perfused hearts according to the Langendorff. ECG from Iso5/21 awaked rats showed in R amplitude decrease in II. lead, but the increase in the I. lead, deepening of S wave for about 60 %, ascending depression of ST segment. The increased voltage criteria in anesthetized animals as well as the longer QT interval were found both in the animals and isolated hearts. Higher incidence of ectopic activity (VPB) was documented in Iso5/21 hearts (116±29 vs 68±5). Myocardial contractility decreased to 54 % of control values and left ventricular (LV) diastolic pressure increased (6.39±0.38 vs 4.89±0.59 mm Hg) compared to control hearts. Ultrastructural analysis of surviving cardiomyocytes in Iso5/21 showed marks of persisted neomyogenesis leading to hypertrophy accompanied with the increased number of newly formed sarcoplasmic processes. In growing areas disorganized masses of myofibrils were found as well as the absence of membrane systems (t-tubules and sarcoplasmic reticulum). It could be concluded that high degree of ultrastructural heterogeneity at the level of individual cells as well as at myocardial tissue could result into symptoms of failing myocardium.

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This work was supported in part by grants SR VEGA 1/4244/07, 2/0174/09, 1/0726/10.

CALCIUM BINDING PROTEINS IN SKELETAL AND HEART MUSCLES OF RATS WITH ALTERED THYROID STATUS AFTER HETEROCHRONOUS ISOTRANSPLANTATION

P. Novák[1], V. Marková[2], V. Sulimenko[2], T. Soukup[1]
[1]Institute of Physiology and [2]Institute of Molecular Genetics, Academy of Sciences, Prague, v. v. i., Czech Republic

We investigated effects of altered thyroid hormone levels on expression of skeletal and cardiac isoforms of calsequestrin (CSQ, 55 kDa), parvalbumin (PV, 12 kDa) and phospholamban (PhL, 5 kDa) in normal and regenerated fast and slow muscles and in the heart of euthyroid (EU), hypothyroid (HY) and hyperthyroid (TH) adult Lewis strain rats. These calcium binding proteins (CaBPs) were determined by SDS-PAGE followed by western blot analysis. Gene expression was assessed using reverse transcription and subsequent real time polymerase chain reaction. We found that the protein levels for CSQ1 were the highest in the fast extensor digitorum longus (EDL), medium in the soleus (SOL) and hardly detectable in the heart. The HY status decreased and the TH status increased expression of CSQ1 relatively to CBB or GAPDH in the EDL, but not in the SOL muscle. At mRNA level, the HY and TH statuses decreased CSQ1 expression in both muscles. The mRNA transcript levels for CSQ2 mRNA were the highest in the heart, medium in the SOL and the lowest in the EDL muscle. We found no obvious differences in CSQ2 between atria and ventricles. The HY status slightly increased CSQ2 mRNA expression in the EDL compared to the EU or TH statuses. The transplanted muscles exhibited protein and mRNA levels of CSQ1 corresponding to the host muscle (after being innervated by the collaterals of the host axons) and not to the source of the graft. PV was expressed only in the EDL and its expression appeared lower in the HY and TH than in the EU rats. We found higher levels of PhL in the SOL and ventricles of the HY compared to the EU or TH rats. While the levels of MyHC isoforms, both at the mRNA and protein levels, are always shifted by the HY status towards slower isoforms and by the TH status towards faster ones, the changes of the analyzed CaBPs were not always in agreement. It remains an open question whether thyroid hormones may alter the calcium homeostasis and thus the excitation-contraction coupling in accord with the MyHC isoforms and muscle fiber types (1,2).

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Supported by MYORES No. 511978, MSMT CR LC554 and GACR 304/08/0256 grants and by the Research project AV0Z 50110509.

MATERNAL SYNCHRONIZATION OF CIRCADIAN RHYTHMS OF THE LABOARTORY RAT DURING PRENATAL DEVELOPMENT

M. Nováková, A. Sumová
Department of Neurohumoral Regulations, Institute of Physiology v.v.i., Academy of Sciences of the Czech Republic, Prague, Czech Republic

Under constant conditions, many biological processes repeat regularly with a period about 24 hour, i.e., exhibit circadian rhythms. For example, these rhythms are in sleep/wakefulness, locomotor activity, hormonal secretion and body temperature. In mammals, circadian rhythms are controlled centrally from the hypothalamic suprachiasmatic nuclei (SCN). At the molecular level, the periodicity of these rhythms is due to the rhythmic expression of clock genes within individual neurons of the SCN. The expression of the clock genes is controlled by transcriptional-translational feedback loops. Circadian clock in the SCN develops gradually during prenatal and early postnatal period. In the rat, this period lasts from around 14th day of gestation till 10th day of the postnatal development. Circadian system of fetuses and pups is entrained mostly by non-photic maternal cues during prenatal and early postnatal development, respectively. Photic entrainment gradually prevails only after the first postnatal week. In this study, we focused on the mechanism of maternal entrainment during prenatal development. The aim of this study was to ascertain whether exposure of pregnant rats to restricted feeding (RF) regime during gestation is able to entrain circadian clock in the SCN of their fetuses. The pregnant rats were kept under LD12:12 or under constant light conditions (LL); the control groups were fed *ad libitum*, the experimental groups had restricted access to food for 6 h during their resting time. Daily profiles of *Avp* and *c-fos* gene expression were examined by *in-situ* hybridization in the SCN of 1-day-old pups. The profiles of *c-fos* and *Avp* expression exhibited significant circadian rhythm in pups born to mothers maintained under LD12:12 and fed *ad libitum* but not in those born to mothers maintained in LL and fed *ad libitum*. Exposure of pregnant rats kept under LD12:12 to RF did not notably affect the daily rhythms in *c-fos* and *Avp* expression in SCN of their 1-day-old pups. However, exposure of pregnant rats kept under LL to RF partly restored circadian rhytmicity of *c-fos* and *Avp* expression in SCN of their 1-day-old pups. The data suggest that under conditions of disrupted maternal circadian system, food intake may play important role in synchronization of fetuses during prenatal ontogenesis.

The work was supported by Grants No. 309080503, 30908H079, LC554, by the 6th Framework Project EUCLOCK No. 018741 and by Research Project No. AV0Z50110509.

THE EFFECT OF MELATONIN ON BLOOD PRESSURE AND NITRIC OXIDE SYNTHASE ACTIVITY IN RATS WITH METABOLIC SYNDROME

J. Parohová, S. Vranková, A. Barta, O. Pechánová
Institute of Normal and Pathological Physiology and Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovak Republic

Melatonin, a multitasking indolamine, seems to be involved in a variety of physiologic and metabolic processes via both receptor-mediated and receptor-independent mechanisms. The aim of this study was to find out whether melatonin can affect blood pressure and nitric oxide synthase (NOS) activity in rats with metabolic syndrome (MS) - obese spontaneously hypertensive rats [SHR/ND mcr-cp (cp/cp)]. Rats were divided into two groups: male 6-week-old rats with MS treated with melatonin (10 mg/kg/day) for next 3 weeks and age-matched controls with MS. Blood pressure was measured by tail-cuff plethysmography every week. NOS activity was determined by measuring the formation of L-[^{3}H] citrulline from L-[^{3}H] arginine in the aorta, heart, kidney, cerebellum, brain cortex and brain stem. Blood pressure was decreased by 10 % already after the second week of melatonin treatment and this decrease persisted till the end of the treatment in comparison to age-matched untreated rats. NOS activity in the aorta, heart as well as in the kidney of rats with MS was not affected by chronic melatonin treatment. However, melatonin treatment increased NOS activity of rats with metabolic syndrome in all brain tissues investigated. As no changes in NOS activity in peripheral organs were determined, we can conclude that nitric oxide produced in brain may be responsible for blood pressure decrease after 3 weeks of melatonin treatment. These data suggested that melatonin might play an important role in the decrease of blood pressure via increasing brain NOS activity in rats with metabolic syndrome.

