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EFFECT OF OMEGA-3 FATTY ACIDS DIET ON MYOCARDIAL CONNEXIN-43 EXPRESSION IN LEWIS RATS WITH ALTERED THYROID STATUS

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It has been established that thyroid hormones (TH) are powerful modulators of heart function *via* both genomic and non-genomic effects. Moreover, clinical and our previous studies suggest that the altered thyroid status may affect susceptibility of the heart to arrhythmias, whereby intercellular connexin-43 (Cx43) channels are likely involved. Since omega-3 fatty acids (omega-3) have been shown to protect the heart in both clinical and experimental settings the purpose of this study was to examine myocardial Cx43 expression in rats with the altered thyroid status and the effects of omega-3. Experiments were performed on adult male Lewis rats that were divided into six groups: 1. untreated controls; 2. rats treated with omega-3 (20mg/100g/day/six weeks); 3. rats treated with T_3 (0.25 mg/kg body weight three times a week/six weeks); 4. rats subjected to T_3 and omega-3 for six weeks; 5. rats treated with 0.05 % methimazole six weeks; 6. rats subjected to methimazole and omega-3 for six weeks. Body, heart and left ventricular (LV) weights as well as blood glucose and serum TH were registered at the end of the experiments. The LVs were used for immunoblotting of Cx43 with primary rabbit antibody (Sigma) and secondary donkey antibody (peroxidase-labeled anti-rabbit immunoglobulin, Amersham). Expression of PKC-epsilon, which directly phosphorylates Cx43, was determined as well. Compared to untreated controls the total myocardial expression of Cx43 was reduced in hyperthyroid while increased in hypothyroid rat hearts and enhanced due to omega-3 supplementation. Moreover, phosphorylated form of Cx43 was suppressed in T_3-treated rats and increased in hypothyroid ones as well as enhanced upon omega-3 treatment. In parallel, the PKC expression was decreased in hyperthyroid but increased in hypothyroid rat hearts and up-regulated by omega-3. It is concluded that there is down-regulation of Cx43 in the hyperthyroid and up-regulation in the hypothyroid state. Moreover, omega-3 treatment affects myocardial expression of Cx43 in the altered thyroid status.

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CHANGES IN PQ AND QT INTERVALS DURING APNOIC EPISODE AND REOXYGENATION IN RAT MODEL. CHRONOBIOLOGICAL STUDY

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Disorders of pulmonary ventilation belong to the group of proarrhythmogenic factors, but the link between disorders breathing and cardiac arrhythmias in the dependence on light-dark (LD) cycle is less studies [1,2]. The aim of this study was to evaluate the effect of apnoic episode and reoxygenation on some ECG parameters in dependence on LD cycle. The experiments were performed in ketamine/xylazine anaesthetized female Wistar rats (100 mg/kg+ 15 mg/kg, i.m.,) after adaptation to a lighted regime of 12:12 h for 4 weeks. The animals were artificial ventilated by respirator at ventilatory parameters: 1 ml/l00 g of body weight and respiratory rate 40-50 breaths/min. The apnoic episode was simulated by switching off the respirator for 2 minutes. PQ and QT intervals were evaluated during each step of the experiment (intact animal, after tracheotomy, artery preparation, thoracotomy, at the end of 5 min. stabilization, after 30., 60., 90., 120 sec. of apnoic episode and after 5., 10., 15. and 20 min. reoxygenation) during the light and the dark periods. The significant LD differences ($p<0.01$) in duration of PQ intervals were found after 30. and 60. sec. of apnoic episode, but this significance was not determined after 90. and 120 sec. Reoxygenation shortened the PQ intervals and recovered significant LD differences ($p<0.01$). Significant differences ($p<0.01$) were found in the QT interval duration only after 90. and 120 sec. of apnoic episode. Reoxygenation recovers parameters to the pre-asphyxic values, but LD differences were eliminated. It is concluded that the predisposition of the myocardium for ventricular arrhythmias result from disorders of the production and impulse conduction is significantly influenced by LD cycle not only in the intact animals but also during the apnoic episode and reoxygenation. Dispersion of the refractory periods, represented by duration of QT interval, is independent on LD cycle. Probably, LD dependence in the dispersion of the refractory periods arises only after serious apnoic and reoxygenation injures.

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2. Bayram NA., Diker E.: Turk. Kardiyol. Dern. Ars., 36(1), s. 44-50, 2008.

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EFFECT OF ISOFLAVONE POMIFERIN AGAINST PROLONGED ISCHEMIA AND REPERFUSION INJURY IN THE ISOLATED PERFUSED RAT KIDNEY

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Bioflavonoids may diminish cold storage-induced injury due to antioxidant and iron chelating activities. This study was designed to delineate the renoprotective mechanisms of bioflavonoid pomiferin of 5 mg/kg dose per day in pre-treated two weeks therapy of reperfusion injury after a 48 hours cod storage. Three groups of animals – treated, placebo and intact. Animals were anesthetized, kidney were immediatlly flushed in situ with 80 ml of a cold preservation solution. After excision, the kidney were stored at 4 °C for 48 hours. After cold storage the kidney were reperfused (mean arterial perfusion pressure of 100 mm Hg), urine and perfusate samples were collected and perfusion flow rate (PFR), diuresis, total protein and malondialdehyde were analysed. The kidney of treated group showed significantly higher PFR in comparison with placebo and intact group. Kidney of placebo and intact group showed significantly lower diuresis and significantly higher total protein concentration than in treated group. Malondialdehyd was increased significantly in both two groups too. Pomiferin medication supported antioxidative system and reduced lipoperoxidation such as participated in the renoprotective mechanisms in pre-treated animals.

SURPRISINGLY LOW BONE DENSITY AT THE LUMBAR SPINE IN MORBIDLY OBESE PATIENTS

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A BMI less than 19 is a major risk factor for osteoporosis. On the contrary, obesity in terms of a skeleton is considered a protective factor. Some studies have shown that only moderate overweight and obesity have beneficial effects on bone mineral density, but severe and morbid obesity (defined as BMI > 35 $kg.m^{-1}$, or BMI > 40 $kg.m^{-1}$) is disadvantageous for the skeleton. The aim of the research is to determine the values of bone mineral density in morbidly obese patients. During six months of 2010 we examined 60 morbidly obese patients enrolled in a pilot study focused on metabolit ganges after sleeve gastric resection. The sample was examined by DXA (Hologic Discowery W) which is considered the gold standard. The results were statistically processed using StatSoft Software Package Version 6. 60 patients (9 men and 51 women, average age 41.3 and 40.5 years) suffering from morbid obesity – average BMI 41.2 for men and 40.1 for women. All women were premenopausal. Patients with osteoporosis risk factors were excluded. The average Z score of patients was 0.84 SD, slightly above average value, but six patients were ranked Z score lower, at three patients from -1 to -2.5 SD ("osteopenia") and at three patients lower than -2.5 SD ("osteoporosis"). One patient suffered from extremely low density (Z score -4.2 SD). BMI over 40 was at all six patients with pathological Z score. In the last four years investigations pointed out the correlation between bone mineral density and body fat and fat-free mass. Mutual communication between fat and bone tissues expresses a homoeostatic feedback system. Our observations show the protective effect of increased body mass on bone mineral density. For some individuals with BMI higher than 40, however, we found very low

bone mineral density. The reasons for this are unknown. Its role will certainly play an influence of cytokines produced by adipose tissue, hypovitaminosis D and secondary hyperparathyroidism.

NIMODIPINE INHIBITST ACTION POTENTIAL FIRING IN CULTURED HIPPACAMPAL NEURONS DUE TO BLOCK OF VOLTAGE-DEPENDENT POTASSIUM CHANNELS

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L-type calcium current (LTCC) is important functional determinant of hippocampal neurons contributing to processes like memory formation and gene expression. In adult mouse CA1 neurons absence of the $Ca_V1.2$ channel affected parameters of repetitive action potential firing[1]. We attempted to test contribution of LTCC to action potential firing of cultured rat neonatal hippocampal neurons using LTCC blocker nimodipine. Ionic currents and action potentials were recorded in whole cell patch clamp. Pipette solution contained (in mM): CsCl, 135; $MgCl_2$, 2; TEACl, 20; Na_2ATP, 3; Na_2GTP, 0.4; EGTA, 3;HEPES, 10; pH 7.4 (with CsOH). Bath solution contained NaCl, 105; KCl, 3; TEACl, 25; $MgCl_2$, 0.5; $CaCl_2$, 2; HEPES, 10; D-glucose, 10; pH 7.4 (with NaOH). In some experiments 2 mM Ca^{2+} were replaced by 2 mM Mg^{2+} to exclude Ca^{2+}-dependent potassium current. 500 ms long current pulse activated firing of series of action potentials (AP). Presence of 10 μM of nimodipine blocked all but first AP in series. This concentration, which is able to block completely LTCC, inhibited about 40 % of total calcium current. Sodium current was not affected. Nimodipine blocked about 60 % of voltage dependent potassium current (K_V current). Ca^{2+}-dependent potassium current was not affected, probably due to weak coupling to LTCC. We concluded that nimodipine suppressed firing of action potentials in cultured hippocampal neurons predominantly due to inhibition of K_V current.

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APOPTOSIS AND PROLIFERATION IN THE ENAMEL KNOT STAGED MOUSE THIRD MOLAR

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The third molar (M3) is the most common missing tooth in human population as well as in mice. Moreover, mouse M3 resembles the human secondary dentition as it also develops postnatally. However, despite the fact that M3 seems to be a great model to study tooth-bone interactions, the prevalence of odontological knowledge is based on findings in the mouse first molar (M1). Therefore, this study aims to supplement recent evidence of the M3 development. The presented data focus particularly on the enamel knot stages when the signaling centres of developing tooth appear and accompany transition of the tooth bud to the cap stage (primary enamel knot, PEK) and further cusps formation. Proliferation and apoptosis are the major morphogenetic events involved. Mouse mandibular M3 were investigated in serial histological sections after hematoxylin-eosin staining to evaluate morphology, imunohistochemistry to localize proliferation (PCNA – proliferating cell nuclear antigen), labelling of DNA breaks to follow apoptosis (TUNEL) and *in situ* hybridisation to detect signalling molecules – markers of the enamel knots (*Shh, Fgf4*). The M3 started to develop perinatally by budding from the stalk of the second molar. Formation of the PEK and gradual elimination by apoptosis was detected at postnatal (P) day 3. Later on, high proliferation confirmed the growing cervical loop. The secondary enamel knots (SEK) were visible during the bell stage by P5 and underwent apoptosis after their signalling mission. *Shh* was found in the whole inner enamel epithelium including the PEK and the SEK, while *Fgf4* expression was restricted to the PEK and SEK. These findings will be used in further comparative studies of tooth morphogenesis and osseointegration.

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RHEUMATOID ARTHRITIS SUSCEPTIBILITY GENES IN POPULATION OF SLOVAKIA - PRELIMINARY RESULTS

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Rheumatoid arthritis (RA) is an autoimmune disease that affects up to 1 % of the general adult population worldwide. Genetic studies in autoantibody-positive RA among subjects of European ancestry have identified multiple risk alleles in the major histocompatibility complex (MHC) region, as well as more than 30 confirmed RA risk alleles in non-MHC loci. It has been estimated that these alleles explain about 20 % of the genetic burden of the disease. To detect the presence of RA risk alleles in Slovak population we conducted a genetic analysis of more than 800 samples (85 % females, 15 % males) with balanced geographic distribution. We selected 4 previously confirmed RA-associated non-MHC SNPs in immune response-related genes. 1858CT polymorphism in PTPN22 gene is unequivocally associated with RA and encodes a protein phosphatase involved in TCR signalling inhibition. Among other genes, CTLA4 encoding a co-stimulator for T-cell activation, STAT4 encoding a transcription factor involved in Th1 cell differentiation, and SNP in TRAF1/C5 locus that includes genes implicated in TNF signal transduction and complement function also belong to established RA risk loci. The presence of risk alleles was determined by real-time PCR using the allele specific TaqMan probes. Our preliminary results suggest significantly higher prevalence of PTPN-22 risk allele among RA patients compared to controls, as well as higher portion of risk allele homozygote phenotypes within the group of patients.

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PROTECTIVE EFFECTS OF PPAR-α ACTIVATION ON MYOCARDIAL ISCHEMIA/REPERFUSION INJURY IN ISOLATED RAT HEART

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Peroxisome proliferator-activated receptor-alpha (PPAR-α) is a nuclear ligand-activated transcription factor expressed in the heart. Growing evidence suggests that PPAR-α activation may reduce ischemia/reperfusion (I/R) injury through metabolic and antiinflammatory effects (1). However, the role of PPAR-α in acute I/R injury remains unclear. Therefore, the study was aimed to investigate effects of PPAR-α activation in myocardial I/R injury. Male Wistar rats were pretreated for 5 days with highly selective PPAR-α agonist WY 14643 (WY) (3 mg/kg/day). Hearts of pretreated rats as well as untreated controls (C) were perfused according to Langendorff and subjected to 30-min global ischemia followed by 40 min reperfusion for the evaluation of postischemic contractile dysfunction (characterized by the recovery of left ventricular developed pressure, LVDP, and pressure-rate product, PRP) and coronary flow (CF) recovery. Gene expression of PPAR-α was determined (RT-PCR) in the heart samples before ischemia and after I/R. All parameters were expressed in % of baseline values. Postischemic restoration of LVDP reached 50 ± 10 % in WY group ($P<0.05$ vs. 25 ± 4 % in C). Recovery of PRP was increased from 26 ± 7 % in C group to 46 ± 8 % in WY group ($P<0.05$). CF was also better restored in the WY-treated group. Measurement of PPAR-α gene expression revealed a significant

increase in cardiac mRNA levels in WY-treated group at baseline and preservation of enhanced PPAR-α expression after I/R in contrast to its marked downregulation in controls. Our findings suggest that activation of PPAR-α is involved in protection of the heart against I/R injury. Improved functional recovery in normocholesterolemic rat hearts indicates a role of lipid-independent effects of WY. However, precise mechanisms involved in cardioprotection caused by PPAR-α activation require further investigation.

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INFLAMMATION MODULATES LOCAL METABOLISM OF GLUCOCORTICOIDS IN SECONDARY LYMPHATIC ORGANS

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Glucocorticoids exert anti-inflammatory and immunomodulatory effects that may be regulated in part by the activities of the glucocorticoid-activating and -inactivating enzymes, 11beta-hydroxysteroid dehydrogenase type 1 (11HSD1) and type 2 (11HSD2), respectively. Previous studies have demonstrated that inflammatory bowel diseases in humans and experimental animals increase glucocorticoid production due to upregulation of 11HSD1 and downregulation of 11HSD2. The objectives in this study were therefore, to determine the effect of proinflammatory cytokines TNF-alpha and IL-1beta on colonic 11HSD1 and 2 and to investigate the effect of inflammation associated with colitis on 11HSD1 in spleen and mesenteric lymph nodes (MLN), i.e. in secondary lymphoid organs that are known to express 11HSD1. Experimental colitis induced by intracolonic administration of 2,4,6 trinitrobenzenesulfonic acid stimulated 11HSD1 activity not only in colon but also in mesenteric lymph nodes and spleen. Analysis of mRNA for 11HSD1 in colon-draining lymph nodes and spleen showed that inflammation upregulated expression of this enzyme in mobile lymphoid cells similar to intraepithelial and lamina propria lymphocytes isolated from colon. Inflammation increased in the intraepithelial and lamina propria lymphocytes also the expression of proinflammatory markers TNF-alpha, IL-1beta and COX-2. To assess whether proinflammatory cytokines are responsible for upregulation of 11HSD1 and downregulation of 11HSD2 in colon, the colonic explant cultures were incubated in the presence of TNF-alpha or IL-1beta. Treatment with TNF-alpha exhibited upregulation of 11HSD1 mRNA whereas IL-1beta downregulated 11HSD2 mRNA. In contrast, both cytokines upregulated COX-2 mRNA. It is inferred that inflammation stimulates reactivation of biologically active glucocorticoids in lymphoid organs and in gut-associated lymphoid tissue.

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EFFECT OF EXPERIMENTAL INFECTION WITH *EIMERIA ACERVULINA* AND DIETARY PLANT EXTRACT SUPPLEMENTATION ON MUCUS DYNAMICS IN THE INTESTINE AND PERFORMANCE OF THE CHICKENS

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Coccidiosis is a common and costly disease in poultry. The effectiveness of antimicrobials in poultry production has reduced its economic losses but the use of antibiotics as growth promoters in animals feeds has been banned in the EU. As a results, phytogenic feed additives with antimicrobial and growth-promoting effects have been proposed to chicken production. Mucus layer in the GIT acts as an important medium for protection, lubrication and transport between the lumen content and epithelial cells. The objective of this study was to determine the effect of feeding diet supplemented with oregano on mucus dynamics in the intestine and performance in chicks infected with *E. acervulina.* Forty, 1-d-old ROSS 308 broiler chicks were divided into 4 groups. Birds of group 1 (control) and 2 (positive control) were fed diet without coccidiostaticum; the chicks of group 3 were fed diet supplemented with oregano (0.707 g/kg) and the chicks of group 4 were fed the diet supplemented with coccidiostaticum (Robenidin hydrochloride -33 mg/kg feed). Chicks were inoculated orally on d 12 with $25x10^3$ *E. acervulina* sporulated oocysts. The thickness of the adherent mucus layer in duodenum was not altered in any group although the *E. acervulina* meronts were detected in that region of intestine. Similarly, however, the meronts were detected in jejunum and caecum, the mucus layer in these regions in animals fed diet supplemented with oregano was similar to those fed the cocidiostaticum supplement on d 3 and 10. The thickness of mucus layer was not altered by treatment in jejunum and caecum on d 17 post infection. The feeding diet supplemented with oregano reduced live b.w. of chicks on d 17 ($P<0.001$); internal organ weight was not affected by treatment. The results of this study indicate that feeding diet supplemented with oregano in *Eimeria* challenge might have a protective effect against coccidiosis infection and affect performance in chickens.

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ACTIVATION OF THE RAT CARDIAC RYANODINE RECEPTOR BY ITS DOMAIN PEPTIDE DP_{CPVT-C}

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The majority of mutations linked to the congenital cardiac diseases such as ARVD and CPVT are localized to one of two regions – the N-terminal and the central domains of the cardiac ryanodine receptor (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 whether a peptide with a sequence identical to that of the central domain should suppress the stability of the closed conformation. We constructed the peptide DP_{CPVT-C} (amino acids 2380 – 2411), corresponding to the region of the central domain of the human RyR2 with the highest incidence of CPVT and ARVD-related mutations. We examined its effect on the resting activity of the RyR2 after application from the cytosolic side of the artificial lipid membrane (BLM) containing a reconstituted rat RyR2 channel. The cis/trans solution contained 90 nM cytosolic Ca^{2+}/ 8 mM luminal Ca^{2+}, pH 7.35. Current amplitude was measured at 0 mV potential for 2 minutes. We found that DP_{CPVT-C} increased the RyR2 open probability by an order of magnitude. The concentration of DP_{CPVT-C} that evoked 50 % of the maximal effect was in the range of 10-20 uM. Our results suggest that DPcpvtC is able to suppress the interaction between the N-terminal and the central domain and that this interaction may participate in the „domain switch"of RyR2.

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HYPERCHOLESTEROLEMIA INTERFERES WITH ENDOGENOUS PROTECTIVE MECHANISMS INDUCED IN RAT HEART BY ACUTE DIABETES

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This preliminary study indicates the trends of changes in membrane fluidity (MF), Mg^{2+}-ATPase activity and the content of conjugated

dienes (CD) in heart MIT membranes of acute DIA and hypercholesterolemic (HCH) rats. Experimental: Male Wistar rats (229±20 g) were used in the experiment. DM was induced by a single dose of streptozotocin (80 mg/kg, i.p.). HCH was induced by application of a fat–cholesterol diet (1 % cholesterol, 1 % coconut oil, 20 g/day). Results: At termination of experiment, on the 8th day after STZ application, the DIA rats exhibited elevated levels of glucose, total cholesterol, HDL, LDL and triacylglycerols. Increase in these variables was even more expressed in the DIA+HCH group ($p<0.05$). In acute DIA hearts MIT exhibited significant elevation in MF and Mg-ATPase activity ($p<0.05$) and non-significant increase in CD. HCH animals exhibited only non-significant changes in all parameters investigated. In respect to controls the DIA+HCH rats exhibited increased-, but in comparison with the DIA group lower fluidization of MIT membranes. MIT ATPase activity in the DIA+HCH group was slightly depressed in comparison to the control and DIA groups while the changes in contents of CD showed an opposite trend. Conclusions: Fluidization of MIT membranes is a positive effect attributed to endogenous protective mechanisms (EPM). It was most expressed in the DIA group. Significant increased cholesterol found in the DIA+HCH group antagonized the EPM, it increased the rigidity of MIT membranes. Consequently, the MIT ATPase activity in DIA+HCH group became also depressed. This pilot study reveals the trends in development of membrane properties and activities that will follow in heart MIT influenced simultaneously by DIA and HCH.

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ACUTE INFLAMMATION-INDUCED ALTERATIONS OF Cx40 EXPRESSION IN AORTA OF HYPERTRIGLYCERIDEMIC RATS

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Abnormalities in direct intercellular communication *via* connexin (Cx) channels observed during pathophysiological conditions can contribute to endothelial integrity alterations and structural remodeling of vascular wall. The aim of present study was to examine Cx40 isoform spatial distribution in aorta of Wistar (W) and hereditary hypertriglyceridemic (hHTG) rats exposed to acute inflammation. Inflammation was induced by simple dose of lipopolysaccharide (LPS, E.coli, 1 mg/kg, i.p.) in adult male rats of both rat strains. After 10 days, cryostat aortic sections were processed for Cx40 immunodetection using confocal microscope, density of Cx40 clusters and their size were morfometrically evaluated in both endothelium and media. Immunoflourescence demonstrated very heterogeneous and locally very rich or very rare Cx40 fluorescent signal in endothelium and media of aorta of individual rats in both strains. Morphometric analysis of Cx40 in endothelium showed significant increase of number of Cx40 clusters/mm^2 by 80 % in hHTG when compared to W, but averaged area of one cluster was smaller by 16 % in hHTG in contrast to W. In media, high levels of TG resulted in growth of Cx40 spots' number by 22 % when compared to W and size of one cluster did not differ between the strains. LPS induced reduction of both, number (by 32 % in W and by 45 % in hHTG) and area (by 25 % in W and by 10 % in hHTG) of endothelial Cx40 when compared to non-inflammatory groups. Media of aorta of inflammatory rats showed lower density of Cx40 by 45 % in both W and hHTG, while larger size of Cx40 clusters by 55 % in W only. LPS had no marked effect on area of Cx40 in hHTG. Our preliminary results suggest that acute inflammation might further modify intercellular communication abnormalities in aorta induced with high levels of TG, thus conducing to progression of vessel injury.

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CA^{2+} AND BA^{2+} IONS COMPETE FOR BINDING TO LUMINAL FACE OF THE RAT CARDIAC RYANODINE RECEPTOR

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Ca^{2+} ions released from the sarcoplasmic reticulum (SR) *via* cardiac ryanodine receptor (RyR2) are the key determinant of cardiac contractility. Mass of RyR2 channel consits of large cytosolic domain and small transmembrane region accessible from the lumen of the SR. Activity of RyR2 channel is tightly controled by cytosolic Ca^{2+} as well as Ca^{2+} stored in the lumen of the SR. Concentration of free Ca^{2+} inside the SR was estimated to be ~ 1 mM. However, single channel experiments with solely 1 mM luminal Ca^{2+} did not provide acceptable signal-noise ratio. Therefore, the current through the channel was increased by mixing luminal Ca^{2+} (1 mM) with luminal Ba^{2+} ions (7 mM). Under these experimental conditions cytosolic agonist caffeine activated RyR2 channel with the similar EC_{50} as it has been observed for 53 mM luminal Ca^{2+}. However, the channel maximal activation (P_{omax}) was decreased to 30 % (1). Aim of our study was to distinguish whether P_{omax} was reduced due to either the presence of low concentration of luminal Ca^{2+} (1 mM) or a potential competition effect between Ba^{2+} and Ca^{2+} on the luminal side of the channel. RyR2 channels were isolated from the rat heart and were incorporated into an artificial planar lipid membrane. Caffeine sensitivity of the channels was examined for various mixtures of luminal Ca^{2+} with luminal Ba^{2+}, while the composition of cytosolic solution was kept constant for all experiments with concentration of Ca^{2+} ~ 90 nM. EC_{50}, P_{omax}, current amplitude and parameters related to the gating kinetics of RyR2 channels after applying caffeine were analyzed. Interestingly, only for P_{omax} we obtained clear anomalous behavior as relative amounts of luminal Ca^{2+} and luminal Ba^{2+} were varied. A deep minimum was observed in mixture of 1 mM Ca^{2+} with 7 mM Ba^{2+}. From our results, we might conclude that revealed reduction in P_{omax} for mixture of 1 mM Ca^{2+} with 7 mM Ba^{2+} was likely due to a competition between both ions from the luminal side of the RyR2 channel.

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EFFECT OF LUMINAL CALCIUM ON GATING KINETICS OF COUPLED CARDIAC RYANODINE RECEPTORS

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In cardiac muscle, ryanodine receptor (RyR) channels play a pivotal role in excitation-contraction coupling. The opening of RyR channels results in a large Ca^{2+} flux from intracellular Ca^{2+} stores that drives cardiac contractility. RyR channels in cardiomyocytes are packed into regular, two-dimensional arrays that exhibit a unique "checkerboard-like" organization. By forming a close contact, neighboring RyR channels might interact with each other and operate as a functional unit. Indeed, two or more RyR channels reconstituted into artificial lipid membrane (BLM) - can open and close simultaneously (coupled gating). Although the physiological relevance of coupled gating phenomenon is still not understood, it has been considered as a one of mechanisms required for termination of local Ca^{2+} release in the cardiac muscle (1). The objective of our work was to further characterize the functional profile of the coupled RyR channels in respect to the single RyR channel. We focused on the effect of luminal Ca^{2+} on gating kinetics parameters. Employing the method of reconstitution of an ion channel into a BLM we showed that coupled RyR channels isolated from the rat heart exhibited less intensive flicker gating inside main open events in the presence of luminal Ca^{2+}. When flickering was ignored, the average open and closed times and the frequency of opening determined for coupled RyR channels as one functional unit were not affected by luminal Ca^{2+}. This is in contrast to the results reported for the single RyR channel where luminal Ca^{2+} significantly prolonged the average open and closed times and reduced the frequency of opening (2). Our results suggest that Ca^{2+} binding site located on the luminal face of each RyR channels could be

somehow protected by the functional interaction between channels recruited into the functional complex and therefore luminal Ca^{2+} is not able to exert the effect on gating kinetics.

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CHARACTERISTIC CHANGES OF BLOOD PRESSURE IN PATIENTS WITH SLEEP DISORDERS BREATING

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Ambulatory blood pressure monitoring and parallel polysomnographic study were performed in 116 adult subjects divided into 6 groups. Thirty blood-pressure (BP) and polysomnographic variables were measured to test their usefulness for screening for both arterial hypertension and obstructive sleep apnea-hypopnea syndrome (OSAHS). The development of severe breathing disorders and hypoxemia during sleep was attributed to OSAHS, when compared with measurements in healthy controls and in patients with arterial hypertension. Such disorders manifested as an increased apnea-hypopnea index, apnea index, duration of arterial oxygen saturation of less than 85 %, and decrease of average arterial oxygen saturation that correlated with nocturnal average diastolic BP ($p=0.0049$; $p=0.0027$; $p=0.05$ and $p=0.05$; respectively). These sleep related respiratory disorders resulted in various nocturnal, rather than diurnal, and diastolic and systolic BP variables. The acute antihypertensive effect of continuous positive airway pressure (CPAP) therapy for OSAHS significantly reduced the episodes of apnea and hypopnea and the secondary component of hypertension caused by excessive sympathetic stimulation. For the OSAHS-induced, dose-dependent component of hypertension that responded to CPAP, the following variables, in decreasing significance, were useful: nocturnal and average diastolic and systolic BP and 24-hour average diastolic and systolic BP. The monitoring of these variables could contribute to early diagnostic and prognostic stratification of complications and adequate therapy of the secondary component of hypertension caused by OSAHS.

OBSTRUCTIVE SLEEP DISORDERS BREATING AND OBESITY

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Obstructive sleep apnoea hypopnoea syndrome (OSAHS) is often associated with central obesity and important changes in blood pressure (BP). Epidemiological studies reported the prevalence of OSAHS adult patients with obesity in 40 %, and 70 % OSAHS patients are obese. The aim of our study was to find dependence between hypertension (AH) patients with OSAHS and body mass index (BMI). 116 adult subjects were divided into 6 groups by overnight polysomnography (Alice 3) and non-invasive parallel discontinuous measurement of BP by Cardiotens 1.34. 1) patients with AH without OSAHS with apnea/hypopnoe index-AHI≤5/h, (n=16, BMI=30.5±5.3 kg/m^2), 2) patients with AH and mild OSAHS (I.degree OSAHS) with 5<AHI≤20/h, (n=25, BMI=29.9±3.8 kg/m^2), 3) patients with AH and moderate OSAHS (II.degree OSAHS) with 20<AHI ≤40/h, (n=23, BMI=33.3±6.9 kg/m^2), 4) patients with AH and severe OSAHS (III. degree) with AHI>40/h, (n=26, BMI=35.2±6.4 kg/m^2), 5) control subjects without AH and OSAHS, (n=15, BMI=26.5±3.2 kg/m^2), 6) special severe OSAHS group treated with CPAP, (n=11, BMI=36.2±7.7 kg/m^2). BMI was increased significantly in all groups compared to the controls: AH ($P<0.05$); AH+I. degree OSAHS ($P<0.05$); AH+II. degree OSAHS ($P<0.01$); AH+III. degree OSAHS ($P<0.0001$); AH+III.degree OSAHS+CPAP ($P<0.01$). Correlation results between BMI and apnea/hypopnoe index ($r=0.322$; $P<0.01$), diurnal systolic (dAvgSTK 140.4±7.0 mm Hg, $r=0.409$; $P<0.001$) and diastolic blood pressure (dAvgDTK 86.8±7.8 mm Hg, $r=0.389$; $P<0.001$) suggest that the obesity is the reason whitch causes the leading to major changes of monitored parameters in hypertensive patients. Of the total number of hypertensive patients with OSAHS were 68.3 % of obese patients. The gradual increase in the number of apnea-hypopnoic episodes and blood pressure in dependance to body mass index led to establishing the relationshop between OSAHS in hypertensive patients and increasing body weight.

INULIN AND NATURAL BIOACTIVE FOOD SUBSTANCES IN COLON CANCER PREVENTION

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Natural bioactive food substances and diet intervention have now been extensively studied to reduce risks of colon cancer, which is one of the major public health problem throughout the world. The aim of the presented experiment was to investigated the effect of prebiotic – inulin by itself and in combination with *Hyppocastani extractum siccum*, and *Lini oleum* virginale in dimethylhydrazine induced colon cancer. Rats were randomly divided into 5 experimental groups of 12 rats each. Rats were fed with high fat (HF) diet containing 10 % of fat, supplemented by prebiotic BeneoSynergy 1 (ORAFTI, Tienen, Belgium) at a dose of 2 % of HF diet itself and in combination with Hyppocastani extractum siccum known as Horse chestnut (Calendula, SR) at a dose of 1 % of diet and Lini oleum virginale (flaxseed oil, Dr.Kulich Pharma, CR) at a dose of 2 % of diet. Two weeks after beginning the diet was applicated dimethylhydrazine (DMH, Merck, DE) injections in dose 20 mg/kg b.w., two times at week interval. The activity of fecal bacterial enzymes, concentration of lipid parameters, bile acids and short chain fatty acids were determined. Treatment with prebiotic and its combination with selected substances significantly decreased activity of bacterial enzymes ß-galactosidase, ß-glucuronidase, and α-glucosidase ($p<0.001$). Bile acids concentration was significantly decreased ($p<0.01$) excepting combination of prebiotic with Horse chestnut. Self-applicated prebiotic decreased ($p<0.001$) lipids parameters (total cholesterol and triacylglycerols), and enhanced short chain fatty acids production – acetic acid, propionic acid and butyric acid. Results of experiment show that ingestion of prebiotics have protective effect and may be the useful candidate agents for colon cancer prevention and treatment. Applied selected bioactive food components supported effect of prebiotics.