The study was supported by VEGA 2/0178/09 and APVV-0538-07.

MAGNESIUM PREVENTS KAINATE-INDUCED EXCITOTOXIC DAMAGE IN CA3a HIPPOCAMPAL PYRAMIDAL NEURONS

A. Pavlik, S. Mohammadi, G. Alexander
Department of Physiology, Faculty of Medicine, Kuwait University, Kuwait

Intracellular calcium elevation seems to be the main cause of excitotoxic neuronal damage in stroke, injury and seizures. However, in neuronal hippocampal culture blocking calcium entry with calcium entry antagonists upon excitotoxic injury failed to prevent extended neuronal depolarization as well as neuronal death (1). Magnesium (which is natural blocker of NMDA channel participating in the induction of excitotoxicity) was recently used for the prevention of neuronal damage (ND) both in experimental and clinical studies. Kainic acid (KA) is inducing well characterized ND in hippocampal CA3 region, which is also preceded by extended neuronal depolarization (2). In this study, we elucidated protective effects of magnesium in a model of KA–induced hippocampal ND (3). Young female rats were anesthetized with urethane (1.25 g/kg) throughout the whole

experiment. KA (0.15 μg) and $MgCl_2$ (50-250 μg) were injected together intraventricularly while antagonists of NMDA-subtype (MK-801: 2.5 mg/kg) and AMPA/KA subtype (CNQX: 15 μg) of glutamate receptors were injected 20 min before KA. Vibratome sections (40 μm) were processed for Hsp70, c-Fos, and OX-42 immunohistochemistry or stained by thionin. Quantitative image analysis with Anova statistics for c-Fos expression and volume of neuronal damage (ND) was used. KA injection induced fast microglial activation in the lateral half of dorsal hippocampus ipsilaterally. ND was restricted to CA3a sub region where the damaged pyramidal neurons attenuated or even lost c-Fos protein expression. Interestingly, there was no induction of HSP 70 in the damaged CA3a pyramidal neurons. Neither MK-801 nor CNQX protected significantly against ND at 4 hrs after KA. However, $MgCl_2$ (200-250 μg) completely rescued CA3a neurons morphology and also maintained their c-Fos expression. Reducing $MgCl_2$ dose attenuated neuroprotection, which was lost at doses between 100 and 50 μg. NaCl and $ZnCl_2$ solutions (in the same tonicity range as those of $MgCl_2$) were not protective. Blockade of NMDA receptors with MK-801 did not alleviate $MgCl_2$ protection. We suggest that magnesium may rescue CA3a neurons by interfering with calcium entry through depolarization-induced non-NMDA channel(s).

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THE CATECHOLAMINE LEVELS IN THE BLOOD PLASMA OF SHEEP AFTER HORMONAL STIMULATION

B. Pástorová, D. Sopková, S. Ondrašovičová, A. Staníková
Institute of Physiology, University of Veterinary Medicine, Košice, Slovak Republic

The effect of hormonal stimulation with PMSG (1500 IU) on the levels of plasma catecholamine (dopamine (DA), norepinephrine (NE) and epinephrine (EPI) was studied by radioenzymatic methods (1) during synchronized estrous (10 days) of the sheep. Catecholamine were determined in the blood plasma before and 1,12,13 days of experiments. It follows from the results that the levels of plasma dopamine increased significantly ($p<0,001$) 13 day of experiment (the time of ovulation). Further more that the levels of dopamine (DA) during the 13 days observed, compared with those of controls before hormonal stimulation, remained a higher level. A statistically significant increase in plasma norepinephrine and dopamine was recorded 24 hours after administration of 1500 IU PMSG. During the other time intervals observed its levels did not differ from the "sham" control values. Plasma epinephrine (EPI) showed a significant increase in day after synchronization. The effect of PMSG was pronouceed especially in the period ovulation. We suggest that our findings could contribute to a better understanding of the effect of superovulatory preparations on the peripheral adrenergic system affecting the levels of plasma catecholamines (2).

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ADIPONECTIN AND LEPTIN IN THE DEVELOPMENT OF THE METABOLIC SYNDROME

D. Petrášová, A. Galovičová[1], J. Koprovičová
Institute of Experimental Medicine and [1]1st Department of Internal Medicine, Faculty of Medicine, Safarikiensis University, Košice, Slovakia

Leptin and adiponectin dispose of a lot of physiological and pathological functions, whereas one of them plays an important role in the development of the metabolic syndrome (MS) and complications. The basic parameters of the lipid and glucose metabolism were examined using the biochemical methods, and concentrations of adiponectin and leptin were determined by the ELISA method. For statistical processing basic descriptive statistics and commercially available computer statistic programs were used. The hypothesis of equality of variances was tested by One-Way Analysis of Variance ANOVA. Fifty persons divided into two groups were involved into the pilot study. In the first group there were 29 patients with metabolic syndrome (21/8, M/F), and in the control group were 21 patients (13/8, M/F) – healthy with overweight. Selected anthropometric parameters – glucose metabolism, concentration of adiponectin and leptin were compared. The patients with MS had a lower concentrations of adiponectin (8.91±0.64 ng/ml) and higher of leptin (15.67±1.88 ng/ml) than those with overweight. However, the results did not reach the statistical significance. The value of the ratio of adiponectin/leptin was significantly decreased in the group with metabolic syndrome ($p<0.05$). This ratio simultaneously showed a significant negative correlation with BMI, waist girth, and concentration of insulin and index of HOMA-IR. A decreased value of adiponectin/leptin can point out the risk individuals, who suffer from the metabolic syndrome, or diabetes mellitus of type 2. The whole series of molecules participate in the development of the metabolic syndrome, diabetes mellitus of type 2, atherosclerosis and their complications. Out of them adipocytokines take a significant place, above all, adiponectin and leptin, which have the pleiotropic effects. Connection of these adipocytokines into the pathophysiological process of the atherosclerosis development can be a new parameter of the risk of its origin. Results of our pilot study stimulate further research of greater set of patients.