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VARIABILITY OF THE SARCOLEMMAL IMPEDANCE OF ISOLATED CARDIAC MYOCYTES

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Impedance parameters of cardiac myocytes determine the activation threshold and the propagation velocity of action potentials in cardiac muscles. Recently, we showed that high resolution impedance measurements on isolated cardiac myocytes are technically feasible by means of the Q-method (1). The Q-method is based on integration of the membrane current response of a myocyte during square wave stimulation by the patch-clamp method in the whole-cell configuration. In this work we focus on fluctuations of impedance parameters of cardiac myocytes isolated from the left ventricles of young adult male Wistar rats. In contrast to its electrical model, passive impedance parameters of myocytes showed spontaneous fluctuations well observed both in the time and the frequency domains. Quantification of fluctuation dynamics in the time domain revealed substantial differences in the variability among individual myocytes during the 5 minutes long recordings. Based on the extent of changes of impedance parameters, we divided the analyzed myocytes into 4 groups. The largest group (S1) contained myocytes with relatively steady membrane capacitance ($\Delta C_M \sim 6$ pF) and variable membrane resistance ($\Delta R_M \sim 580$ MΩ). The second largest group (S2) involved myocytes with $\Delta C_M \sim 20$ pF and relatively stable membrane resistance $\Delta R_M \sim 60$ MΩ. In the S3 group, profound changes in dynamics were observed, both in membrane

capacitance and membrane resistance ($\Delta C_M \sim 12$ pF, $\Delta R_M \sim 160$ MΩ). One of myocytes (S0) displayed both parameters stable ($\Delta C_M = 2$ pF, $\Delta R_M = 8$ MΩ) during the recording. Correlation analysis of fluctuations revealed both dependent and independent dynamics of membrane capacitance and membrane resistance changes. We conclude that dynamics of the sarcolemmal impedance reflects complex processes regulated at the level of single myocytes. The variability of the basic impedance parameters of myocytes might represent a useful measure for characterization of the sarcolemmal dynamics in physiological experiments.

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PREDICTIVE EQUATIONS FOR NUTRITIONAL INTAKE OF ENERGY AND SUBSTRATES OF CZECH PREGNANT WOMEN

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The maternal diet must provide sufficient energy and nutrients to meet the mother's usual requirements, as well as the needs of the growing fetus, and enable the mother to lay down stores of nutrients required for fetal development as well as for lactation (1). Equations linking nutritional intake of energy and substrates (NIES) during pregnancy to anthropometry as expression of dietary pattern (2) of Czech pregnant women are currently unknown. A total of 152 randomly-recruited healthy pregnant Czech women (nonsmokers, not users of chronic medications or abusers of alcohol or drugs, normoglycemic, and not anemic) were divided into two cohorts: group 1 (n=31) was used for determination of the equations for NIES during pregnancy and group 2 (n=121) for cross-validation of these equations. Anthropometry and the resting energy expenditure of the women in both study groups were examined by indirect calorimetry after 12 h of fasting during four phases of pregnancy. NIES was evaluated from self-reported dietary intake records over 7 days. Strong relationships were found between NIES and anthropometric parameters, especially the difference between pregnancy body weight and ideal body weight (W-IBW). By correlation analysis and linear regression, new predictive equations were derived for NIES during pregnancy using the variable (W-IBW). We observed high concordance between values from the predictive equations and the actual assessed values of NIES in group 2. Used method for derivation of predictive equations as expression of dietary patterns in other studies can be applied.

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CALCIUM SPIKES IN RAT CARDIAC MYOCYTES HAVE A QUANTAL CHARACTER

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During excitation-contraction coupling in cardiac myocytes, Ca^{2+} ions needed for contraction are released from the sarcoplasmic reticulum *via* calcium release units (CRU) composed of a large number of ryanodine receptors (RyRs). In subsarcolemmal CRUs, amplitudes of Ca^{2+}-release fluxes were related to only a small number of RyRs activated during a spark (1). In this work, we directly measured local Ca^{2+}-release fluxes as Ca^{2+} spikes triggered by Ca^{2+} current to estimate the number of RyR channels activated in intracellular CRUs. Ca^{2+} currents of isolated rat ventricular myocytes were activated by 80-ms voltage pulses from -50 mV to 0 mV under whole-cell patch clamp conditions. Ca^{2+} spikes were measured by laser scanning confocal microscopy using Ca^{2+} indicator Fluo-3, and their parameters were determined by fitting their time course with a theoretical function (2). Out of 305 analyzed release sites, 13.4 % responded to the stimulation by two subsequent spikes. Maximum likelihood fitting of amplitude distributions of the set of the early (single and first) spikes revealed 4 quantal levels with equal amplitudes of 1.87 $\Delta F/F_0$, while fitting the set of the second spikes revealed 2 quantal levels with a smaller quantal amplitude of 1.31 $\Delta F/F_0$. The probability of occurrence of the second spike decreased with increasing quantal size of the first spike. The amplitude distribution of early and second spikes could be well approximated with the binomial distribution, suggesting that spike amplitudes differ due to a different number of simultaneously open RyRs (3). We conclude that two calcium spikes may be generated by the same dyad if a smaller number of ryanodine receptors randomly activates during the first calcium spike.

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THE EFFECT OF FUNGICIDE TOLYLFLUANID ON OVARIAN BIOMETRY AND CONCENTRATIONS OF OVARIAN STEROIDES DURING ASSISTED OESTRUS CYCLE OF EWES

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The tending ewes on grass pastures can be exposed to effect of pesticides/fungicides, which influence physiological activities by cumulation in organism. Tolylfluanid (TLF) is phenylsulphamide fungicide with mycostatic and mycolytic impact for mildews as potential resource of mycotoxins. Aim of this work was to follow the effect of TLF on biometry of ovaries and concentration of ovarian steroids (progesteron – P_4, oestradiol 17β – E_2) during assisted oestrus cycle of sheep breed Improved Valachian. Group 1 (n=5) was control. Ewes of group 2 (n=5) were subsequently induced to oestrus with 25 mg of Fluorogeston acetate (FGA) per head/13 days (intravaginal sponges). After withdrawal of sponges animals were treated by i.m. injection of 500 IU equine chorionic gonadotropin (eCG). TLF was applied to the ewes of group 3 (for 30 days) in dose of 20-fold of NOEL. During TLF application ewes were treated by combination of FGA+eCG as group 2. Blood sampling was realized in time October-December. The concentrations of ovarian steroids (P_4 and E_2) were determined in blood serum using RIA. Then animals were undergone laparotomy and ovariectomy. We achieved partial biometric measurements of ovaries and fixed them in 4 % formalin. The ovaries were processed by current histological method. The results were statistically evaluated by ANOVA (mean ± S.E.M) and considered significant at the level $P<0.001$. Means were compared by Tukey test. The biometric parameters (length, breadth and height) showed no significant differences in all groups. In group, where TLF with combination FGA+eCG was applied, significant changes of ovarian steroids concentrations ($P<0.001$) were achieved at comparison with control and group treated by FGA+eCG. Our partial results showed on fact that though TLF is no toxic for animals but can influence concentrations of ovarian steroids**,** including oestral cycle and folliculogenesis.

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THE CONCENTRATIONS OF OVARIAN STEROIDS DURING OESTRUS INDUCTION AND SYNCHRONIZATION OF ANOESTROUS EWES BY OVSYNCH PROTOCOL

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Anoestrous ewes have deficient releasing of gonadotropins from hypothalamus by absence of oestrous cycle. OvSynch protocol is biotechnical method using combination of i.m. application gonadotropin

(GnRH) with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) for induction and synchronization of oestrus and ovulation in non-breeding season. In our study we investigated changes of the ovarian steroids concentrations (progesterone – P_4 and oestradiol 17β – E_2) in blood serum during OvSynch protocol treatment in anoestrous ewes (June). The ewes of Improved Vallachian breed were separated into two groups: 1st group - control (C, n = 6) and 2nd group was experimental (E, n = 6). Consequently, ewes of experimental group were treated according to OvSynch protocol: induction of oestrus with GnRH (0.0125 mg/head), after 5 days ewes were synchronized with $PGF_{2\alpha}$ (0.0375 mg/head) and after forty-eight hours second dose of GnRH (0.0125 mg/head) was injected. The blood samples were obtained from the animals to determine concentrations of ovarian steroids before performed interventions and after ending of treatment. The concentrations of P_4 and E_2 in blood serum were determined using RIA. The analysis of P_4 concentration of control in comparison with experimental group showed no significant changes before (1.407 ± 0.539 $ng.ml^{-1}$ and 1.686 ± 0.381 $ng.ml^{-1}$, respectively) and also after treatment (1.750 ± 0.645 $ng.ml^{-1}$ and 0.828 ± 0.213 $ng.ml^{-1}$, respectively). Concentration of E_2 was significantly increased ($P<0.001$) in experimental group (111.1 ± 17.31 $pg.ml^{-1}$) in comparison with control (15.03 ± 1.832 $pg.ml^{-1}$) after treatment. Before treatment the changes were no significant (C: 18.05 ± 3.304 $pg.ml^{-1}$; E: 66.49 ± 10.45 $pg.ml^{-1}$). Our results show that the GnRH application can stimulate secretion and releasing of gonadotrophins (FSH, LH) in anoestrous ewes and thus positive effect on the increase of E_2 concentrations and folliculogenesis. Prostaglandin $F_{2\alpha}$ has specific luteolytic effect producing good conditions for start of oestrus and ovulation.

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ANTIOXIDANT PROPERTIES OF THE TEAS: COMPARATIVE ANALYSIS

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In this work session we study antioxidant activities of some species of *Camellia sinensis* using a system of unilamellar liposomes. Peroxidative process was initiated by Fenton reaction which produces free radicals damaging lipid bilayer membrane. Longevity (black) tea, Tie Guan Yin (oolong) tea, Special Chunmee (green) tea, Pai Mu Tan Superior (white) tea and Matcha tea (Japan powder tea) are species of plant *Camellia sinensis* whose leaves and leaf buds are used to produce tea for almost 5000 years [1]. In summary, we have compared antioxidant activities of these natural products with well-known antioxidant α- tocopherol (vitamin E). Antioxidant activity of teas was monitored by encapsulating them in a different concentration into the structure of liposomes and measuring changes of Klein peroxidation index [2] with concentration of tea extracts. Obtained results imply that antioxidant activity of *Camellia sinensis* compared with α-tocopherol decrease in a sequence: α-tocopherol > green tea > white tea > oolong tea > matcha tea > black tea.

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BISPHENOL A INHIBITS RECOMBINANT T-TYPE CALCIUM CHANNELS

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Bisphenol A (BPA) is a chemical estrogen widely used in the food-packaging industry and baby bottles (1). BPA is a single hydrocarbon molecule that binds with other molecules to form polymers, such as polystyrene and polycarbonates (2). In rodents, BPA is associated with early sexual maturation, altered behavior, and effects on prostate and mammary glands. In humans, BPA is associated with cardiovascular disease, diabetes, and male sexual dysfunction in exposed workers (3). The aim of our study was evaluate effect of bisphenol A to $Ca_v3.1$ and $Ca_v3.2$ channels. We have used whole cell configuration patch-clamp method. The experimental objects were HEK 293 cells permanently transfected with cDNA encoding main subunit for $Ca_V3.1$ and $Ca_v3.2$ calcium channels. The bath solution contained (in mM): HEPES 10, CsCl 5, $CaCl_2$ 2, $MgCl_2$ 1, NaCl 135; pH 7.4 with NaOH. The pipette solution contained: (in mM): CsCl 130, Na-ATP 5, EGTA 10, HEPES 10, $MgCl_2$ 5, TEA-Cl 10; pH 7.4 with CsOH. Stock solution of bisphenol A was prepared in methanol in 100 mM concentration and was diluted to experimental concentration (10 μM a 100 μM) in a bath solution prior to experiment. Effect of BPA was evaluated at holding potential –100 mV. Bisphenol A inhibited current through both $Ca_V3.1$ and $Ca_v3.2$ calcium channels. 10 μM concentration of drug inhibited 25 % of the current amplitude through the $Ca_v3.1$ channel and 50 % through the $Ca_v3.2$ channel. BPA in concentration of 100 μM inhibited fully calcium current through $Ca_v3.1$ and $Ca_v3.2$ channels. These concentrations are higher than those expected during chronic environmental exposure. They could be reached during acute intoxication only.

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STRESS AND ITS HORMONAL CONTROL IN INSECTS

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Various stress situations in insects are controlled by adipokinetic hormones (AKHs), which are synthesised, stored and released by neurosecretory cells from the corpora cardiaca, a neuroendocrine gland connected with the brain (1). A major function of these peptides is control of insect metabolism, however, the AKHs are pleiotropic, with a number of actions attached to their metabolic role that combat the stress problems and suppress processes that are momentarily less important (2). Two AKHs were isolated and characterized from a model species - the firebug *Pyrrhocoris apterus* where they control a mobilization of lipid stores and stimulate a general locomotion (3, 4). Recently, it has also been proven in this species that AKHs are involved in the activation of antioxidant protection mechanisms because a positive feed back regulation between an oxidative stressor action and the level of AKH has been recorded (5). Thus the AKH application increased a level of reduced glutathione - a metabolite responsible for the destruction of reactive oxygen species. Additionally, the AKH modulated (probably indirectly) the activity of catalase that reduces hydrogen peroxide to water and oxygen. It has also been found that AKHs participated on antistress reaction elicited by insecticides: surprisingly, AKH significantly increased the lethal effect of the insecticides alone (6). It is supposed that AKH-induced increase of metabolism accelerated an exchange of metabolites including a faster penetration of the insecticides into tissues. The results show that AKHs provide an initiation of the complex antistress response that involves both biochemical and physiological protective mechanisms.

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APOLIPOPROTEINS AND LIPIDS IN METABOLIC SYNDROME

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Metabolic syndrome accelerates the course of atherosclerosis. Apolipoproteins (apo) mainly $apoB_{100}$, apoA-I and apoB/ apoA-I ratio

are strong independent predictors of early atherosclerosis and initial coronary events. Several indices related to apoC-II and apoC-III blood serum levels have been proposed to reflect TG metabolism more accurately than the serum level of TG. The hypertriacylglycerolemia is a rare hereditary disorder usually closely associated with abdominal obesity. 27 subjects with metabolic syndrome have been selected for the realisation of the pilot study (MS). 21 healthy subjects with normal body weight, without any hereditary lipid disorder and with normal lipid and apolipoprotein profile were designed as the control subjects. ApoA-I, B_{100}, C-II and C-III serum levels were determined by the RID and EIA methods using the standards and antibodies from Japan and Germany. The TG, TC and HDL-C serum levels were assessed by using Czech biochemical sets. The LDL-C and non HDL-C serum levels were calculated. In the group MS against C the mean of the apoC-III: 15.4±4.8 mg/dl ($p<0.002$), apoC-II: 7.4±1.5 mg/dl ($p<0.001$), $apoB_{100}$ serum level: 111.2±18.2 mg/dl ($p<0.001$), apoB/apoA-I ratio ($p<0.001$), TG, TC and LDL-C has been found significantly increased versus C. The mean of the apoA-I and HDL-C has been found significantly decreased versus C. Increased BMI in group MS about 3.1 $kg.m^{-2}$ against C is a biologically important gain, what associates with significant changes in the atherogenic apolipoprotein and lipid serum levels in the subjects with metabolic syndrome.

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COMPUTATIONAL DESIGN OF COMBINED ELECTROPORATION AND MAGNETOFECTION DEVICE FOR CELL TRANSFECTION

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Effective transfection of the cells by functionalized macromolecules is one of the problems in the field of biomedicine and biotechnology. Besides biological targeting, attention is focused also on physical techniques, when the specificity is ensured by localized application of physical forces [1], whether mechanical, electrical or magnetic, photonic or thermic effects. Magnetofection [2], technique when superparamagnetic particles with reversibly bonded effective compounds are focused to target place whit high gradient and high intensity magnetic field, is one method which was concerned. Magnetofection by itself supports cell transfection, but not by cell membrane permeabilization and tranction compounds into the cells, but accumulation complexes magnetic particle-effective compound to the cells surface. For cell membrane permeabilization for macromolecules was magnetofection combined with electroporation, technique using short high intensity electric pulses for pore formation, which remain open order of hundreds milliseconds to several seconds. We have computationally simulated motion of superparamagnetic micro as well as nanoparticles in several sources of high gradient magnetic field like magnetic quadrupoles [3, 4] or originally homogenous magnetic field affected by ferromagnetic discs and shown that capture time of motion of magnetic particles is sufficiently short, comparable with time of existence of membrane pores. A new flow-through electroporation cuvette combined with cell separation and magnetofection was computationally designed.

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GENETIC MARKERS OF SEX HORMONES EFFECTS IN AUTISTIC GIRLS. A PILOT STUDY

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Autism is a neurodevelopmental disorder characterized by social deficits, impaired communication and repetitive behavior. One of the theories potentially explaining autism etiology is the extreme male brain theory (Baron-Cohen *et al.* 2005). According to this theory autism represents an extreme of the male pattern. Possible mechanisms of autism pathogenesis involve effects of higher testosterone levels during the prenatal period resulting in hyperandrogenic brain development. Genetic factors involved in the regulation metabolism of testosterone may play an important role in the pathogenesis of autism. The aim of this study was to investigate genetic factors related to testosterone metabolism in autistic girls in Slovakia in comparison to healthy controls. Seventeen girls with diagnosed autism (ICD 10) and 63 age-matched healthy girls attending general elementary schools were recruited into the study. DNA was isolated from buccal swabs and particular gene sequences were amplified by PCR. We examined four polymorphisms in genes encoding the androgen receptor (AR), 5α-reductase (SRD5A2), estrogen receptor alpha (ESR1) and sex hormone binding globulin (SHBG). These polymorphisms were examined using restriction fragment length polymorphism or capillary electrophoresis. There were no significant differences in genotype distribution in polymorphisms in SRD5A2, ESR1, SHBG and AR genes between autistic girls and controls. The G allele of the analyzed SHBG polymorphism was slightly higher prevalence in autistic girls. Further studies with larger cohorts are needed to determine the role of genetic factors in female autism.

Baron-Cohen S, Knickmeyer RC, Belmonte MK. Sex differences in the brain: implications for explaining autism. Science 2005, 310: 819-823.

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CASPASE-3 IN MOUSE LIMB DIGITALIZATION AND IMPACT OF PHARMACEUTICAL INHIBITION

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Limb digitalisation is the most striking example of embryonic apoptosis as it leads to separation of individual digits and final limb shaping. Apoptosis in interdigital segments occurs in caspase dependent manner. However, alternative cell death initiation was shown in case of general caspase inhibition. Caspases, as cystein proteases, become activated in a proteolytic cascade starting by receptor-ligand interaction or release of pro-apoptotic factors from mitochondria. This work focuses on caspase-3 as the central caspase of both pathways of apoptosis activation. Localisation of active caspase-3 in interdigital regions during *in vivo* development was performed by immunohistochemistry (IHC) of paraffin-embedded mouse front limbs, embryonic day (E) 12.5-15.5. These days correspond to the digitalisation, which was simultaneously followed morphologically and according to proliferation (PCNA IHC) and apoptosis (TUNEL assay). As expected, temporospatial distribution of active caspase-3 clearly correlated with apoptotic (positively) and proliferating (negatively) cells. As the highest activity of caspase-3 was found at E13.5, mouse limbs staged E12.5 and E13 were used in the modulation experiment. Caspase-3 was inhibited in limb explants cultures by addition of pharmaceutical fluoromethylketone inhibitor (R&D System) administered in the culture medium. Correct growth of explants cultures was monitored by PCNA evaluation, samples were taken every 24 h up to 3 days. Our results showed a distinct role of caspase-3 in interdigital webbing regression. Nevertheless, compensatory mechanisms and/or alternative cell death become

activated after caspase-3 inhibition. These processes are recently under study.

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PREMOVEMENT COMPONENTS OF THE SLOW POTENTIAL RESPONSE EVOKED BY SELF-PACED MOVEMENT REPRESENT DISTINCTIVE BRAIN OPERATIONS

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Though consisting of an early and a late component, the potential which precedes a voluntary movement (Readiness Potential – RP) is considered mostly as a unitary phenomenon. Our endeavor was to demonstrate that the components are electrophysiological correlates of distinct brain operations. The RPs recorded in 44 epilepsy surgery candidates (26 men, 18 women; age from 18 to 43 years) during self-paced clenching movements of the hand opposite to the explored hemisphere were investigated in the study. Microdeep (DIXI Besançon) intracerebral 5-15 contact platinum electrodes were used. The averaged curves were calculated from approximately 30 trials in each case. All the records were taken with a binaural reference. The total number of recording contacts was 1305; the event-related premovement potentials were observed in 123 more or less restricted areas. Sixty-four of these RPs exhibited signs of local generation (amplitude gradient or polarity reversal between neighboring contacts of an electrode), the remaining 59 responses were far-field potentials. Three types of premovement responses were observed: (i) RP with both components; (ii) RP with the late component only; (iii) RP with the early component only. In the subgroup of complete RPs, the polarity and amplitude gradient of both components varied independently of each other. Two types of structures generating the RP with the early component only were found: (i) structures with responsiveness during movement and (ii) structures without responsiveness during movement. The generators of the premovement responses were localized in 21 distinct anatomical structures. The following conclusions could be formulated from the results: Electrophysiological characteristics, such as polarity and amplitude gradient, are not identical in early and movement RP components; the RP is a heterogeneous phenomenon. Three types of structures are activated during a voluntary movement in the premovement period. These structures differ in their integrative potential. "Voluntary" mechanisms from two different networks could be considered to participate in a self-paced movement task; the first seems to be directly linked with the network sustaining movement planning and execution, the second could be engaged in higher level voluntary tasks.

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ENHANCED RESISTANCE TO ISCHEMIC INJURY AND DIFFERENTIAL EFFECTS OF ISCHEMIC PRECONDITIONING IN ISOLATED FEMALE RAT HEARTS

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Gender differences represent one of the important factors that may determine lower susceptibility to ischemia/reperfusion (I/R) injury in females, however, experimental data on the mechanisms of cardioprotection are not always consistent. Phosphatidylinositol 3-kinase (PI3K)/Akt, the system that plays one of the key roles in the protection against ischemia/reperfusion (I/R) injury conferred by ischemic preconditioning (IPC), has been found activated in the female heart (1). Therefore, the present study was designed to investigate whether potential resistance to acute myocardial I/R injury in adult Wistar female rats can be further facilitated by IPC. Isolated Langendorff-perfused hearts of female and age-matched male rats were subjected to 30-min LAD coronary artery occlusion without or with prior IPC (1 cycle of I/R, 5 min each) for the measurement of ischemia-induced ventricular arrhythmias followed by 2-h reperfusion for the evaluation of the infarct size (IS; TTC double staining) expressed as % of the area at risk (AR) size. Lower susceptibility to I/R in female hearts was documented by decreased infarct size (IS/AR 20.3 ± 0.6 % vs. 34.3 ± 0.4 % in the male hearts; $P<0.05$). In addition, the total number of premature ventricular complexes (PVC) and duration of ventricular tachycardia (VT) was significantly smaller in female than in male hearts (81 ± 15 and 3.2 ± 1.7 s vs. 550 ± 60 and 42 ± 8 s, respectively; $P<0.05$). IPC reduced IS/AR by 67 % and 55 % in both, males and females, respectively. In contrast, suppression of PVC and VT by IPC occurred only in males, while no further antiaarhythmic effects were observed in females. Adult female rats are more resistant to acute myocardial infarction and severe ventricular arrhythmias than male rats of the same age. IPC confers efficient antiinfarct protection in both genders, however, antiarrhythmic protection is not facilitated by IPC in females. These differences may reflect differential role of PI3K/Akt in antiinfarct and antiarrhythmic protection.

Bae S, Zhang L. J Pharmacol Exp Ther. 2005 Dec;315(3): 1125-35.

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EFFECT OF MANGANESE ON INNATE IMMUNE SYSTEM IN GOATS

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Neutrophils after activation produce reactive oxygen (ROS) and reactive nitrogen species (RNS) together with microbicidal peptides and proteases. Thus, generation and release of ROS contribute to innate immune response against bacterial infection. Manganese (Mn) is an essential trace element in all forms of life. The classes of enzymes that contain manganese cofactors are very broad. The best known is Mn-containing superoxide dismutase (Mn-SOD) which is a major scavenger of ROS. Mn uptake is essential for the innate immune system. The experiment was conducted to determine the effect of adequate level of manganese supplementation on innate immune response of goats. Twenty four kids after weaning (69 days) were divided into four groups: (1) control (no supplemental Mn), (2) inorganic form (manganese sulphate), (3) organic form (manganese proteinate) and (4) organic form (manganese glycinate). The blood samples were investigated at the age of 90 days of kids. The mothers (age of 6 years) were divided into the same tested groups and received the same doses of Mn as their kids. 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 calcium ionophore A23187 (Cal-I), opsonised zymosan (OZP) and phorbol-12-myristate-13-acetate (PMA). Our data suggested that organic form of Mn can be beneficial for neutrophil function of kids and their mothers. The production of ROS was higher in both the organic groups and also phagocytic activity and phagocytic index (as a marker of the immune function) were higher in organic-Mn-treated groups. Considerable differences were found in the count of leukocytes, as well as in other observed parameters between kids and their mothers. There is little evidence about which form of manganese (organic vs. inorganic) is accepted better by ruminants. Our data on the innate immune response indicate that organic form of Mn can be suitable for enhancement of innate immune system in goats.

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DENSITY OF DENDRITIC SPINES IN HIPPOCAMPUS AFTER FLUROTHYL INDUCED SEIZURE

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Short single seizure elicited by flurothyl vapours causes impairment of learning in Morris water maze. It could be prevented by preceding (3 days) hypobaric hypoxia or application of melatonin (60 min before the seizure). As a consequence of epileptic seizure both the increase and decrease of the density of dentritic spines at the apical dendrites of pyramidal cells in the hippocampus were described. Hippocampus is closely related to spatial memory formation. Therefore we used our model of flurothyl seizures. Adult male Wistar rats were subjected to flurothyl vapours until they exhibited tonic-clonic seizure. Animals were sacrificed 24 hours later (together with control animals) and their brains were impregnated (Golgi-Moliner) to visualize the dendritic spines. In controls we found 7.7 spines/10 μm and 8.9 spines/10 μm in experimental animals. The increase was highly significant (ANOVA $p<0.001$). We suppose that learning is related to both the slight increase as well as to decrease in dendritic spine density. When the seizure shifts the density to maximum or minimum, then the freedom in oscillation decreased and learning could be impaired. This structural alternation is probably not the only cause of the learning impairment after flurothyl seizure.

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c-MYB MAY PLAY A ROLE IN HARD TISSUE MINERALISATION

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c-Myb transcription factor is involved in the control of cell proliferation, survival and differentiation. As these processes accompany morphogenesis of developing teeth, we investigated the possible role of c-Myb during odontogenesis. c-Myb expression in developing teeth and correlation of c-Myb expression with proliferation and apoptosis were reported earlier (1, 2). This work pays a special attention to the mineralization stage of tooth development. The first mouse molar was used as a model system to follow the expression of c-Myb at RNA and protein levels using methods of immunohistochemistry and *in situ* hybridization. Abundant c-Myb expression is often associated with immature stages of cellular differentiation and decreases during organ development and tissue differentiation. However, the link does not completely apply in odontogenesis as c-Myb positive cells were also found in differentiated cells of the tooth germ (ameloblasts, odontoblasts). One of possible function supported by our findings could be a role for c-Myb in calcium metabolism. Therefore, c-Myb might regulate the calcium level in odontoblasts or ameloblasts and contribute to the mineralization of dentin or enamel. This hypothesis can be supported by the fact that also osteoblasts and osteoclasts were c-Myb positive. Thus, c-Myb may be predicted to participate also in bone remodelling during early embryonic osteogenesis and tissue interactions between bone and teeth during alveolus formation. However, only limited information has been published about the role of c-Myb in osteogenesis and further work is necessary to confirm our findings.

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MOUSE EXPLANT CULTURES: HANDS-ON EXPERIENCE IN PHYSIOLOGICAL EDUCATION

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Animal experiments are still an important background for the rapid progress in biomedicine, however, alternative methods are preferred to follow 3R principles (reduction, refinement, replacement) also in education. Organ explant cultures eliminate animal suffering during experiments but simultaneously allow investigations of organ, tissue and cell systems with preserved cell-cell interactions. Explant cultures refer to *ex vivo* systems and a mouse model is widely used in research. The aim of this work was to introduce mouse explants culture approaches and following biomedical applications into physiology education in Czech as well as international undergraduate program at the University of Veterinary and Pharmaceutical Sciences in Brno. The students recently apply mouse explants cultures to investigate several physiological processes and following pathophysiological consequences, particularly: 1) Normal/abnormal development: digitalization staged mouse embryonic limbs are used to clearly follow morphogenesis and digit formation, PCNA immunohistochemistry to detect tissue proliferation; moreover, PCNA is an important marker for cancer progression/regression evaluation; in addition, proliferation markers allow to demonstrate correct development of the cultures. 2) Cell cycle regulation: PCNA along with other cell cycle markers (particularly S-phase) such as Ki67 are used to follow physiological tissue renewal using the duodenum as an example of rapidly proliferating cell populations; consequences of different chemotherapeutics are demonstrated using these markers. 3) Programmed cell death: apoptosis can be followed in normal tissues (e.g. regression of interdigital webbing of the limbs) or after modifications, e. g. caspase inhibition or chemotherapeutical treatment of the cultures. These topics are presented on the background of related Nobel Prizes in Physiology or Medicine (particularly 1995, 2001, 2002). Corresponding guidelines were issued at the University providing students with printed and electronic manuals and knowledge related to these topics.