ENTRAINMENT OF CIRCADIAN CLOCKS WITHIN THE RAT LIVER AND GUT DURING ONTOGENESIS

L. Polidarová[1], M. Nováková[1], M. Soták[2], J. Pácha[2], A. Sumová[1]
Departments of [1]Neurohumoral Regulations and [2]Epithelial Physiology, Institute of Physiology v.v.i., Academy of Sciences of the Czech Republic, Prague, Czech Republic

The aim of the present study was to detect daily profiles of clock gene expression in the rat gut during ontogenesis. The next aim was to ascertain whether exposure of rats to restricted feeding (RF) regime during pregnancy may synchronize circadian clocks within the liver and gut of their 1-day-old pups. In the first part of the experiment, pregnant rats and newborn pups with their mothers were maintained under regime with 12 h of light and 12h of darkness (LD12:12) with free access to food and water. At embryonic day 20 and postnatal days 2, 10, 20, 30, the fetuses or pups were sampled during the 24-h cycle. In the second part of the experiment, the pregnant rats were kept under LD 12:12 or under constant light conditions (LL); the control groups were fed ad libitum and the experimental groups had restricted access to food for only 6 h during the resting time (RF). Daily expression profiles of clock genes Per1, Per2, Bmal1 and Rev-erb-α were examined by real-time RT-PCR. In the first part of the study it was found that circadian rhythmicity of clock genes in the gut was detected only since P20. At P30, all studied clock genes were expressed rhythmically with amplitude and phase identical to the adults. The second part of the study revealed that in 1-day-old pups born to rats maintained under LD12:12, the clock gene expression profiles in the liver and gut did not exhibit circadian rhythmicity under ad libitum feeding regime, but a significant rhythm in Rev-erb-α gene expression was detected in both tissues under the RF regime. In 1-day-old pups born to rats maintained under LL and fed ad libitum, none of the clock gene exhibited circadian rhythm in their expression. Exposure of pregnant rats maintained under LL to RF regime affected the daily gene expression profiles differently in the pup's liver and gut: In the liver, only expression of Rev-erb-α exhibited circadian rhythm, while in the gut all studied clock genes were expressed rhythmically. Our data demonstrate that in the rat gut, rhythmic expression of clock genes develops gradually during ontogenesis. Under conditions of synchronized maternal circadian system, RF regime affects expression of Rev-erb-α gene in the pups liver and gut. Under conditions of disturbed maternal circadian system via exposure to LL, the RF regime restores rhythms in expression of all clock genes studied. The data suggest that under conditions of disrupted maternal circadian system, food intake may play important role in synchronization of gastrointestinal rhythmicity during prenatal ontogenesis.

Supported by grants 309080503, 305090321, 30508H037, Research Projects AV0Z 50110509, LC554 and by the 6th Framework Project EUCLOCK 018741.

SPONTANEOUSLY DIABETIC RATS BENEFIT FROM OMEGA-3 FATTY ACIDS SUPPLEMENTATION DUE TO SUPPRESSION OF CARDIOVASCULAR DISEASES RISK FACTORS AND UP-REGULATION OF MYOCARDIAL CONNEXIN-43

J. Radosinska[1,2], B. Bacova[1], V. Dosenko[3], M. Barancik[1], H. Lin[5], I. Imanaga[5], J. Navarova[4], N. Tribulova[1]
[1]Institute for Heart Research, SAS and [2]Faculty of Medicine, Comenius University, Bratislava, SK, [3]Bogomoletz Institute of Physiology, Kyiv, Ukraine, [4]Institute of Experimental Pharmacology and Toxicology, SAS, Bratislava, Slovak Republic, [5]Fukuoka University School of Medicine, Fukuoka, Japan

Background: Contractile dysfunction and heart rhythm disturbances are frequent complications of diabetes mellitus in human whereby our previous studies using insulin-deficient (STZ) diabetic rats suggest that abnormal cell-to-cell communication due to hyperphosphorylation of connexin-43 (Cx43) channels can be involved. Goal of this study was to investigate whether myocardial Cx43 mRNA and protein expression are altered in insulin-resistant diabetic rats and whether they may benefit from omega-3 fatty acids (omega-3 FA) supplementation. Design and Methods: Experiments were conducted on male adult spontaneously diabetic Goto-Kakizaki rats and age-matched healthy Wistar-Clea rats. Animals were divided into un-treated and treated for 2mth with omega-3 FA (Vesteralens, Norway, 200mg/kg/day). At the end of experiments biometrical and biochemical parameters of serum were registered. Left ventricular heart tissues were used for determination of Cx43 mRNA and protein expression using qRT PCR and Western blotting. Isoform-specific protein kinase C (PKC) phosphorylation of Cx43 was analysed by immunoblotting. Key results: Blood glucose, cholesterol and triglycerides were increased in diabetic rats while significantly reduced upon treatment with omega-3 FA. Body and heart weights were lower in diabetic compared to healthy rats and these parameters were not affected by omega-3 FA. Myocardial Cx43 mRNA level was higher in diabetic than non-diabetic rats and omega-3 FA caused its marked increase in both groups. Ratio of phosphorylated to non-phosphorylated Cx43 protein was lower in diabetic versus healthy rats. In contrast, a significant elevation of phosphorylated forms of Cx43 was detected upon omega-3 FA in diabetic and to lesser extent in healthy rat heart ventricles. It was associated with increased expression of PKC-epsilon. Conclusions: Rats with type-2 diabetes benefit from omega-3 FA supplementation because of suppression CVD risk factors and particularly due to up-regulation of Cx43. It may affect intercellular communication and consequently heart function and its susceptibility to malignant arrhythmias. Findings support the role of non-pharmacological approaches in primary and secondary CVD prevention.

EVENT-RELATED POTENTIALS RECORDED IN A SIMPLE SENSORIMOTOR TASK IN EPILEPTIC PATIENTS (STEREO-ELECTROENCEPHALOGRAPHIC STUDY)

R. Roman[1], J. Chládek[3], M. Brázdil[2], I. Rektor[2], P. Jurák[3], M. Kukleta[1]
[1]Department of Physiology, Medical Faculty, Masaryk University, Brno, [2]Department of Neurology, St. Anne's Hospital, Masaryk University, Brno, [3]Institute of Scientific Instruments, Academy of Sciences, Brno, Czech Republic.

Scalp-recorded ERPs in a simple sensorimotor task show that stimulus processing for a consecutive executive task is performed by different evaluating networks (1). The present paper attempted to demonstrate the existence of various evaluating systems by means of intracerebral electrodes implanted in different brain regions. In four right-handed patients with medically intractable epilepsies, depth electrodes were implanted to localize the seizure origin prior to surgical treatment. A total of 300 sites of the frontal, parietal and temporal lobes were investigated. Each subject was instructed to respond to the 1 kHz tone by pressing a microswitch button in the dominant hand. It was not necessary to respond as quickly as possible. ERPs components longer than 250 ms were analyzed both in stimulus-locked and response-locked averages, which were separately averaged for fast and slow responses. Local generation of ERPs was demonstrated in 33 sites. Three different types of time relationship of ERPs components were found: 1) time-locked to the stimulus - in 10 cases (middle and inferior temporal gyri, hippocampus, middle frontal gyrus and frontal operculum), 2) time-locked to the motor response - in 8 cases (precentral gyrus, inferior parietal lobulus, medial frontal gyrus and superior temporal gyrus), and 3) with ambiguous time relationship to the stimulus and the motor response - in 15 cases (hippocampus, superior, middle and inferior temporal gyri, middle frontal gyrus, amygdala, cingulate and postcentral gyri). It appears that more partial intracerebral processes have to be involved to cope with simple sensorimotor tasks. Three types of cognitive components could be interpreted as a manifestation of various types of stimulus evaluation networks.
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Supported by the grant MSM0021622404.