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DETECTION OF APOPTOSIS IN SPECIFIC TISSUE BOUND CELL POPULATIONS ISOLATED BY LASER CAPTURE MICRODISSECTION

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In mammalian development, apoptosis occurs in several embryonic tissues, starting at the blastocyst. Later apoptosis plays a role in sculpting tissues and organs. Several specific cells may play critical roles in correct embryogenesis as signalling centres. However, investigation of such small populations bound within the tissues use to be difficult. The repertoire of methods for single cell analysis of tissue bound populations has been revolutionized by laser capture microdissection (LCM) technique. Cells identified in histological sections can be harvested as a homogenous population by a laser catapult directly into the test-tube. However, due to limited amount of the material, nucleic acid are mostly studied as amplification methods are available. Recently, a novel approach based on LCM and flow cytometry was introduced for protein analysis and apoptosis detection (1). Sensitive procedures for protein evaluation in LCM samples have been further developed. Caspases represent important molecules responsible for apoptotic cell machinery. As caspases become activated posttranslationally by enzymatic cleavage (caspase cascade), they are often difficult to be examined, particularly in fixed histological sections. Recently, ELISA of LCM samples was successfully applied for active caspase-3 detection in mouse embryogenesis. The primary enamel knot as a signalling centre of developing teeth and digitalization staged

mouse limbs were investigated. Further studies of caspase activation during embryogenesis are in progress using these novel approaches.
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CASPASE-7 AND DENTAL APOPTOSIS

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The key proteins involved in major apoptotic pathways are caspases, a large family of cysteine aspartate proteases. At least 15 mammalian caspases being involved in apoptosis or/and inflammation have been reported so far. Initiator caspases (caspase-8, -9, and -10) are activated during the first steps of apoptosis *via* death adaptor molecules proximity and oligomerization induced proteolytic processing. Once activated, the initiator caspases cleave other members of the family called effector caspases (caspase-3, -6, -7), which subsequently cause degradation of several cellular polypeptides that are essential for cell survival (lamin, PARP) resulting to DNA fragmentation, cytoskeleton break-down, and cell death. The molecular machinery of apoptosis in mammals is very complex and involves many redundant molecules. Caspase-3 and caspase-7 were long-time considered as functionally redundant. As caspase-3 was found important in dental apoptosis (1) this work aimed to investigate engagement of caspase-7 in tooth development using immunohistochemistry of active caspase-7, biochemical detection of DNA breaks (TUNEL assay) and knock-out approaches. The stage of the primary enamel knot characteristic by a distinct population of apoptotic cells was in particular focus. Caspase-7 deficiency did not cause any alterations in the primary enamel knot apoptosis (presence of apoptotic bodies, TUNEL positivity). Interestingly, active caspase-7 was found in osteoclasts in areas forming the bony socket of developing molar tooth. Moreover, active caspase-7 was found also in osteoclasts not undergoing apoptosis (TUNEL negative). These findings suggest possible involvement of caspase-7 in osteoclast differentiation as reported for caspase-3 (2) and/or at later stages of tooth development (recently under investigation).
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TIME-COURSE OF CHANGES IN ERYTHROCYTE DEFORMABILITY, MEMBRANE RIGIDITY AND SOME BIOCHEMICAL VARIABLES IN PATIENTS WITH BREAST CANCER TREATED BY RADIOTHERAPY

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Impairment of erythrocyte membrane elasticity has been detected under several pathological conditions (diabetes, sepsis, anaemia etc.), and also in the radically treated patients (1). The changes in haemorheological properties of erythrocytes have been examined in patients with advanced breast cancer, who were subjected to radiotherapy. Since both the disease and ionizing radiation can adversely influence erythrocyte deformability (ED), we examined changes in ED and selected relevant variables, (erythrocyte membrane rigidity – EMR, haematological and selected biochemical variables as well as acid-base variables) in 15 patients, mean age 55 years (range 36-75), in pre-treatment, during treatment and post-treatment periods. ED was analyzed by filtration of erythrocyte suspensions, EMR was measured with a fluorescence probe, diphenylhexatriene, on isolated erythrocyte membranes. Our results revealed that, on average, ED was significantly decreased in the pre-radiation and radiation periods. Up to the 3rd week after external radiotherapy, ED gradually increased in contrast to decreased EMR, with a subsequent decline in ED associated with increased rigidization of the membrane in the 4th week. The time relationship was described by Spearman's rank correlation coefficient (Rho) near to minus one; $P = 0.042$. We found significant or nearly significant correlation between ED and pH or pCO_2 of the blood ($Rho = 0.42$; $P = 0.043$ and $Rho = -0.40$; $P = 0.078$, respectively). Study of ED, which is a factor of key importance in microcirculation for securing tissue perfusion and oxygenation, provides new possibilities for evaluation of clinical status in patients.
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DIFFERENTIAL ROLE OF PI3K/AKT IN THE MECHANISMS OF INCREASED TOLERANCE AGAINST ISCHEMIA-REPERFUSION INJURY INDUCED BY PRECONDITIONING

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Acute activation of phosphatidylinositol 3-kinase (PI3K) and its effector protein kinase B (Akt) during the preconditioning (PC) procedure plays an important role in cardioprotection against ischemia/reperfusion (I/R) injury by mediating antiapoptotic effects (1). However, most of the studies focused on the postischemic phase of I/R only. In the present study, PI3K/Akt inhibitor wortmannin (W; 100 nM) administered 15 min prior to test ischemia was used to elucidate a role of PI3K/Akt in the effect of PC on ischemic as well as postischemic phase of sustained I/R. Langendorff-perfused non-PC rat hearts and hearts preconditioned with one cycle of I/R (5 min each) were subjected to either 30-min LAD coronary artery occlusion for the study of ischemic arrhythmias or 30-min global ischemia followed by 2-h reperfusion for the infarct size (IS) determination (in % of the area of left ventricle, LV) and assessment of postischemic contractile dysfunction and reperfusion-induced ventricular arrhythmias. Pretreatment with W modified neither arrhythmogenesis nor IS in the non-PC hearts. Bracketing of PC with W did not abolish antiarrhythmic protection during ischemia (total number of premature ventricular complexes, PVC: 92 ± 25 vs. 166 ± 40 in the untreated PC hearts). On the other hand, W exacerbated arrhythmias during reperfusion (PVC: 650 ± 103, incidence of ventricular fibrillation: 57 % vs. 249 ± 147 and 25 %, respectively, in the untreated PC group; $P<0.05$) and blunted IS-limitation (IS/LV 24 ± 1.2 % vs. 9 ± 0.6 % in the untreated PC hearts; $P<0.05$). In addition, W attenuated the improved postischemic recovery of LV developed pressure in PC hearts (44 ± 4 % of preischemic values vs. 71 ± 11 % in the untreated PC group; $P<0.05$). Conclusions: in contrast to postischemic injury, enhanced resistance to ischemic arrhythmias does not appear to be mediated by PI3K/Akt suggesting that PI3K/Akt activity may play distinct roles in different end-points of PC-induced cardioprotection.
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EFFECT OF ISOFLAVONE OSAJIN AGAINST PROLONGED ISCHEMIA AND REPERFUSION INJURY IN THE ISOLATED PERFUSED RAT KIDNEY

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Bioflavonoids may diminish cold storage-induced injury due to antioxidant and iron chelating activities. This study was designed to delineate the renoprotective mechanisms of bioflavonoid osajin of

5 mg/kg dose per day in pre-treated two weeks therapy of reperfusion injury after a 48 hours cod storage. Three groups of animals – treated, placebo and intact. Animals were anesthetized, kidney were immediatlly flushed in situ with 80 ml of a cold preservation solution. After excision, the kidney were stored at 4 °C for 48 hours. After cold storage the kidney were reperfused (mean arterial perfusion pressure of 100 mm Hg), urine and perfusate samples were collected and perfusion flow rate (PFR), diuresis, total protein and malondialdehyde were analysed. The kidney of treated group showed significantly higher PFR in comparison with placebo and intact group. Kidney of placebo and intact group showed significantly lower diuresis and significantly higher total protein concentration than in treated group. Malondialdehyd was increased significantly in both two groups too. Osajin medication supported antioxidative system and reduced lipoperoxidation such as participated in the renoprotective mechanisms in pre-treated animals.

EFFECT OF PIRINIXIC ACID ON MYOCARDIAL ISCHEMIA/REPERFUSION INJURY IN ISOLATED RAT HEART

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Pirinixic acid, a hypolipidemic drug WY 14643 (WY), is a synthetic exogenous ligand selectively activating the nuclear receptors PPAR-α. Beneficial effects of treatment with hypolipidemics are caused not only by their antihypercholesterolemic effects but by their pleiotropic actions. Since positive effects of synthetic PPAR agonists on cardiovascular diseases have not yet been sufficiently validated, our study was designed to examine the lipid-independent effects of WY in protection against acute myocardial ischemia, specifically its effects on the size of myocardial infarction and incidence of reperfusion-induced arrhythmias in normocholesterolemic rat hearts. Adult Wistar rats were administered WY for 5 days (3 mg/kg/day, p.o.). On the sixth day, isolated hearts of pretreated and control rats underwent 30-min global ischemia and subsequent 2-h reperfusion for the assessment of infarct size (IS, in % of the size of the risk area, TTC staining) and incidence of reperfusion-induced arrhythmias. After 5-day treatment with WY, a significantly smaller IS (20 ± 3 %) was observed in the treated group as compared with the control group (42 ± 2 %; $P<0.05$). In addition, the suppressed occurrence of ventricular fibrillation and reduced duration of ventricular tachycardia had a positive impact on the overall severity of reperfusion arrhythmias (reduced arrhythmia score). Our results suggest that administration of WY alleviates lethal ischemia-reperfusion myocardial injury manifested by a smaller size of infarction and reduces the incidence and severity of arrhythmias during reperfusion indicating that lipid-independent effects of PPAR activation are involved in cardioprotection in this model of myocardial ischemia.

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EFFECT OF CARVEDILOL ON MYOCARDIAL REMODELING INDUCED BY REPEATED LOW DOSES OF ISOPROTERENOL

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Functional studies of the rat heart revealed, that repeated administration of low doses of isoproterenol (Iso) (5 mg/kg s.c. for 21 days) induced high mortality of animals (83 %) as well as symptoms and signs of the failing heart. Addition of carvedilol (Car) (10 mg/kg s.c. for 20 days) to Iso rats decreased the weight and dimensions of the heart and left ventricle, lowered the amplitude of R wave, shortened both QT and QTc intervals, suppressed the myocardial arrhythmic activity, and did not improve contractility of left ventricle of isolated hearts perfused according to the Langendorff. Despite the "therapeutic" effect of beta-blocker carvedilol, the recovery of the observed parameters was incomplete and did not rich the control characteristics before Iso-induced myocardial remodeling. Ultrastructural analysis of Car-treated cardiomyocytes showed marks of reduced of hypertrophy accompanied with the decreased number of caveolae in plasma membranes but increased number of vesicles under the plasma membrane in comparison to myocytes affected by Iso. The membranes of T-system showed vesiculation, which was present in the dyads, too. Electron microscopic analysis revealed substantial changes in the mitochondrial population. The arrangement of mitochondria was irregular**,** occasionally, clusters of mitochondria were found as well as the signs of mitochondrial fusion**.** Cristae of mitochondria were disintegrated or fragmented. It could be concluded that ultrastructural changes in myocardium observed in microdomains at the level of individual cells and remodeling of E-C units after carvedilol-treatment could result into persisted symptoms of failing myocardium.

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A COSINOR ANALYSIS OF 24-HOUR AMBULATORY BLOOD PRESSURE MONITORING IN YOUNG ADULTS; THE EFFECT OF ANTHRACYCLINE THERAPY

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The 24-hour ambulatory blood pressure monitoring (ABPM) allows us to measure circadian variability in systolic and diastolic blood pressures (SBP, DBP). A cosinor analysis adjusts, by least squares, a sinus function to a measured data and provides an estimate of MESOR (a rhythm-adjusted mean defined as the average value of a rhythmic function fitted to the data), amplitude (half the total predictable change between the night and day values of SBP and DBP), acrophase (the lag from a midnight to the highest value on a sinusoidal curve). Anthracycline antibiotics are used in antitumour therapy, but their cardiotoxicity limits their use. To find out the impact of anthracycline therapy on blood pressure values and cosinor analysis parameters. ABPM was evaluated in 88 healthy controls (group C, age: 19 to 22) and in 19 patients with acute lymphoblastic leukemia (ALL) after anthracycline therapy (group A; age: 19 to 22; total cumulative dose of anthracyclines: 227±32 mg/m^2; average time after treatment: 9.2±3.8 yrs.). The ABPM data were considered by cosinor analysis. A comparison of the blood pressures between groups C and A discovered a statistically significant decrease of values in SBP (01:00, 02:00, 03:00, 04:00, 12:00, 13:00, 14:00, 16:00, 24:00), DBP (01:00, 14:00, 15:00). The decrease of BP was clearer in the subgroup of women than in that of men. A cosinor analysis discovered a significant difference in the MESOR of SBP (C vs. A; 119 ± 9.6 vs. 112 ± 7.6 mm Hg; $p<0.01$). The study confirmed late negative effects of anthracycline therapy on blood pressure values.

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SINGLE HIGH DOSE OF ISOPROTERENOL LEADS TO REDUCTION OF MORPHOLOGICAL QUALITY OF DYADIC MICRODOMAINS

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Experimental myocardial injury (MI) induced in rats by a single high dose of isoproterenol (ISO) manifested in isolated ventricular myocytes by changes in calcium spikes activated by calcium current (1).The aim of this study was to compare ultrastructural changes of dyadic microdomains from the point of excitation-contraction coupling. Male Wistar rats was treated with a single dose of ISO (150 mg/kg b.w.) to evoke MI and processed on day 15 after ISO administration. Samples of non ischemic tissue from the endocardial layer of the left ventricular free wall were dissected and processed for electron microscopy. The basic types of dyads were defined and their occurrence was analyzed in randomly selected regions about 5 μm from the sarcolemma, in correspondence to regions were calcium spikes are measured by confocal microscopy. Morphological analysis revealed five types of dyads in myocytes of both the control and the ISO-treated group. The

differences involved t-tubules (appearance of extrusions and caveolae), terminal cisterns (fragmentation and detachment from central t-tubule), and their mutual arrangement in dyadic microdomains. The effect of ISO manifested in changes of the relative occurrence of specific types of dyadic distortions. While in control rats the occurrence of single dyads reached about 61 %, in ISO myocytes their occurrence was reduced to 25 %. Dyads with distorted t-tubules represented about 9 % and 15 % in the control and the ISO myocytes, respectively, while dyads with distorted cisternal membranes represented about 30 % in the control but up to 60 % in ISO myocytes. We conclude that the myocardial injury caused by high dose of ISO is accompanied by substantial reduction in the morphological quality of dyadic complexes. It is very likely, that the observed distortions in the sites of calcium release are responsible for the reduced quality of excitation contraction coupling.
Zahradnikova A jr. et al., J. Physiol. Sci. 59: Suppl. 1, p. 313 (2009).

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INFLAMMATION-INDUCED CHANGES OF SPATIAL EXPRESSION OF CONNEXIN-40 IN RAT AORTA

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Connexin (Cx) channels allow direct chemical, metabolic and immunological coupling of two adjacent cells. In a vascular wall, channels connect cells of endothelium, smooth muscle and endothelial-smooth muscle cells, regulating tissue homeostasis, endothelial permeability and vasomotor tone. Our previous studies demonstrated abnormalities of major connexin (Cx)-43 isoform expression in rat aorta during hypertriglyceridemia and hypertension. Both of them have low inflammatory level. Inflammation is accepted as integral part of coronary heart disease and atherosclerosis, but unanswered question regards spread of inflammation in the vessel wall. We examined, whether inflammation itself can affect Cx40 expression in a vascular wall. Inflammation was induced by lipopolysaccharide (LPS) (E.coli, single dose, 1 mg/kg, i.p.). Spatial expression of Cx40 in aortic endothelium and media of adult Wistar rats after 10-day-lasting inflammation was studied using confocal immunofluorescence (IF) and evaluated by morphometric analysis. We measured also some selected physiological and biochemical parameters. LPS induced significant decrease of endothelial Cx40 spots' density and their area compared to controls. This indicated changes in endothelial permeability, allowing transendothelial migration of leukocytes to subendothelial layer. Contrary, density of Cx40 spots and their area in media of rats with LPS tended to be increased, but not significantly. In addition, LPS impaired NO-dependent relaxation of the aorta and induced increase of NO synthase activity in aorta. LPS increased specific activities of MDA and NAGA in plasma. Our preliminary results indicate that inflammation may induce abnormalities in intercellular communication via Cx channels in aorta, providing new insight on Cx channels as potential regulators in spread of inflammation in vascular wall.

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INITIAL NEURONAL HSP70 NON-RESPONSIVENESS TO EXCITOTOXICITY ATTENUATES NEURONAL SURVIVAL

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Heat shock protein Hsp70 is recognized as a major stress protein affording significant protection against many cellular stress types like hyperthermia, ischemia, trauma and toxicity. However, we reported that whole body hyperthermia did not induce Hsp70 protein either in most forebrain neurons or in astrocytes (1). On the contrary, preconditioning with Hsp70 induction effectively protects against above mentioned stresses, since Hsp70 attenuates both apoptotic as well as necrotic neuronal cell death (2). In this study we used a model of excitotoxic damage in hippocampal CA3 to elucidate Hsp70 induction (Mohammadi *et al.* 2009). Young female rats were anesthetized with urethane (1.25 g/kg). Glutamate (GLU: 10-700 mM), kainate (KA: 0.15 µg), NMDA (3 µg), MK-801 (15 µg), CNQX (25 µg), cyclothiazide (CTZ 10 µg), dihydrokainic acid (DHK: 10 µg) were injected intracerebroventricularly (icv) in 5µl of phosphate-buffered saline. In addition, MK-801 (2.5 mg/kg) and CNQX (30 mg/kg) were also given systemically. Animals were sacrificed 4 hours post injection by perfusion with fixative. Vibratome sections were processed for Nissl staining, Hsp70, c-Fos, OX-42 and GFAP immunohistochemistry (IHC) and electron microscopy (EM). Sections were evaluated quantitatively by image analysis for c-Fos expression and volume of neuronal damage in the hippocampus and Anova statistics. PBS-injected controls showed neither expression of Fos/Hsp70 nor neuronal degeneration in the hippocampus while there was a significant Fos expression in other regions. GLU, supposedly the main neurotransmitter causing excitotoxic damage, did not induced Hsp70 in any concentration used (10-700 mM). Administering GLU with DHK (blocker of glial glutamate transporter) or CTZ (inhibitor of receptor desensitization) did not result in Hsp70 induction. Also the agonists of ionic GLU receptor subtypes did not show Hsp70, despite causing substantial CA3a neuronal damage in case of KA and AMPA injection. We also tried MK-801, the blocker of NMDA receptors, which induces Hsp70 in some limbic regions, but again without response. The possibility that forebrain neurons are generally Hsp70 non-responsive to excitotoxic drugs was excluded since there was extensive induction of Hsp70 at 24 hours after systemic or icv injection of KA or AMPA, but not after GLU and NMDA. We conclude that hippocampal neurons apparently do not react to excitotoxic stress by early Hsp70 induction, which may be undermining their ability to cope with non-NMDA-receptor conveyed neuronal damage.
(1) Pavlik A. et al.: Brain Res., 973, 179-189, 2003.
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AUTO-CPAP IS AN EFFICIENT METHOD TO COMMENCE THE VENTILATORY TREATMENT OF SEVERE SLEEP APNEA IN OBESE MALE ADULTS IN SLEEP LABORATORY

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It has been shown previously that obstructive sleep apnea is a frequent and underdiagnosed sleep-related breathing disorder. An increasing body of evidence suggests that continuous positive airway pressure (CPAP) is the gold standard for treatment of moderate to severe sleep apnea. Our aim was to assess the acute effect of automatic CPAP (auto-CPAP) device in male subjects with severe sleep apnea. A retrospective chart review was performed on 52 consecutive male patients who underwent a baseline and auto-CPAP titration in our lab over the past 18 months. All records were scored by Alice 4 software and were visually evaluated. The exclusion criterion was a baseline apnoe-hypopnoe index (AHI) < 30 events/hour. The primary outcome variables included: AHI, ODI (3 % oxyhemoglobin desaturation index) and meanSaO_2 (mean oxyhemoglobin saturation during sleep). The average age was 52±10 (mean±SD) years, BMI 32.8±4.7 and Epworth Sleepiness Scale 10.7±5.1. The AHI decreased from 62.35 (45.7-70.9) events/hour [median (interquartile range)] to 3.25 (1.05-11.15) events/hour, ODI decreased from 62.5 (43.2-72.5) events/hour to 10.65 (5.45-17.25) events/hour and meanSaO_2 increased from 91 (90-93) % to 94 (92.5-95) % (P<0.001 for each parameter). All subjects tolerated the auto-CPAP well. The use of the auto-CPAP machine during the overnight CPAP titration proved to be safe and efficient in improving all of the observed outcome variables. These findings add a further support to the recognized contribution of auto-CPAP titration compared to a traditional CPAP machine.

THE SELECTED RISK FACTORS OF METABOLIC SYNDROME IN ROMANY AND MAJORITY CHILDREN POPULATION

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The aim of our work was to find the incidence of overweight, obesity and risk factors of metabolic syndrome in Romany and majority children population. Methods: 282 children, aged 2-15 years from the East Slovak region were examined. There were 187 Romany children and 95 children of the majority population. We observed their anthropometric parameters, body weight and height from which the body mass index - BMI (kg/m^2) was calculated. The systolic and diastolic blood pressures was calculated as the average of three blood pressures. Of the biochemical parameters the serum concentration of total cholesterol (TC), HDL-cholesterol (HDL-C), triacylglycerol (TG) were determined by commercial sets of Fy Pliva-Lachema (Czech Republic). The concentration of LDL-cholesterol (LDL-C) were calculated by the Friedewald formula. Concentration apolipoprotein B-100 (apo B-100) was evaluated by electroimmuno-diffusion using standards Fy Behringwerke, Marburg. The systolic and diastolic blood pressures in Romany 2-6-year-old children were significantly higher ($p<0.01$) than non-Romany children. At comparison of the body weight values of the majority population with Romany one a significance in the age group 2-6 years ($p<0.01$). The BMI values in percentiles were Romany boys higher than those in Romany girls. Romany children show riskier values determining a degree of obesity and malnutrition. Concentration of TC in Romany 10-15-year-old children was significantly lower ($p<0.05$). The serum concentration of TG was in most Romany children within the limits with exception for the youngest age group. In the Romany children of the youngest age group, the concentration of TC and apo B-100 correlated significantly ($r=0.55$, $p<0.001$, $n=100$) as well as in the children of the majority population ($r=0.46$, $p<0.001$, $n=50$). Prevention of children from getting to obesity in adulthood is directed prevention, because this along with decreasing the obesity risk in adulthood will also reduce the development of the negative consequences of the latent metabolic syndrome.

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CHANGES IN ADIPOCYTES SIZE IN PERIPARTURIENT COWS WITH RELATIONSHIPS TO SELECTED SERUM PARAMETERS

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A negative energetic balance develops in periparturient dairy cows, which energy release from the fat tissues is essential for covering of. The aim of this study was to evaluate the mean size of adipocytes of the subcutaneous fat tissue together with their changes in relation to biochemical markers of lipomobilization. A total of 32 Holstein cows of the mean parity after calving of 2.4 were included into the study. From each animal was collected a pair of samples of blood and the subcutaneous fat, which was obtained from the *regio clunis* using bioptic forceps. The first sampling was performed 2 to 20 days prior to the delivery (a.p.), while the second 1 to 12 days after the delivery (p.p.). The fat tissue was collected into 10 % buffered formalin, treated using a routine histological technique. Sections of the paraffin blocks were stained in a standard way. The size of a fat cell in each sample was computed using image analysis as the mean area (μm^2) of one adipocyte calculated from 20 cells bearing a nucleus (Soft Imaging System Cell F, OLYMPUS, Japan). Blood sera were examined for the levels of non-esterified fatty acids (NEMK) and β-hydroxybutyrate (BHB). Statistical analysis included the evaluation of the relation between the studied parameters using the Spearman´s test and comparison of paired samples of blood and fat cells areas in whole pre- and post-parturient groups (Wilcoxon test). The mean area of a adipocyte amounted to 7 371 μm^2 a.p. and 5 831 μm^2 p.p. with a highly significant difference ($p<0.001$). While the level of NEMK was higher in p.p. animals ($p<0.001$) (0.10 and 0.94 mmol/l, respectively), no difference was in the level of BHB (0.59 and 0.62 mmol/l, respectively). The area of adipocytes a.p. was in a negative correlation with the level of BHB a.p. ($r= -0.49$; $p<0.01$). The rate of decrease in fat cells size was in correlation with levels of NEMK and BHB a.p. only; with NEMK in a positive way ($r=0.43$; $p<0.05$) and with BHB in a negative way ($r= -0.38$; $p<0.05$). BHB and NEMK were negatively correlated a.p. ($r= -0.50$; $p<0.01$), and positively correlated p.p. ($r=0.36$; $p<0.05$). The results confirmed that fat cells are decreasing in size in periparturient dairy cows and that there is a possibility of using standard biochemical markers because of their relationships to adipocytes size for the evaluation of activity of the fat tissue.

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EXPERIMENTS FOR ANALYSIS OF RESPIRATORY SINUS ARRHYTHMIA BY MEANS OF SPECTRAL METHODS

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Short-term heart rate changes related to respiration, termed as respiratory sinus arrhythmia, is well known component of heart rate variability (HRV) that contribute to high-frequency region of HRV spectrum. In order to study this phenomenon and related signal processing aspects in more detail, we have developed measurement system and signal processing tools for Matlab. Both heart rate and respiration signals are derived from single-channel electrocardiogram (ECG) recorded by means of designed biopotential amplifier connected to universal data acquisition board AD512 (Humusoft). ECG-derived respiration is based on QRS amplitude modulation as a result of cardiac axis deviations due to respiration. Respiration and HRV signals were evaluated by means of: (a) auto-spectral density, (b) coherence function and (c) smoothed pseudo Wigner Wille distribution. Various approaches were considered to obtain auto-spectral densities, including tachogram interpolation, Berger method, spectrum of counts and Lomb-Scargle periodogram. Measurements were conducted in three situations: (1) spontaneous breathing, (2) paced breathing at common respiration rates, (3) paced breathing at elevated rate exceeding mean heart rate halved. In the cases of paced breathing, the measured subjects were asked to breath in synchrony with a moving bar displayed on the computer screen. Synchronous breathing at an elevated rate caused the phenomenon known as cardiac aliasing. We have observed that under such conditions, the Lomb-Scargle periodogram and the spectrum of counts are most suitable methods for detection of components that would be considered to be beyond information limit from the viewpoint of uniform sampling theory.

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ALTERATIONS OF CELL-TO-CELL COUPLING PROTEIN, CONNEXIN-43, DURING VENTRICULAR FIBRILLATION AND SINUS RHYTHM RESTORATION IN PERFUSED RAT HEARTS

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Ventricular fibrillation (VF) is life-threatening arrhythmia, which occurrence precedes development of myocardial arrhythmogenic substrate resulting from either chronic or acute pathophysiological conditions. Our previous and other studies suggest that down-regulation of cell-to-cell coupling protein, connexin-43 (Cx43), facilitate occurrence of malignant arrhythmias (1). We hypothesize that VF itself deteriorates Cx43 alterations and it may hamper cardioversion into sinus rhythm. The purpose of this study was to

examine whether myocardial expression and phosphorylation status of Cx43 is altered during VF and during sinus rhythm restoration. Experiments were performed using male and female Wistar rats. Isolated Langendorff-mode perfused heart was subjected to: (1) ten minutes stabilization, (2) electrically-induced VF lasting two minutes, (3) electrically-induced VF lasting ten minutes, (4) two min VF followed by its termination due to stop perfusion resulting in sinus rhythm restoration. The hearts were snap frozen in liquid N_2 at each stage (1,2,3,4) and ventricular tissues were taken for immunobloting of Cx43 using mouse antiCx43 MAB (Zymed) to detect only un-phosphorylated form (noP) of Cx43 and rabbit antiCx43 MAB (Sigma-Aldrich) to detect phosphorylated (P) as well as noP forms of Cx43. Compared to male rat heart at control stage there was a significant increase of noP forms of Cx43 due to VF lasting 2 or 10 min, while much less at the moment of sinus rhythm restoration. Different to male only 10 min lasting VF induced significant increase of noP forms of Cx43 in female rat hearts. Total Cx43 expression did not change during experiment. However, P forms of Cx43 as well as ratio of P to total Cx43 were significantly decreased due to 2 min and 10 min lasting VF and much more during sinus rhythm restoration in male rat hearts, while not in females. It appears that there is down-regulation of Cx43 due to VF. Whether changes in phosphorylation status of Cx43 are involved in cardioversion induced by stop perfusion should be elucidated more in details.

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ISCHEMIC TOLERANCE IN THE DIABETIC HEART: THE ROLE OF OXIDATIVE STATE AND PRO-SURVIVAL PATHWAYS

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Research suggests that besides higher vulnerability to ischemia/reperfusion (I/R), diabetes mellitus (DM) may evoke development of adaptation leading to paradoxically enhanced ischemic tolerance proposed to share some molecular pathways with preconditioning in non-diabetic myocardium, in particular, those related to oxidative state and activation of pro-survival pathways. We investigated changes induced by 1-wk DM in adult male rats (STZ, blood glucose > 20 mM) in myocardial ROS formation (TBARS), protein expression of nuclear factor κB (NFκB) and eNOS. In addition, we evaluated the involvement of phosphatidylinositol 3-kinase (PI3K)/Akt in preconditioning-like effect in the Langendorff-perfused diabetic hearts subjected to I/R and explored a potential link between cardiac response to ischemia and gene expression of transcription factors PPAR involved in different aspects of lipid metabolism and inflammation (RT-PCR). Results: enhanced resistance to I/R in the diabetics was documented by a 2-fold higher postischemic functional recovery, 60 % decrease of infarct size and lower arrhythmogenesis. This was associated with increased baseline levels of endogenous antioxidants, eNOS expression and unchanged TBARS and NFκB expression. Infarct size-limiting effect was PI3K/Akt-dependent and coupled with reduced ROS production during ischemia and markedly enhanced mRNA levels of PPARs at baseline and post-I/R, in contrast to their down-regulation in non-diabetics. In conclusion, adaptive processes induced in the diabetic myocardium in the acute phase might be associated with the changes in PPAR gene expression and attenuation of oxidative stress during I/R. Hyperglycemia might contribute to lower susceptibility to I/R *via* mechanisms involving PI3K/Akt and its downstream target eNOS.

Malfitano C. et al., Eur. J. Heart Fail. 12: 659-67, 2010.

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AGE RELATED CHANGES OF BLOOD PRESSURE AND HEART RATE IN CHILDREN WITH RESPECT TO GENDER AND BODY BUILD

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The aim of the study was to explore the dynamism of age related changes of blood pressure (BP), heart rate (HR) and of the double product (DP) in children of Slovakia with respect to gender and body build and to assess the impact of obesity on these parameters in different developmental periods. The study group consisted of 3041 boys (B) and 3003 girls (G), aged 3 to 18 years (y), from different regions of Slovakia. At the preventive investigations, basic somatic parameters and the casual sphygmomanometric BP three times in a session were measured, the average of the second and third BP measurements was computed. Only subjects with BP<140/90 mm Hg were enrolled. Obesity was determined according to the WHO reference values. ANOVA and correlation analysis were used to test the significance of the observed developmental changes and intergroup differences in BP. Within the age and gender groups BP values were strongly associated with height and BMI. The tightness of correlation between BP and height decreased with age. The most rapid rise of BP was found in preschool age and during the period of growth acceleration (11-13 y. in B, 10-12 y. in G). The HR deceleration preceded the BP increase by 3-4 y. The age dependent increment of BP stopped in G at the age of 13 y., while in B it continued. 50 % of B and 25 % of G reached the optimal BP limit according ESH 120/80 mm Hg, at the age of 16 y. Gender differences started to be significant at 14 y. with higher values in B. Minimal values of DP were found at the age of 8-10 y., the maximal were achieved at the age of 13-15 y. In obese subjects the actual BP corresponded to the values pertinent to the approximately 3 y. older age-group. Their HR was only slight increase, but the DP was consistently higher and significant since the age of 10 y. In the effort to lay out the age dependent BP reference values for children and adolescents it is of importance to pay regard to the dynamism of the somatic development in the critical age periods. Impact of obesity as a risk factor of hypertension increases with age, with starting significance at the school age.

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THE SENSITIVITY TO METHAMPHETAMINE IN ADULTHOOD IS DIFFERENT IN MALE AND FEMALE LABORATORY RATS PRENATALLY EXPOSED TO THE SAME DRUG

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Our previous study showed that in male rats prenatal exposure to methamphetamine (MA) increases the sensitivity to the same drug in adulthood. The aim of the present study was to find out whether responsiveness to MA challenge in adulthood is different in male and female rats prenatally exposed to the same drug. Pregnant dams were injected daily with MA 5 mg/kg or saline (SAL) subcutaneously (s.c.) during the whole gestation. Behavior of their adult male offspring was tested in a Laboras apparatus (Metris B.V., Netherlands) for 5 consecutive days, 1 h daily. To test the responsiveness to MA in adulthood, MA 1 mg/kg or SAL were used as a challenge every day immediately prior to testing. Locomotion, exploratory behavior, immobility, distance traveled and speed were monitored and automatically evaluated. Our results showed that acute MA challenge in adulthood increased locomotion, rearing, average speed and distance traveled and decreased immobility in both sexes, regardless of prenatal exposure. Moreover, chronic MA administration in adulthood further increased the psychomotor activation during the 5 days' testing period and thus, elicited behavioral sensitization. Interestingly, the behavioral sensitization was significantly stronger in females than in males. In contrast, while males prenatally exposed to MA were more sensitive to the same drug in adulthood than males prenatally exposed to SAL, there were no differences in females with different prenatal exposure.

In conclusion, we demonstrated different response of males and females to both prenatal and adult administration of MA.