ONTOGENETIC PROFILE OF 11β-HSD1 AND H6PDH IN LYMPHOID TISSUES

L. Řeháková, P. Ergang, J. Pácha
Institute of Physiology v.v.i., Academy of Sciences of the Czech Republic, Prague, Czech Republic

It is known that glucocorticoids modulate immunological processes by influence of development and effector functions of immune system. The effect of glucocorticoids depends on the concentration of circulating glucocorticoids, the number of their receptors and the local metabolism of glucocorticoids predominated by 11β-hydroxysteroid dehydrogenases type 1 and 2 (11β-HSD1, 11β-HSD2). 11β-HSD1 reduces predominantly 11-keto forms (cortisone, 11-dehydrocorticosterone) to active glucocorticoids (cortisol, corticosterone) whereas 11β-HSD2 operates strictly as an oxidase that converts biologically active glucocorticoids to their inactive 11-keto forms. It is suggested that 11β-HSD1 uses NADPH which is generated by hexose-6-phosphate dehydrogenase (H6PDH) in the lumen of endoplasmic reticulum. Evidence suggests that glucocorticoids play essential roles during development and differentiation of some kinds of immune cells, so we aimed our study to determine whether the developmental pattern of immune organs correlates with changes of local glucocorticoid metabolism. Therefore, we studied the expression of 11β-HSD1 and H6PDH in thymus, spleen, lymph nodes and in liver of Wistar male rats during suckling, weaning, prepubertal and adult life period. The abundance of mRNAs was measured by qRT-PCR using TaqMan probes. PCR reaction was performed as a duplex measurement of the gene of interest and the housekeeping gene (GAPDH). We demonstrated that in the early postnatal life the expression of 11β-HSD1 increases in thymus, spleen and liver but decreases in mesenteric lymph nodes. The expression of H6PDH correlated with the expression of 11β-HSD1 in spleen and mesenteric lymph nodes (R=0.69 and R=0.45 respectively).

The project was supported by Czech Science Foundation GACR 305/07/0328.

PRENATAL EXPOSURE TO METHAMPHETAMINE INCREASES SENSITIVITY TO ACUTE AND CHRONIC APPLICATION OF THE SAME DRUG IN ADULTHOOD

B. Schutová, L. Hrubá, R. Rokyta, R. Šlamberová
Charles University in Prague, Third Faculty of Medicine, Department of Normal, Pathological and Clinical Physiology, Prague, Czech Republic

Methamphetamine (MA) is a psychostimulant drug with high potential for abuse. While acute administration of psychostimulants increases psychomotor activity, intermittent exposure to these drugs was shown to induce behavioral sensitization in adult animals. Since half of MA users are women of reproductive age, there is a risk of prenatal MA exposure of their children. Therefore, the aim of the present study was to investigate whether prenatal exposure to MA is able to affect behavior and reactivity to MA challenge in adulthood. Behavior of adult male rats prenatally exposed to MA (5 mg/kg) or saline was tested in a Laboras apparatus (Metris B.V., Netherlands). For 5 consecutive days, each rat was placed into the apparatus for 1h period during the dark phase of the light/dark cycle. Subucutaneously administered MA (1 mg/kg) or saline were used as challenge immediately prior to testing.

Locomotion, exploratory behavior, immobility, distance traveled and speed were monitored and automatically evaluated. Our results showed that prenatal MA exposure did not alter any of the measured behavioral parameters. In contrast, acute MA dose increased locomotion, rearing, distance traveled and speed, but decreased immobility. This stimulatory effect of acute MA in adulthood was higher in rats prenatally exposed to MA than in rats prenatally exposed to saline. Chronic MA application further enhanced psychomotor activation and thus, elicited behavioral sensitization. This behavioral sensitization was also greater in rats prenatally exposed to MA when compared to prenatally saline-exposed rats. In conclusion, the present study demonstrates that although prenatal exposure to MA does not influence the baseline behavior, it increases the sensitivity to acute and chronic MA challenge in adulthood, and thus is able to elicit long-lasting alterations in brain development.

Supported by: GACR 305/09/0126, IGA NS10509-3/2009 and MSM 0021620816.

STRESS MARKERS LEVEL IN ANIMAL CATHETERISATION EXPERIMENTS

H. Skarlandtová[1], E. Medová[1], H. Kazihnítková[3], L. Zamrazilová[3], M. Mlček[1], V. Hrachovina[1], P. Neužil[2], M. Bičíková[3], O. Kittnar[1]
[1]Institute of Physiology, First Faculty of Medicine, Charles University in Prague, Czech Republic, [2]Cardiology Department, Na Homolce Hospital, Prague, Czech Republic, [3]Institute of Endocrinology, Prague, Czech Republic

Measurement of stress markers is very interesting in many fields of biological studies. In medicine (human and veterinary) the knowledge of stress hormones levels is useful for detection of stress situations and for improving some conditions, e.g. operation protocol. In our catheterisation experiments, we are using nine pigs (*Sus scrofa domestica*). Four markers were measured in blood during catheterisation: cortisol, cortisone, DHEA and DHEA-S. Cortisol is stress hormone majority in mammals, cortisone is it's inactive form, DHEA and sulfate DHEA-S work on as counterbalance of stress hormones. We collected blood in four different stages of operation. The first was collected in farm, in non-stress domestic conditions, second after intubation and induction of anesthesia, third after heart stimulation and the last after operation, before animal has been sacrificed. For statistical analysis we used non-parametric Friedman test. Tests were performed for changing levels during operation, for three groups of pigs. For group 1) catheterisated in morning hours, group 2) catheterisated in afternoon and group 3) both groups together. There are no statistical significant changes for cortisol and DHEA-S in any of groups, but there are statistical significant results for cortisone ($p<0.05$ in group 1) and 2), $p<0.01$ in group 3) and DHEA ($p<0.05$ in group 1); $p<0.01$ in group 2) and 3). These results shows pigs are not stressed during catheterisation, but rising inactive form cortisone and "anti-stress" DHEA could mean, that pigs have elevated defence in face of stress during our experiment.

Supported by grant: 259233 118209/2009 GAUK.

BOVINE LYMPHOCYTE APOPTOSIS DURING EXPERIMENTAL INFLAMMATORY RESPONSE INDUCED BY MURAMYL DIPEPTIDE

P. Slama
Department of Animal Morphology, Physiology and Genetics, Mendel University in Brno, Brno, Czech Republic

The aim of this study was to determine whether apoptosis of bovine mammary gland lymphocytes is modulated during an inflammatory response of bovine mammary gland induced by muramyl dipeptide (MDP) as the minimal structural unit of peptidoglycans in Gram-positive bacteria. Samples of cell populations were obtained by lavage of the mammary gland at 4 intervals (24, 48, 72 and 168 h) following stimulation with MDP. Intramammary instillation of MDP resulted in a significant increase in portion of apoptotic lymphocytes in comparison to the control. The percentage of apoptotic lymphocytes peaked at 48 h following stimulation. In previous studies, there was demonstrated that apoptosis of bovine mammary gland lymphocytes is delayed during an experimentally induced infection of bovine mammary gland with *Staphylococcus aureus* and *Streptococcus uberis* (1) and during an *in vitro* cultivation with MDP (2). Contrary to that, results of this study suggest that MDP intramammary instillation induces apoptosis of lymphocytes.

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AMP-ACTIVATED PROTEIN KINASE α2-SUBUNIT DEFICIENCY DOES NOT INFLUENCE CARDIOPROTECTIVE EFFECT OF CHRONIC HYPOXIA

K. Slámová, J. Neckář, J. Kopecký, F. Kolář
Institute of Physiology, Academy of Sciences of the Czech Republic and Centre for Cardiovascular Research, Prague, Czech Republic

AMP-activated protein kinase (AMPK), an important sensor and regulator of cellular energy status, is activated in response to ischemic stress and is implicated in ischemic preconditioning (1). The aim of our study was to analyze the role of AMPK in increased ischemic tolerance of chronically hypoxic mouse hearts. To find out whether α2-subunit of AMPK is involved in this form of protection, we compared infarct size in transgenic AMPK $\alpha2^{-/-}$ and wild-type (WT) mice adapted to either continuous normobaric hypoxia (CNH; FIO_2 = 0.1; 3 weeks) or intermittent hypobaric hypoxia (IHH; PO_2 = 8.6 kPa; 8h/day, 5 weeks). Isolated Langendorff-perfused hearts were subjected to 45-min global no-flow ischemia and 60-min reperfusion. Infarct size, determined by TTC staining and normalized to the size of the left ventricle, was similar in both normoxic strains (WT 39.6±1.2 %; AMPK $\alpha2^{-/-}$ 42.5±1.3 %). Adaptation to CNH and IHH decreased infarct size in both WT (34.6±1.5 % and 31.4±1.1 %, respectively) and transgenic animals (37.9±2.5 % and 31.2±1.3 %, respectively). The protective effect of IHH was stronger than that of CNH, but no significant difference was observed between the strains. The results suggest that the α2-subunit of AMPK does not play a role in the infarct size-limiting mechanism of chronic hypoxia.