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ALTERED CIRCADIAN CLOCK IN MOUSE AZOXYMETHANE-INDUCED COLON CANCER

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Circadian clocks govern various processes in organism. The core of the clock is organized as transcriptional-translational feedback loop, where positive elements (*Clock*, *Bmal1* genes) enhance expression of negative elements (*Per*, *Cry*, *RevErb* genes), which inhibit expression and activity of positive elements. Whole loop takes approximately 24 hours and is entrainable by various stimuli such as light or feeding regime. Beside the central clock localized in suprachiasmatic nuclei of hypothalamus, almost all organs including colon possess peripheral clocks which can be differentially regulated. As disruption of circadian clock by shift work or nocturnal light exposure is associated with increased cancer risk there is hypothesized that disruption of clock increases the susceptibility to cancer. The aim of this study was to characterize the circadian clock in neoplastic and surrounding normal tissues in colitis-associated azoxymethane-induced model of colorectal cancer. ICR mice were treated intraperitoneally with a single dose of azoxymethane (AZO, 10 mg/kg) and subsequently treated with 2 % dextran sodium sulfate in drinking water for 5 days (treatment was repeated 5 times with 2 weeks of recovery between treatments). Mice were kept in standard light conditions (LD 12:12) and with *ad libitum* access to food and water. Six months after AZO treatment the mice were euthanized and samples of tumor and surrounding normal looking tissues were collected, total RNA isolated, reverse transcribed and transcript levels of clock genes were measured by quantitative real time PCR. Rhythmic expressions of main clock genes, particularly *Per2* and *Bmal1* were found in both healthy and tumor tissue. The phase of the clock was identical in normal and neoplastic tissue but amplitude and mesor were different. The rhythm of clock gene expression was strongly attenuated in tumor compared to surrounding tissue. We demonstrated that colonic tumorigenesis is associated with altered expression of clock genes and that the established model of azoxymethane-induced colon cancer in ICR mouse is a suitable model for studying participation of clock in colon tumorigenesis.

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EFFECT OF ATORVASTATIN ADMINISTRATION ON ENDOTHELIAL FUNCTION OF THE SUPERIOR MESENTERIC ARTERY OF HYPERTRIGLYCERIDEMIC RATS

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Hypertriglyceridemia is an independent risk factor for coronary artery disease and it participates in the development of atherosclerosis and hypertension in human. Statins are most widely prescribed drugs in treatment of dyslipidemia. However, data about efficacy of statins in relation to age and gender are missing. The aim of the study was to compare the effect of atorvastatin (ATO) on endothelium-dependent relaxation of the superior mesenteric artery (SMA) of male *vs* female rats, as well as young (5-mth-old) *vs* old (13-mth-old) HTG rats. ATO was administered *p.o*. for two months in the dose of 0.15 mg/100g/day (young rats) or 0.30 mg/100g/day (old rats). Control rats (C) received vehiculum. At the end of the experiment, blood pressure, body weight and plasma lipids were registered and endothelial function of SMA was tested *in vitro* under isometric conditions. We evaluated response of phenylephrine-precontracted rings to acetylcholine (ACh) before and after NO synthase inhibition with N^G-nitro-L-arginine methyl ester (NO-resistent relaxation). We found that endothelium-dependent relaxation was greater in young female than in male SMA. However in old animals, the relaxation response of SMAs to Ach was more pronounced in males than females. While responses of SMA to ACh of old male rats were not statistically different compared to young rats, responses of old female rats were much less than those of young ones. Administration of ATO resulted not only in improvement of lipid profile of HTG rats, but also it improved endothelium-dependent relaxation of animals. The most pronounced enhancement of Ach relaxation after the ATO treatment was recorded in old female rats. NO-resistent relaxation was not influenced either by gender and age of animals, or by treatment. In conclusion, our results showed the influence of age and gender on endothelial function of SMA of HTG rats. It appears, the worse endothelial function the more effective ATO treatment.

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CONSERVED EXPRESSION PATTERN OF RECEPTORS FOR NEUROTROPHIC FACTORS IN VAGAL NOCICEPTIVE NERVE SUBTYPES

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The inflammation in visceral tissues is often associated with local production of neurotrophic factors that have high potential to alter the phenotype and induce hypersensitivity in the nociceptive primary afferent nerves (nociceptors). This neural plasticity depends on the expression of the receptors for neurotrophic factors in the nociceptors. We have previously shown that the vagus nerves provide two functionally distinct subtypes of nociceptors to the esophagus: neural crest-derived jugular and placodes-derived nodose nociceptors. Here we addressed the hypothesis that the jugular and nodose nociceptors innervating the esophagus differ in expression profile of receptors for neurotrophic factors of the NGF and GDNF families in two relevant laboratory species. Single cell RT-PCR detection of multiple targets in a single neuron was performed on the vagal afferent neurons retrogradely labeled from the esophagus in the mouse (n=172) and guinea pig (n=25). The nociceptive neurons were idenfied by the expression of the capsaicin receptor TRPV1. We found that the mouse neural crest-derived jugular nociceptors co-expressed GFL co-receptor subunit GFRα3 (for artemin) and neurotrophin receptor TrkA (for NGF), but rarely expressed TrkB (for BDNF). In stark contrast, the mouse placodes-derived nodose nociceptors lacked GFRα3 and TrkA, but expressed TrkB. The expression of GFRα1 subunit (for GDNF) was slightly higher in the mouse nodose nociceptors. The remaining tested receptors TrkC and GFRα2 subunit were expressed rarely and did not discriminate the phenotypes. The expression pattern of receptors for neurotrophic factors in the guinea pig vagal nociceptors was essentially identical to the expression pattern detected in mouse. We conclude that the vagal neural crest- and placodes-derived nociceptors innervating the esophagus differ in expression pattern of receptors for the key neurotrophic factors. Our similar finding in the mouse and guinea pig indicate that this expression pattern is conserved among the species.

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THE EFFECT OF P2X7 RECEPTOR BLOCKERS ON ATP RELEASE FROM RAT SUPRACHIASMATIC NUCLEI

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In mammals, circadian rhythms are driven by a pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The rhythm continues in constant darkness and is dependent on cell-cell communication, involving both neurons and glia. Previous studies have shown that rhythm of total ATP content and extracellular level negatively correlate with electrical activity and arginine vasopressin (AVP) secretion

rhythm, indicating that ATP and AVP are released from different cells. Here we tested a hypothesis that ATP acts as extracellular messenger in the SCN and is released by plasma membrane purinergic P2X7 receptor. We measured circadian release of ATP from the SCN organotypic slice and examined the effect of *N*-[2-[[2-[(2–hydroxyethyl)amino]ethyl]amino]-5-quinolinyl]-2-tricyclo[3.3.1.13,7]dec-1-ylacetamide dihydrochloride (AZ 10606120) and 3-[[5-(2,3-dichlorophenyl)-1*H*-tetrazol-1-yl]methyl] pyridine hydrochloride (A 438079), which inhibit P2X7 receptor, and tetrodotoxin (TTX), which inhibits voltage-gated sodium channels. Coronal sections (~ 250 μm thick) containing the SCN were removed from rats of 14-15 postnatal days of age between CT 2 and CT 8. In our experimental protocol spanning for 52 hours, samples of medium above slices were collected every 4 hours and ATP was measured by bioluminescence assay. Control group of organotypic SCN slices exhibited circadian rhythm in ATP release with peak between CT 18 and CT 22 which was disrupted in the presence of AZ 10606120 and A 438079, indicating involvement of P2X7 receptor in maintaining the rhythm. Application of TTX also disrupted ATP release rhythm indicating involvement of neuronal activity. The fact that ATP rhytm is ihibited by P2X7 blockers might indicate that ATP is primarily stored and released from glia through activated P2X7 channel, inhibition by TTX suggests that glia function is under control of activity of neurons. Further studies are required to clarify the mechanism of ATP release.

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DOES PRENATAL METHAMPHETAMINE EXPOSURE AFFECT THE DRUG-SEEKING BEHAVIOR OF ADULT MALE RATS?

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Methamphetamine (MA) is one of the most frequently used illicit drugs in the Czech Republic and also one of the most common drugs abused by pregnant women worldwide. There are studies demonstrating that repeated administration of psychostimulants such as MA enhances locomotor activities in response to treatment of the same or related drugs in rodents. This phenomenon is defined as behavioral sensitization or reverse tolerance. There are, however, no studies investigating possible sensitizing effect of prenatal MA exposure. Our most recent studies demonstrated that prenatal MA (5 mg/kg) exposure makes adult rats more sensitive to acute injection of the same drug. One way how to examine drug-seeking behavior or how to show "the animal's own will" to take the drug is to use the Conditioned place preference (CPP) test. Prenatally MA-exposed adult male rats and their controls were tested in the CPP test for drug-seeking behavior. The following psychostimulant drugs were used: MA (5 mg/kg), amphetamine (5 mg/kg), cocaine (10 mg/kg). All psychostimulant drugs induced increased drug-seeking behavior in adult male rats. However, while MA and amphetamine-induced increase in drug-seeking behavior did not differ based on the prenatal drug exposure, prenatally MA-exposed rats displayed tolerance effect to cocaine in adulthood. This finding is surprising because we expected sensitizing effect of prenatal MA-exposure. In addition, there was increased defecation during days of conditioning when the psychostimulants were administered relative to days without drug administration. This finding suggests the effect of stress caused by psychostimulants, which is independent on prenatal MA exposure. In conclusion, our results did not confirm our hypothesis that prenatal MA exposure increases drug-seeking behavior in adulthood in the CPP test.

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PRENATAL IRRADIATION CAUSES IMPAIRMENT OF HIPPOCAMPAL NEUROGENESIS AND BEHAVIORAL CHANGES IN ADULT RATS

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The intrauterinal development in mammals represents a very sensible period of life in relation to many environmental factors, including ionizing radiation. The aim of our work was to study the possible influence of a very low prenatal dose of radiation on selected behavioural parameters and on the level of hippocampal neurogenesis in the adult age in rats. Pregnant females of Wistar-strain rats were irradiated on the 17th day of gravidity with a whole-body dose of 1 Gy. The three months old offspring of irradiated and non-irradiated mothers was tested in behavioral tests. Basic locomotion, exploratory activities and anxiety were tested in open field test and in elevated plus maze (EPM). Learning and memory functions were tested in passive avoidance test and in the Morris´s water maze (MWM). After finishing the testing, the level of hippocampal neurogenesis was determined by immunohistochemical methods. The irradiated rats showed significantly lower locomotory and exploratory activities in the open field and EPM in comparison with controls. The effect of radiation in the open field test was more pronounced during the light period of the day, whereas in the EPM test during the night. The significant reduction of the time spent in the open arms of elevated plus maze indicates an increase of anxiety in irradiated rats. Prenatal irradiation caused significant impairment of spatial memory in MWM test, but no such effect was found in passive avoidance test. The deficit in spatial memory may be associated with the impairment of hippocampal neurogenesis: the number of proliferating cells in *gyrus dentatus* of hippocampus was significantly lower in irradiated animals in comparison with controls.

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CHRONOBIOLOGICAL ASPECTS OF APNOIC EPISODE IMPACT ON MYOCARDIAL PARAMETERS IN RAT EXPERIMENTAL MODEL

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Clinical studies suggest a circadian link between sudden death from cardiac causes and obstructive sleep anoea syndrome. The aim of study was to refer to chronobiological aspects of the changes in some heart parameters and acid-base balance after the apnoic episode and during reoxygenation in *in vivo* rat model. Experiments were performed in female Wistar rats in ketamine/xylazine anesthesia (100 mg/kg+15 mg/kg,i.m.) after adaptation on the light-dark (LD) cycle 12:12 h. Parameters of normal artificial ventilation/reoxygenation (1 ml/100 g, 50 breaths/min.) Apnoic episode was simulated by the switching off respirator for 2 minutes. Apnoic episode significant ($p<0.001$) decreased electrical stability of the heart in both lighted parts of the day and eliminated LD differences. In the dark part of the day, electrical stability of the heart increased with the duration of reoxygenation, whereas oposite tendency was seen in the light one. The heart rate decrease was indentical till to end of apnoic episode in both lighted parts, with the preservation of LD differences. Although reoxygenation significantly ($p<0.001$) increased heart rate in the both lighted parts, LD differences were eliminated in duration reoxygenation. The calculation of the correlation coefficients between heart rate and electrical stability of the heart did not reveal any relationship, except apnoic episode in the light part, where moderate negative correlation ($r= -0.47$) was found. Although, from the chronobiological point of view, apnoic episode produces higher acidity, hypercapnia and hypoxia in the light part of the day, these changes do not depend from LD cycle. The recovery of the pulmonary ventilation changes pH and blood gases by manner depending on LD cycle. It increases pH till to alkalic level, decreases pCO_2 gradually till to hypocapnia and increases pO_2 and O_2 saturation only in the dark part in contrast to the light one. It is concluded that electrical stability of the heart and heart rate are decreased by apnoic episode, independently on LD cycle. Reoxygenation changes electrical stability of the heart by the manner

dependent on LD cycle. Antiarrhythmogenic effect was found in the dark part and proarrhythmogenic effect in the light one.

TONE OF THE AUTONOMIC NERVOUS SYSTEM IN CONDITIONS OF KETAMINE/XYLAZINE AND PENTOBARBITAL ANESTHESIA IN WISTAR RATS. CHRONOBIOLOGICAL STUDY

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The aim of this experimental work is to demonstrate the chronophysiological aspects of changes in the activity of the autonomic nervous system in female Wistar rats, after administration of two anesthetics - ketamine/xylazine (ketamine 100 mg/kg + xylazine 15 mg/kg, im. and pentobarbital (40 mg/kg, ip.) during spontaneous breathing. To assess the effect of light and darkness, the animals were adapted to a light regime of 12:12 hours over four weeks. In the dark (active) part of the day regime, the parasympathetic division (HF component) was dominant in pentobarbital anesthetized rats. Changes in heart rate were mainly influenced by the parasympathetic division (r=0.82), although a moderate correlation with sympathetic activity (VLF component) (r=0.55) was also found. In ketamine/xylazine anesthesia, the heart rate changes are the result of baroreceptor activity (LF component) (r=0.52). The light (inactive) part of the day regime has revealed no changes in heart rate dependency on the autonomic nervous system tone in pentobarbital anestetized animals. In ketamine/xylazine anesthetized rats, changes in heart rate were only under the regulatory influence of the parasympathetic (HF component) (r=0.59). Pentobarbital eliminated light-dark differences in the VLF component. Ketamine/xylazine did not disturb the light-dark differences in RR interval and HF component, except the LF and VLF components. Administration of different anesthesia affects the autonomic nervous system activity in qualitatively and quantitatively different ways by changing the activity of both autonomic nervous system divisions, depending on time of day.

MODULATION EFFECT OF CYTOSOLIC Ca^{2+} ON THE ATP-ACTIVATED CARDIAC RYANODINE RECEPTOR

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In cardiac muscle, a small entry of Ca^{2+} through L-type Ca^{2+} channels is considered to be the main mechanism triggering the opening of RyR2 channels and the subsequent Ca^{2+} release from the sarcoplasmic reticulum (SR), essential for cardiomyocyte contraction. Adenosine triphosphate (ATP), present in millimolar quantities in cardiomyocytes, is an effective co-activator of the RyR2 channel (1); however, the mechanisms of its action are not fully understood. In our study, we examined the modulation effect of cytosolic Ca^{2+} on ATP-activated RyR2 channels. In all experiments, luminal Ca^{2+} was kept constant and equal to the physiological concentration of Ca^{2+} in the lumen of SR (1 mM). We used the method of reconstitution of RyR2 channels from the rat cardiac microsomes into a planar lipid membrane. Our study was based on the finding (2) that at 90 nM cytosolic Ca^{2+}, the dependence of ATP potency on luminal Ca^{2+} was already saturated at 1 mM luminal Ca^{2+}. In the present study, we kept luminal Ca^{2+} constant (1 mM) and increased cytosolic Ca^{2+} from 90 nM to 300 nM. Under these conditions, EC_{50} for ATP activation was significantly shifted to lower concentrations and the maximal level of activation (P_{max}) was substantially enhanced. Interestingly, the parameters of gating kinetics over the whole range of open probability (P_o) were similar to those found for the RyR2 channel solely activated by ATP. Because cytosolic Ca^{2+} in the tested range was not able to activate the RyR2 channel itself, our results suggest that cytosolic Ca^{2+} modulates the sensitivity of the RyR2 channel to ATP.