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Supported by grants GA CR 305/07/0875 and IAAX01110901.

IMPROVEMENT OF HEALTH IN CZECH POPULATION BY LIFESTYLE

J. Slavíček, O. Kittnar, G. E. Fraser[1], E. Filatova, H. Skarlandtová, R. Žižka[2], J. Konečná[2], A. Dohnalová, Z. M. Trefný
Institute of Physiology, First Medical Faculty, Charles University, Prague, Czech Republic, [1]Center for Health Research, School of Public Health, Loma Linda University, California, U.S.A., [2]Country Life International, Nenačovice, Czech Republic

The lifestyle is capable to improve the morbidity and mortality of cardiovascular diseases without pharmacotherapy to 50 per cent (1). In the present work we measured the parameters of some risk factors before and after a one week the Loma Linda University NEW START rehabilitative retreat (2). 2088 volunteers, 468 men and 1586 women, mean age 52.9±14.7 (S.D.) years, participated in 50 rehabilitative retreats from 1999-2009 in the Czech Republic, using a low-fat, low-energy, lacto-ovo vegetarian diet and exercise, in a stress-free environment. Body weight, height, BMI, blood pressure, heart rate, serum cholesterol and blood glucose were measured. Body weight decreased in 1921 measured persons from 72.0±14.77 (S.D.) to 71.2±14.44 kg ($p<0.0001$), BMI (1855 measured persons) from 25.3±4.75 to 25.1±4.61 kg/m^2 ($p<0.0001$), systolic blood pressure (1906 persons) from 130.2±22.64 to 125.0±21.79 mm Hg ($p<0.0001$), diastolic blood pressure (1898 persons) from 79.8±12.03 to 78.2±11.00 mm Hg ($p<0.0001$), heart rate (1784 persons) from 72.5±11.81 to 71.2±11.61. min^{-1} ($p<0.0001$), serum cholesterol (1648 persons) from 4.87±0.91 to 4.38±0.74 mM ($p<0.0001$), blood glucose (663 persons) from 4.28±1.58 to 3.81±1.28 mM ($p<0.0001$). Similar results were obtained in the measurement of the same parameters in men and women separately. In the sample of 233 volunteers participating in 6 rehabilitative retreats in 2009 (49 men, 183 women, mean age 56.8±15.3-SD-years) the same parameters were decreased during one

week of the stay ($p<0.001$). Our results repeatedly confirmed that the intake of a low-fat, low-energy diet, over the course of one week in a stress-free environment, decreased the risk factors of cardiovascular diseases in the sample of Czech population.

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Supported by grant No. 259223-118209 GAUK.

INFLUENCE OF BIOCATALYSIS ON VALUES OF AMINO ACIDS IN MUSCLES OF POULTRY EXPOSED TO STRESS FACTORS

D. Sopková, R. Vlčková, S. Ondrašovičová
Institute of Physiology, University of Veterinary Medicine, Košice, Slovak Republic

Dominance of catabolism in animals suffering from shock is known for a long time. When in shock, up to 20 to 30 g of nitrogen can be wasted within 24 h which corresponds to loss of 180 g of proteins and 600 to 100 g tissue decrease. An increase in some aminoacids and decrease in the level of albumins was observed in individuals suffering from shock. Catabolism resulting from shock supports fasting. Biocatalysts can contribute to overcoming the somatic and psychic overloading of animals in intensive farming. The amino acids content was determined in the breast muscle of layers which were kept under experimentally simulated stress conditions and consumed feed mixtures fortified with vitamins C, AD_3 and E using conventional column chromatography method with ion exchangers. The results indicated that the poultry supplied with vitamins at the appropriate level were able to cope more successfully with the stress. The values of amino acids in poultry fed with fortified feed increased in comparison with the control. A significant increase ($p< 0.001$) was recorded for proline, arginine and glycine. Poultry fed with common feed mixtures showed decrease in values of amino acids ($p<0.05$) threonine, glycine, alanine, isoleucine, leucine, lysine and arginine after the exposure to stress factors. The stress reaction encompasses increased secretion of STH (somatotropic hormone) induced by dopaminergic stimulation (1,2). The biological significance of elimination of STH during the stress consists in proteoanabolism compensating the action of glucocorticoids (3). STH increases proteosynthesis by means of which the positive nitrogen balance in an organism could be documented. A decrease in the amino acid serum level occurs in parallel. Further, STH ensures the transport of amino acids into muscles and supports the production of proteins in the muscles through stimulation of RNA and DNA synthesis (4) which can explain on increase in the level of some amino acids in the stressed poultry which consumed feed supplemented with vitamins.

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CIRCADIAN EXPRESSION OF TRANSPORTERS AND CHANNELS ENGAGED IN RAT INTESTINAL SODIUM CHLORIDE ABSORPTION

M. Soták[1], L. Polidarová[2], A. Sumová[2], J. Pácha[1]
[1]*Department of Epithelial Physiology,* [2]*Department of Neurohumoral Regulations, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic*

Introduction: Organisms are living in an environment with rhythmic changes and possess molecular clocks in central nervous system as well as in peripheral tissues, including liver and segments of alimentary tract. Central clocks are dominantly entrained by day and night light changes and synchronize peripheral clocks, which can be uncoupled and differentially regulated by changes in feeding regime. Many other physiological processes in gastrointestinal tract exhibit circadian rhythms such as activities of hydrolyses, glucose and peptide transport. Some observations suggest circadian rhythms in sodium transport. There was demonstrated diurnal rhythmicity in expression of renal and colonic Na(+)/H(+) antiporter *Nhe3* and direct role of *Per1* clock gene on aldosterone-dependent stimulation of renal Na(+) channel *ENaC*. *Aim:* Therefore the goal of the study was to elucidate possible circadian regulation of rat intestinal sodium chloride absorption by determining circadian expression of genes involved in sodium absorption. There was studied particular expression of the following genes in colon and duodenum, *Dra*, *Ae1*, *Nhe3* and *ENaC* γ subunit. *Methods:* Adult male Wistar rats were kept in light-dark regime LD 12:12. Sample collection was provided every 4 hours among the 24-hour cycle, when mucosal layers of particular gut segments were scrapped and RNA was examined by quantitative real-time RT-PCR. Results were expressed in arbitrary units as the ratio of the target gene to the level of verified housekeeping gene. *Results:* We demonstrated functional peripheral clocks by determining rhythmic expression of clock genes mRNA in colon and duodenum samples. Further we showed rhythmic expression of *Dra*, *Ae1*, *Nhe3* and *ENaC* γ subunit mRNA in colon and *Nhe3* in duodenum. *Conclusion:* We determined diurnal variations in genes involved in intestinal sodium chloride absorption. These data suggest that intestinal sodium absorption can be regulated in circadian manner. Further analysis will be needed to dissect the detailed role of intestinal clocks in regulation of intestinal ion transport.

Supported by grant from the Ministry of Health no. NS 9982-4 and by grant from the Academy of Sciences no. A 500110605.

ENHANCED TOLERANCE TO ISCHEMIA IN THE DIABETIC HEART IS NOT SUPPRESSED BY β1-ADRENERGIC INHIBITION WITH METOPROLOL

D. Stakheev[1], J. Matejíková, D. Pancza, V. Ledvényiová, F. Kolář[1], T. Ravingerová
Institute for Heart Research, Centre of Excellence for Cardiovascular Research of the Slovak Academy of Sciences, Bratislava, Slovak Republic; [1]*Institute of Physiology, Academy of Sciences of the Czech Republic, Centre for Cardiovascular Research, Prague, Czech Republic*

Despite higher vulnerability to ischemia/reperfusion injury (I/R) in diabetics, experimental studies revealed paradoxically enhanced ischemic tolerance in the diabetic myocardium (1,2) that is believed to share some molecular pathways with other forms of endogenous cardioprotection, such as ischemic preconditioning (IPC) and/or adaptation to chronic intermittent hypoxia (CIH; 1,3). In the non-diabetic (ND) heart, protective mechanisms in some models of IPC and adaptation to CIH have been found to involve β1-adrenergic receptor (β1-AR) activation (4,5). The present study was designed to invesitigate the role of β1-AR in the mechanisms of ischemic tolerance in the diabetic heart using a selective β1-AR blocker metoprolol (M, 50 mg/kg/day) given for 10 days prior to acute I/R challenge. 24 h after M withdrawal, Langendorff-perfused hearts of M-treated and untreated diabetic rats (STZ 65 mg/kg, i.p., 1 week, blood glucose >20 mmol/l), as well as of age-matched ND control animals were subjected to 30-min occlusion of the LAD coronary artery for the measurement of ischemia-induced ventricular arrhythmias and 2-h reperfusion for the determination of the infarct size (IS). Results: Lower susceptibility to I/R in the diabetic hearts was documented by a 65 % reduction of the IS (expressed in % of area at risk) from 48±4 % in ND controls to 17±2 % associated with a decreased total number of premature ventricular complexes (PVC) from 402±86 in controls to 198±38 ($P<0.05$) and shorter duration of ventricular tachycardia. In the ND hearts, chronic treatment with M modified neither the IS (43±2 % ; $P>0.05$) nor arrhythmogenesis. In the diabetic hearts, M failed to reverse both, antiinfarct and antiarrhythmic protection (IS 20±3 %; PVC 167±49; both $P>0.05$ vs. respective controls). Conclusions: The results suggest that the activity of β1-AR is not involved in the induction of the improved cardiac ischemic tolerance during the acute phase of STZ-induced diabetes mellitus in rats.

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Supported by grants VEGA SR 2/0173/08, APVV-SK-CZ-0049-07, GACR 305/07/0875, and IAAX01110901.

ETIOPATHOGENETIC FACTORS IN THE DEVELOPMENT OF NEUROGENIC PULMONARY EDEMA

J. Šedý[1,2,3], J. Kuneš[3,4], A. Hejčl[1,2], J. Zicha[3,4]
[1]Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic, [2]Center for Cell Therapy and Tissue Repair and Department of Neuroscience, Second Faculty of Medicine, Charles University, Prague, Czech Republic, [3]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic, [4]Center for Cardiovascular Research, Prague, Czech Republic

Neurogenic pulmonary edema (NPE) is an acute life-threatening complication following the injury of spinal cord or brain. The role of nitric oxide in the development of NPE in rats after balloon compression of spinal cord has not been examined yet. We therefore pretreated Wistar rats with NO synthase inhibitor N^G-nitro-L-arginine (L-NAME) either acutely (just before the injury) or chronically (for four weeks prior to the injury). Acute L-NAME administration enhanced NPE severity, leading to the death of 83 % of animals within 10 min after injury. Chronic L-NAME treatment also caused the death of 50 % animals due to severe NPE. Pretreatment with either ganglionic blocker pentolinium (to lower blood pressure rise) or muscarinic receptor blocker atropine (to diminish heart rate reduction) prevented or attenuated NPE development in these rats. We did not observe any therapeutic effects of atropine administered two minutes after spinal cord compression. Our data indicate that NPE development is dependent upon a marked decrease of heart rate under the conditions of high blood pressure elicited by the activation of sympathetic nervous system. These hemodynamic alterations are especially pronounced in rats subjected to acute NO synthase inhibition. In conclusion, nitric oxide has partial protective effect on NPE development because it attenuates sympathetic vasoconstriction and consequent baroreflex-induced bradycardia following spinal cord injury.

CHRONOBIOLOGICAL COMPARISON OF EFFECT OF TWO ANAESTHETIC AGENTS ON ACID-BASE BALANCE AND HEART RATE IN EXPERIMENTAL RAT MODEL

P. Švorc, I. Bačová, P. Švorc, Jr.
Department of Physiology, Medical Faculty UPJS, Kosice, Slovak Republic

Stability of the internal environment plays key role in maintenance of correct functions in organism. Problem is that parameters of the acid-base balance are comparised with the averaged reference values, where we do not often know the conditions of blood sampling (in wakeful state or in anaesthesia), often irrespective of the circadian dependence and in not least cause also time of the goining in vivo experiments or adaptation of animals on the lighted regime. The aim of our study was refered to some parameters of the acid-bace balance (blood gases, pH, O_2 saturation) and heart rate in ketamine/xylazine (100 mg/kg/ 15 mg/kg, i.m.) and in pentobarbital anaesthetized rats (40 mg/kg, i.p.) at the spontaneous pulmonary ventilation in the light-dark dependence. In the first phase of the experiments, the animals were adapted to the lighted regime 12:12 hours, with the dark part from 18.00 to 06.00 hour. In the second phase of experiments, parameters were analyzed after the inverse setting, with the dark part from 06.00 to 18.00 hour. Followed parameters of the acid-base balance and heart rate were analyzed after cca 20 min. from the anaesthetic agent administration. Parameters of the acid-bace balance did not show any significant light-dark differences at both types of anaesthesia, except the heart rate in ketamine/xylazine anaesthesia (HR light 225±29 vs. HR dark 268±31 beats/min., $p < 0.05$). The differences between single parameters of the acid-base balance were not significant in the light period, but differences were detected for pO_2 (ketamine/xylazine 6.052±1.07 kPa vs. pentobarbital 9.283±2.85 kPa, $p<0.02$) and O_2 saturation (ketamine/xylazine 78.42±5.34 % vs. pentobarbital 92.52±5.52 %, $p<0.002$) with the significantly higher values in pentobarbital anaesthetized rats in the dark period. HR was significant higher in pentobarbital anaesthetized rats vs. in ketamine/xylazine anaesthesia in the both lighted periods (light-ketamine/xylazine 225±29 beats/min. vs. pentobarbital 348±32 beats/min., $p<0.001$; dark-ketamine/xylazine 268±31 beats/min. vs. pentobarbital 357±29 beats/min., $p<0.001$). It is concluded that the both types of anaesthesia produce acidosis, hypoxia and hypercapnia independently on the heart rate and the light-dark cycle and disturb the light-dark differences at once from the start of in vivo rat experiments.

Supported by VEGA grant 1/4303/07.