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ANALYSIS OF RYANODINE RECEPTOR CHANNEL ACTIVITY IN THE FREQUENCY DOMAIN

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Activity of ryanodine receptor calcium release channels (RYRs) at the level of single channels is effectively studied by the technique of planar lipid bilayers. For technical reasons, the experimental conditions should be set so that individual openings of the channel are unambiguously identified by the software for automatic analysis. A major disadvantage of this approach is that the channel cannot be studied under physiological or near-physiological conditions. In this work we test the applicability of the FFT spectral analysis to characterize RYR behaviour in the frequency domain. Cardiac RYR2 channels of male Wistar rats were reconstructed in planar lipid bilayers by fusion of microsomal vesicles as previously described (1). The single RYR2 channel activity was recorded by the MultiClamp 700B amplifier, digitized by Digidata 1440A, and analyzed in Clampfit 10 (Axon Instruments) and Origin (Ver. 8, OriginLab). The Fast Fourier transform in the range of 0.5 to 2000 Hz was applied on 20 segments, each of 4096 points (2 s), dissected sequentially from about 2 min long continuous current records and averaged to reduce the smear of spectra. The currents recorded from the electrical model, from a pure bilayer membrane, from a membrane with an incorporated vesicle without RYR activity, and from a membrane with an incorporated RYR either at low or at high open probability were analyzed and compared. The contributions of individual conditions to the overall noise were estimated and compared. It was found that the major contribution to the power spectra comes, as expected, from the transitions of the RYR2 between open and closed states. Nevertheless, subtle differences were observed in the noise of the open and the closed states of the RYR2, respectively. Interestingly, the contribution of fast, incompletely resolved open/close transitions of the channel could be identified. These results confirm that the fast Fourier transform has a potential of a simple and effective tool to study RYR activity in isolation under conditions closer to the physiological, when fully resolved channel openings are buried in the noise of the system.

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DOG ERYTHROCYTE ANTIGEN (DEA) 1.1 HAS NO INFLUENCE ON ERYTHROCYTE OSMOTIC FRAGILITY

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The aim of this study was to investigate the influence of dog erythrocyte antigen (DEA) 1.1 on osmotic fragility. Peripheral blood was collected from 22 adult dogs. Complete clinical, hematological, biochemical and coagulation tests found no abnormalities. Blood group was typed using gel card test ID-Canine DEA 1.1. Osmotic fragility was measured within 6 hours by adding 25 µl of lithium-heparin blood into 2.5 ml NaCl solution of decreasing concentrations: 0.9 %, 0.8 %, 0.75 %, 0.7 %, 0.65 %, 0.6 %, 0.55 %, 0.5 %, 0.45 %, 0.4 %, 0.35 %, 0.3 %, 0.25 %, 0.2 % and 0.1 %. After 30 minutes of incubation samples were centrifuged and absorbance was measured at 540 nm using ELISA reader. Cumulative curve of hemolysis was drawn from the obtained values. The concentrations of NaCl solution corresponding to the minimum (less than 5 % hemolysis), medium (50 % hemolysis) and maximum (more than 90 % hemolysis) osmotic fragility were calculated. From 22 dogs, 10 were DEA 1.1 positive

and 12 were DEA 1.1 negative. Between these two groups no significant differences were found in minimum, medium and maximum osmotic fragility. P values obtained from Student's T test were 0.12 (minimum osmotic fragility), 0.27 (medium osmotic fragility) and 0.54 (maximum osmotic fragility), respectively. The results indicate no influence of DEA 1.1 on osmotic fragility in dog erythrocytes.

THE ISCHEMIA-REPERFUSION INJURY IN ISOLATED RAT HEART - EFFECT OF MILD HYPOTHERMIA

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During the past decade, the efficacy of hypothermia to treat emergency cases of ongoing ischemia such as stroke, myocardial infarction, and cardiac arrest has been studied by many researchers. Results are promising but mechanisms are still not clear. We investigated whether mild hypothermia during ischemia-reperfusion could modulate Ahnak (protein involved in cytoskeleton stability and intracellular calcium homeostasis) content, cardiac functional recovery, susceptibility to reperfusion arrhythmias, and membrane oxidative damage. Hearts from male adult Wistar rats were isolated and perfused using Langendorff technique at 37 °C or at 33 °C. The protocol consisted of 15 min stabilization, 25 min global ischemia and 30 min reperfusion. Functional parameters (coronary flow, left ventricular pressures, contractility and relaxation index) were recorded. Arrhythmias were measured in spontaneously beating hearts during the first 10 min of reperfusion. In parallel experiments, heart tissue sampling was performed before ischemia, after ischemia and after reperfusion. Ahnak content was evaluated using Western blot technique and conjugated dienes (representing lipid membrane oxidative damage) were determined by spectrophotometry. Reducing the temperature of the perfusion buffer to 33 °C improved the recovery of functional parameters, but also increased the incidence of severe reperfusion arrhythmias. Membrane oxidative damage was attenuated and the content of Ahnak was preserved at 33 °C as compared to its decline at 37 °C. Mild hypothermia during ischemia-reperfusion prevented the decline of Ahnak content in isolated rat hearts. Preserved level of Ahnak might differently influence myocardial functional recovery and electrical stability after ischemia.

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DOES RED PALM OIL PROVIDE PROTECTION OF SPONTANEOUSLY HYPERTENSIVE RATS?

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Previously we have shown that SHR benefit from n-3 unsaturated fatty acids supplementation (1,2), which is known to reduce cardiovascular diseases and sudden cardiac death in humans. Recently, cardioprotective effect of red palm oil (RPO) containing saturated fatty acids, carotenoids, tocoferol and tocotrienols has been reported (3,4). The purpose of this study was to examine effects of RPO supplementation in SHR. SHR and WKY rats were fed with a standard rat chow plus RPO (200 ul/day) for 5 weeks and compared with untreated controls. Systolic blood pressure (SBP) was monitored using tail-cuff plethysmography. Plasma cholesterol (CH), triglycerides (TG) and blood glucose (BG) were registered at the end of experiment. Nitric oxide synthase (NOS) activity was determined in the left ventricle and aorta, which was also submitted for functional examination. Isolated perfused heart was used for the examination of postischemic reperfusion-induced arrhythmias and ventricular fibrillation (VF) inducibility. Results showed that RPO reduced significantly BP (160±13 *vs* 184±20 mm Hg) in SHR and BG in SHR as well as WKY (4.48±0.2 *vs* 5.61±1.1 and 5.5±0.4 *vs* 6.38±0.8 mmol/l), while there were no significant differences in plasma CH and TG among the groups. Body and heart weights were not affected by RPO as well. Compared to WKY rats the activity of NOS was higher in SHR heart and aorta (4.67±0.3 and 3.18±0.5 *vs* 3.02±0.3 and 1.54±0.1 pmol/mg/min). RPO increased NOS activity in the aorta but reduced NOS in the heart of both SHR and WKY while supressed relaxation of the aorta. Duration of reperfusion-related bradykardia was markedly shorter in SHR *vs.* WKY and prolonged due to RPO, which also supressed reperfusion-induced VF in both strains and reduced incidence of electrically-induced VF. Results suggest beneficial effects of RPO in SHR and challenge to elucidate a possible mechanisms involved.

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ACTIVITY OF PARAOXONASE IN RELATION TO THE PROGRESSION OF ATHEROSCLEROSIS IN PATIENTS WITH DIABETES MELLITUS

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Diabetes mellitus (DM) is associated with the alterations in lipid metabolism and also with increased risk of atherogenesis. Several reports have shown that oxidative stress may contribute to the pathogenesis of atherosclerosis (1). Paraoxonase 1 (PON1) is a high-density lipoprotein (HDL) associated enzyme which plays a key role in the protection of a low-density lipoprotein (LDL) from oxidative modification (2). Paraoxonase and arylesterase activities of this enzyme are responsible for antioxidant action of HDL. The aim of this study was to examine basal/salt-stimulated paraoxonase and arylesterase activities in the serum from patients with DM Type 2 and compared to control group. We studied 30 subjects with DM Type 2 and 30 healthy subjects matched for age, sex and duration of diabetes. Basal (in the absence of NaCl) and salt-stimulate (in the presence of NaCl) paraoxonase activities (using paraoxon as substrate) as well as arylesterase activity (using phenyl acetate as substrate) were measured spectrophotometrically. We found that basal and salt-stimulated paraoxonase and arylesterase activities were significantly lower in patients with DM Type 2 in comparison with control group ($p<0.05$). The results of this study showed a significant decrease in paraoxonase and arylesterase activities in patients with DM Type 2. Altered activities of PON1 may play an important role in the pathogenesis of atherosclerosis through decreased the ability of HDL to protect LDL from oxidative modification. Further studies are needed to confirm the possible mechanisms of altered activities of PON1 in the relationship to HDL and LDL. We concluded that impaired characteristics of these parameters may contribute to the progression of atherosclerosis in diabetic patients.

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THE EFFECT OF VARIOUS OESTRUS SYNCHRONIZING TREATMENTS ON THE MORPHOLOGICAL STATE OF THE EWE OVARIES

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OvSynch protocol is a method for oestrus and ovulation induction and synchronization using exogenous GnRH. Good oestrus synchronized effect is reached by using the OvSynch protocol in cyclic animals (1). We tested this method in Improved Vallachian ewes during deep anoestrous season in the spring and observed some morphological changes on the ovaries. Animals were divided into four groups (A, B, C, D; n=6 for each). Ewes of Group A and B were treated with intravaginal sponges (FGA 20 mg/sponge and head) for 11 days and in the time of their withdrawal 1000 and 500 UI eCG were injected i.m., respectively. Ewes of Group C were intact control group. The first dose of GnRH (lecirelinum 0.0125 mg/head) was injected i.m., five days later $PGF_{2\alpha}$ (cloprostenolum-D 0.0375 mg/head) was injected i.m., and the second dose of GnRH (the same dose as the first) two days later – Group D (OvSynch protocol). Laparotomy with following ovariectomy was realized 48 hrs after treatment in all groups. Sizes of the ovaries (length, width, and height) and follicles (≥5mm, ≥3mm, ≥1mm) were measured and numbers of follicles of mentioned diameters were counted. All these parameters were insignificant, except for the length of the ovaries of Groups A and C ($P<0.05$; mean ± S.E.M; 2.17±0.10 and 1.75±0.07, respectively). Our findings show that the OvSynch protocol has very similar effect on the morphologic parameters of the ovaries in tested Slovak sheep breed.

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PSYCHOSTIMULANTS AND THEIR EFFECT ON NOCICEPTION IN RATS

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Amphetamine, methamphetamine and cocaine are psychostimulant drugs which affect central nervous system by releasing dopamine, norepinephrine and serotonin. All these neurotransmitters are important modulators of pain transmission. Although drugs of abuse are known to have analgesic properties, there exists no experimental study which would compare antinociceptive effect of different psychostimulant drugs with classical analgesic standard – morphine. Nociception was tested in 3-4 months adult male Wistar rats (weight 400 ± 67 g). Latencies of withdrawal reflexes of forelimbs, hind limbs and the tail on thermal nociceptive stimuli (Plantar Test, Ugo Basile, Italy) were repeatedly measured before injection of drugs and then in 15-min intervals after the application of 5 mg/kg *s.c.* of amphetamine, methamphetamine, cocaine or morphine. Last measurement was performed 45 min after the injection. Each group consisted of 7-8 animals. Both on hind limbs and the tail, amphetamine was more potent antinociceptive drug than metamphetamine, whereas cocaine was practically without any effect. On the hind limbs, effect of morphine did not differ from that of cocaine. On the other hand, in the tail-flick test, morphine and amphetamine induced the strongest analgesia. All psychostimulant drugs, irrespective of absolute level of analgesia, had comparable antinociceptive effect if expressed relatively as tail/paw ratio (approximately 1.5 longer latencies on tail than on hind limbs during last measurement). In morphine group this ratio was nearly four. Thus, our findings indicate that different body sites might be controlled by different analgesic mechanisms. Although psychostimulants and morphine share common feature of increasing extracellular concentration of dopamine in nucleus accumbens (either directly or indirectly), pharmacodynamics and pharmacokinetics should also be taken in account.

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RYANODINE RECEPTOR RECRUITMENT AND CONSTRUCTION OF CALCIUM RELEASE SITES IN CARDIAC MYOCYTES

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Local character of calcium release in cardiac myocytes implies independent recruitment of calcium release units/dyads by triggering stimuli. It was shown that individual CRUs may release calcium in small quanta. This quantal character has been interpreted as recruitment of small cohorts of independent RYRs (1, 2), or as recruitment of small cohorts of RYR clusters with coupled RYRs gating in synchrony (3). We tested both interpretations on a set of experimental data with a model of virtual calcium release units (vCRUs) consisting of multiple RYR clusters. Simulations were performed in Mathematica (Wolfram Research, Ver. 7.0) and Origin (Ver. 7SR4, OriginLab). Virtual release units consisting of 1-10 clusters were constructed from virtual RYR clusters in accordance with the experimentally observed cluster size distribution (4). If RYR gating was independent, the calcium release flux of any vCRU had quantal character, and the quantal character of all independent vCRUs was similar. vCRUs consisting of 3 or more clusters provided a good agreement between the model and experimental data (1, 5). If RYR gating was coupled, the calcium release flux of vCRUs did not provide the quantal structure that would correspond to *in situ* observations. The model of RYR gating (2) combined with the model of independent RYRs in vCRUs (2) observed the calcium dependence of calcium spark frequency (6) under all conditions. However, the Mg^{2+}-binding parameters of RYRs were in accordance with the single-channel observations (7) only for vCRUs composed of 3 or more clusters. In conclusion, these results favor independent over fully coupled RYR gating *in situ*, and predict the presence of at least 40 RYRs per cardiac calcium release unit.

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CHANGES IN DHPR-RYR CROSSTALK DUE TO MYOCARDIAL INJURY

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The isoprenaline (ISO) model of myocardial injury evokes changes in excitation-contraction coupling at the level of both, RyR channel activity and DHPR-RyR communication. In this work, we analyse the changes in the crosstalk between DHPR and RyR calcium channels of single Ca^{2+} release units (CRUs), characterized by parameters of the local Ca^{2+} release activation and of the whole cell Ca^{2+} current inactivation. Myocardial injury was induced in young adult Wistar rats by a single subcutaneous injection of ISO (150 mg/kg b.w., MI group) or vehicle (5 % ascorbic acid, CONT group). On day 15 after injection, cardiac myocytes were isolated from left ventricles. Ca^{2+} currents (I_{Ca}) were activated by 70 ms voltage pulses from –50 to 0 mV using whole-cell patch clamp. Local Ca^{2+} releases evoked by the stimuli were detected by the laser scanning confocal microscopy as Ca^{2+} spikes, using 1 mM fluorescent Ca^{2+} indicator OG-5N and 4 mM EGTA to limit Ca^{2+} diffusion (1). While latencies of Ca^{2+} spikes of control myocytes were homogeneous, the distribution of latencies of MI myocytes consisted of two components, which is consistent with the electron microscopic observation of release sites with partially detached SR cisterns in MI myocytes (2). Most CRUs responded to I_{Ca} by a single Ca^{2+} spike; however, ~7 % produced also secondary spikes. Neither the fraction of CRUs exhibiting secondary spikes nor the delay between the primary and the secondary spikes were affected by MI. While the extent of I_{Ca} inactivation was significantly larger

in MI than in CONT cells, no correlation between the extent of secondary release and the extent of I_{Ca} inactivation was observed in CONT or MI cells. These data suggest that secondary Ca^{2+} spikes do not play a substantial role in the inactivation of Ca^{2+} current.

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DYNAMIC PROPERTIES OF FINGER ARTERIES

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Several methods of studying the properties of the arterial wall of big arteries have been used in clinical practice. Methods for the study of small arteries are almost unavailable except work [1] of J. Penaz. The aim of the present study was to follow dynamic properties of finger arteries by means of a frequency analysis of the responses of the volume of finger arteries elicited by externally applied pressure. The cuff with a source of infrared (IR) light is placed on the middle phalanx of the third finger. A sensor on the other side of the finger measures the IR signal, which is proportional to the volume of the corresponding segment of finger arteries. External stationary pressure is applied in discrete steps from 20 to 200 mm Hg together with an alternate pressure (40-120 Hz). The obtained curves of the relation between the amplitude of the volume changes and the external pressure frequency correspond to the assumed properties of finger arteries. The signal-to-noise ratio is too low. Improvement of the signal-to-noise ratio by technical means is necessary for the application of the method in clinical practice.

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