LONG-TERM MELATONIN AND SLOVINOL TREATMENT OF YOUNG RATS WITH METABOLIC SYNDROME

J. Török, A. Zemančíková, S. Vranková, A. Barta, M. Tabačeková, O. Pecháňová
Institute of Normal and Pathological Physiology, Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovakia

Hypertension is very prominent feature in the metabolic syndrome and together with insulin resistance, glucose intolerance, dyslipidemia and central obesity represents a major cardiovascular risk factor. The obese spontaneously hypertensive rat (SHR/NDmcr-cp) (SHR-cp) is a rat strain with obesity used as an animal model for the metabolic syndrome (1). The aim of the study was a) to characterize relaxation responses to acetylcholine and neurogenic responses to electrical stimulation of isolated vessels from rats with metabolic syndrome; b) to investigate the effect of long treatment of these rats with melatonin and slovinol – both strong antioxidants – and compare it with this in SHR. Experiments were performed on 6-8-week-old rats divided into three groups: Wistar rats, spontaneously hypertensive rats (SHR) and SHR-cp. SHR and SHR-cp received melatonin for a period of 3 weeks. Moreover, some SHR-cp received slovinol for the same period. Rings of isolated thoracic aorta and mesenteric artery were suspended in organ baths containing modified Krebs solution and connected to a force-displacement transducer for the recording of isometric tension. Neurogenic contractile responses were elicited by electrical stimulation of perivascular adrenergic nerves. In control SHR-cp, endothelium-dependent responses of the thoracic aorta induced by acetylcholine were not significantly different from those in control SHR and Wistar rats. Neurogenic contractions were increased in SHR-cp, especially at low frequencies of electrical stimulation (1 – 8 Hz), in comparison with those in SHR and Wistar rats. Melatonin significantly improved endothelium-dependent relaxation at high concentration of acetylcholine and reduced neurogenic contractions of mesenteric artery, but it had no effect on these responses in SHR-cp. Similarly slovinol did not influence neither acetylcholine-induced relaxation of the aorta nor neurogenic contraction of the mesenteric artery. These results showed that metabolic syndrome is associated, at least at low frequencies of stimulation, with increased vascular sympathetic activity.

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Supported by VEGA grant No. 2/0193/09.

POSSIBLE MECHANISMS INVOLVED IN PROARRHYTHMIC AND ANTIARRHYTHMIC EFFECTS OF THYROID HORMONES

N. Tribulova[1], J. Radosinska[2], B. Bacova[1], V. Knezl[3], T. Soukup[4]
[1]Institute for Heart Research, Slovak Academy of Sciences, Bratislava, [2]Faculty of Medicine, Comenius University, Bratislava, [3]Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Bratislava, Slovakia, [4]Institute of Physiology, Academy of Sciences, Prague, v.v.i., Czech Republic

Thyroid hormones (TH) play an important role in cardiac electrophysiology and Ca^{2+} handling through both genomic and non-genomic mechanisms of action. We tested our hypothesis that chronic or acute elevation of plasma TH levels may affect not only heart function, but also susceptibility of the heart to arrhythmias. We also examined whether alterations of intracellular free Ca^{2+} ($[Ca^{2+}]i$) and cell-to-cell coupling protein connexin-43 (Cx43) may be involved. Series of experiments were performed using young and old Wistar rats for long-term treatment with T_3 (10μg) or T_4 (50μg/100g b.w./day, 2 weeks) while old guinea pigs were used for acute 5 min-lasting application of T_3 (1nM-10μM). Isolated heart Langedorff preparation was used to test

susceptibility to atrial (AF) or ventricular fibrillation (VF) in rats and to examine [Ca]i alterations in guinea pigs. Myocard from the rats upon long-term treatment with TH was processed for Cx43 expression. Results showed that TH-treated old rats were much prone to electrically-induced AF, while young rats were more sensitive to hypokalemia-induced VF compared to age-matched controls. However, ventricular premature beats were reduced regardless the age. The hearts prone to AF and VF exhibited significantly reduced myocardial expression of Cx43 and its phosphorylated form. Acute administration of T_3 in range of 1-100 nM delayed, while in the higher 1μM concentration facilitated the occurrence of VF. Moreover, T_3 in range 0.01-1μM caused dose-dependent decrease of elevated diastolic $[Ca^{2+}]i$, whereas Ca^{2+} overload was not abolished but enhanced due to higher 10 μM concentration. It is concluded that down-regulation of myocardial Cx43 by TH increases susceptibility of the heart to malignant arrhythmias and their occurrence or prevention is most likely determined by acute dose-related modulation of Ca^{2+} handling by TH (1,2).

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This work was supported by APVV 51-059505, VEGA 2/0049/, GACR 304/08/0256 grants and AV0Z 50110509.

MOXONIDINE REDUCES SELF-SIMILARITY OF HEART RATE VARIABILITY

M. Turcani
Department of Physiology, Faculty of Medicine, Kuwait University, Kuwait

Moxonidine, an imidazoline I1 and alpha-2-adrenergic receptor agonist, effectively reduces blood pressure, particularly in patients with metabolic syndrome X. However, its administration to heart failure patients was associated with increased mortality despite significant reduction of sympathetic overactivity. Changes in non-linear properties of cardiovascular oscillation are strong predictors of all-cause mortality in heart failure patients. Hypothesis was tested that central sympatholysis causes break down of fractal properties as well as decline in complexity of cardiovascular fluctuations. Wistar male rats were implanted with telemetric transmitters to monitor blood pressure and ECG. Moxonidine alone or in the combination with yohimbine or efaroxan was applied subcutaneously. Self-similarity and complexity of interbeat intervals and systolic blood pressure time-series were estimated by short and long-range scaling exponents, approximate entropy, Lempel-Ziv entropy, symbol dynamic entropy and percentage of forbidden words.All doses of moxonidine (0.04, 0.12, 0.36, 1.08, 3.24 mg/kg) were sympatholytic and made short-range interbeat interval fluctuations less correlated. The largest moxonidine dose that is also parasympathomimetic caused long-range interbeat interval fluctuations to be more similar to the 1/f noise. This dose increased the complexity of interbeat interval fluctuation but reduced the entropy of systolic pressure oscillations. Moxonidine has no effect on symbol dynamic with the exception of the largest dose that increased the symbol dynamic entropy and reduced the number of forbidden words. All changes were blocked by the pretreatment either with yohimbine, an alpha-2 adrenoceptor antagonist, or with efaroxan, an imidazoline I1 receptor blocker. Moxonidine broke down the short-range fractal organization of interbeat intervals. Fluctuations of interbeat intervals became more random, more similar to the uncorrelated white noise. These changes were mediated by both, imidazoline I1 and alpha-2 adrenergic receptors.

Supported by Kuwait University Research Grant No. MY02/04.

ANTHRACYCLINE INDUCED CARDIOMYOPATHY AND CARDIOTROPHIN-1

K. Turčeková, H. Černecká, L. Mesárošová, M. Snopková, P. Ochodnický, J. Kyselovič, P. Křenek, J. Klimas
Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia

Cardiotrophin-1 (CT-1) is a member of the interleukin-6 (IL-6) family of cytokines and was presented as pro-hypertrophic signaling molecule. CT-1 acts predominantly in the heart, where it is synthesized and secreted via the coronary sinus into the peripheral circulation (1). It has a various functions in different pathological models of cardiovascular disease such as cardioprotective effects, haemodynamic effects and endocrine properties (2). The aim of this work was to study the expression of CT-1 in the early stage of anthracycline induced cardiomyopathy. Male Wistar rats were treated with daunorubicin (six doses of 3 mg/kg, i.p., every 48 hours). Control rats received vehicle. To assess cardiac function, left ventricular catheterization was performed under general anaesthesia and tissue preparations were examined histologically. RT-PCR method was used to determine the level of CT-1 mRNA in left ventricular tissue. Application of daunorubicin led to decrease of heart weight ($p<0.05$) impaired left ventricular hemodynamics (significant decrease of left ventricular pressure and rates of contraction and relaxation, $p<0.05$) and damage of cardiac tissue with decrease of cell width and loss of cardiomyocytes ($p<0.05$). However, CT-1 mRNA expression was increased more then twofold ($p<0.05$) indicating an activation of pro-hypertrophic mechanisms. In conclusion, daunorubicin activated the increase of pro-hypertrophic CT-1 expression but the tissue showed a rather hypotrophic response. We propose that increased CT-1 expression might be an adaptation response to anthracycline-induced cardiac injury.

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Supported by grant No. VEGA 1/0377/09 and VEGA 1/0357/09.

THE INFLUENCE OF MONOVALENT CATIONTS ON TRIMERIC G-PROTEIN ACTIVITY

M. Vošahlíková, K. Surá, L. Bouřová, P. Svoboda
Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

The characteristic features of agonist stimulation of G-protein activity were measured in HEK293 cells stably expressing pertussis-toxin (PTX)-insensitive DOR-$G_i1\alpha$ (Cys^{351}-Ile^{351}) hybrid molecule/fusion protein. Maximum DADLE-stimulated [^{35}S]GTPγS binding was 1.2x higher in PTX-treated cells than in untreated cells. This result was manifested as the ratio between total (B_{total}) and basal (B_{basal}) level of binding as well as the net-increment of DADLE stimulation $\Delta = B_{total} - B_{basal}$. The affinity of agonist response was also higher in PTX-treated than in PTX-untreated cells, EC_{50} = 5.37 x 10^{-8} and 1.01 x 10^{-7} M, respectively. Normalization of G-protein activity to the receptor number has not removed this difference. We have also analyzed the effect of monovalent cations on the ability of δ-opioid receptor agonist DADLE to stimulate [^{35}S]GTPγS binding in membranes isolated from both PTX-treated and untreated cells. In PTX-treated cells, the basal level of [^{35}S]GTPγS binding was inhibited with maximum effect at 200 mM NaCl. The sodium-induced inhibition of G-protein activity was removed by increasing DADLE concentrations, but there was no difference among NaCl, KCl and LiCl when inhibiting the basal level of binding. Contrarily, sodium was clearly more efficient than potassium and lithium in PTX-untreated cells. Thus, endogenous G-proteins of G_i/G_o family exhibited preferential sensitivity to sodium while $G_i1\alpha$ covalently-bound to DOR was sensitive to ionic strength only. Our data indicate that besides constitutive features of receptor activity, DOR covalently bound to the cognate protein $G_i1\alpha$ exhibit higher efficacy towards "in frame" expressed $G_i1\alpha$ protein than towards the endogenously expressed pool of PTX-sensitive G-proteins. Sodium cations acting like inverse agonists alter the intrinsic efficiency of OR drugs.

Supported by MSMT (LC554 and LC06063), IAA 500110606, GD305/08/H037 and AV0Z50110509.

MODIFIED NITRIC OXIDE SYNTHASE ACTIVITY IN BORDERLINE HYPERTENSIVE RATS: THE EFFECT OF NF-{KAPPA}B INHIBITION

S. Vranková, A. Barta, J. Parohová, O. Pechánová

Institute of Normal and Pathological Physiology and Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovak Republic

Since involvement of nuclear factor kappa B (NF-κB) in the upregulation of endothelial nitric oxide synthase (eNOS) in hypertension induced by N^G-nitro-L-arginine methyl ester was demonstrated, the goal of our study was to analyze an effect of NF-κB inhibitor, JSH-23 (4-methyl-N^1-(3-phenylpropyl)benzene-1,2-diamine), on blood pressure and NOS activity in borderline hypertensive rats (BHR). Rats were divided into four groups: 6- and 12-weeks old BHR treated with JSH-23 (10μM, i.v.) for next 2 weeks and age-matched controls. Blood pressure was measured by tail-cuff plethysmography every week. NOS activity was determined by measuring the formation of L-[^{3}H] citrulline from L-[^{3}H] arginine in the aorta, heart, kidney and brain. At the end of treatment blood pressure was decreased by 24 % in young and 11 % in adult BHR treated with JSH-23 in comparison to age-matched untreated rats. NOS activity in the young control BHR was significantly higher in comparison to the adult control BHR in all tissue investigated. JSH-23 treatment decreased NOS activity of young BHR while it increased the activity in adult BHR in all tissues investigated. We hypothesized that in young BHR, NF-κB activation leads to increased NOS expression followed by increased activity which may represent a compensatory mechanism activated due to the blood pressure increase. After blood pressure stabilization NF-κB activation was decreased following by decreasing NOS activity. In the condition of sufficient NO production, inhibition of NF-κB led to decreased NOS activity, while in NO deficiency it elicited the opposite effect. Since NF-κB inhibition led to decreased blood pressure in both young and adult BHR, the involvement of NF-κB in blood pressure regulation should include more than NO-mediated mechanism.

The study was supported by APVV-0586-06 and APVV-0538-07.

IDIOTHETIC NAVIGATION OF RATS IN TRIANGULAR POOL

S. Wittnerová, J. Bureš
Department of Neurophysiology of Memory and Computational Neuroscience, Institute of Physiology Academy of Sciences of the Czech Republic v.v.i., Prague, Czech Republic

The role of the hippocampus in learning and memory has been the focus of numerous neuroscience studies since the discovery that the H.M. and other patients with hippocampal damage suffer from devastating learning and memory deficits (Scoville and Milner, 1957). Dysfunction of the hippocampal formation manifests as impaired relational learning and memory in humans and animals. One of the most frequently applied relational learning paradigms in animals is the Morris Water Maze (MWM), in which the subjects is required to learn complex spatial relationships of visual cues (Morris, 1981). Present study focus on characterization of learning, search strategies, acquired memory traces and cognitive maps in three geometric different shapes constructed as Morris Water Maze pools. Naive Long-Evans hooded rats were trained 9 days in light and 6 days in dark conditions in the large circular, equilateral triangular and middle-sized circular pools. In all of the three pools the orientation in light conditions is according to visual stimuli that´s why the rat placed to the pool in any position of the body immediately turns to the direction, where the platform is located and swims. In the equilateral triangular pool in dark conditions first day first trial in darkness the animals showed the quickest performance because they repeated the strategy they acquired according to the allothetic stimuli in light conditions. Since the second trial in darkness the animals started to explore the whole area of the pool first as random and selective search til the quickest direct search according to self-motion signals from the body as stimuli from proprioreceptors, efferent copies of motor commands and vestibular system. In both sizes of the circular pools the animals had to find the logical idiothetic principle how to find the platform already since the first trial in darkness. The run in dark conditions is about two times longer then in light conditions in every geometric enclosure of the pool. First day first trial in darkness 7 rats from 8 found the platform as direct search in the equilateral triangular pool however only 1 from 8 in the large circular pool and 3 from 8 in the middle–sized circular pool. All experiments complied with the Czech law and with the directive of the European Communities Council No. 86/609/EEC on protection of laboratory animals.

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Supported by research project AV0Z 50110509, GACR grant 309/09/0286, MSMT CR 1M0517 and LC554