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**MECHANISMS INVOLVED IN WORTMANIN-INDUCED MODULATION OF ISCHEMIC TOLERANCE IN RAT HEARTS**

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PI3K/Akt kinase pathway is a key signaling system implicated in cell survival and metabolic control via phosphorylation of proteins in various cell types. Wortmanin (WT) is a specific inhibitor of this kinase pathway. The aim of the study was to investigate the mechanisms involved in WT-induced modulation of ischemic tolerance. In the study isolated Langendorff-perfused rat hearts subjected to 25 min global ischemia and 35 min reperfusion (test ischemia, TI) were used. Short adaptation to ischemia (ischemic preconditioning- IP) was evoked by 2 cycles of short ischemia/reperfusion before TI. The levels and activation of proteins were determined by immunoblot assay. The activities of matrix metalloproteinases (MMP) were measured by zymography. We found that IP procedure leading to increased cardiac tolerance against ischemia/reperfusion induced also an activation of Akt kinase (Akt). To examine the role of this activation in modulation of ischemic tolerance by IP we administered WT before IP procedure. This application of WT was connected with modulation of ischemic tolerance and with inhibition of IP-mediated Akt activation. Moreover, the actions of WT were linked with modulation of MMP-2 activities and levels of some heat stress proteins (Hsp90). Our results suggest that activation of PI3K/Akt kinase pathway is involved in the cardioprotective mechanisms of IP in the rat heart. The results also point to possible relationship between Akt kinase and modulation of MMP-2 activities.
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**THE EFFECT OF QUERCETIN ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF NORMOTENSIVE AND HYPERTENSIVE RATS**

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Quercetin is a plant-derived bioflavonoid with proposed positive effect on cardiovascular system. The aim of the present study was to examine effect of quercetin supplementation on some biochemical parameters of rat hearts and also its effect on physiological parameters of isolated perfused rat hearts (normotensive and SHR).
Rats received quercetin at the 4 weeks age (20mg/kg/day) during next 4 weeks and blood pressure of the rats was measured once a week. After quercetin treatment hearts from both Wistar and SHR rats were used for Langendorf perfusion or for biochemical analysis. Our results showed that quercetin reduce blood pressure in SHR rats, and increase expression of IP3 receptors, SERCA and RYR2 in the hearts. However, quercetin has no significant effect on physiological parameters of isolated perfused hearts from both normotensive and SHR rats. Thus quercetin protects SHR rats against hypertension but independently on contractility of the heart. On the other hand, increased levels of IP3 receptors, SERCA and RYR2 indicate that calcium homeostasis in the heart may be affected by quercetin. However, more studies are necessary to understand mechanism of positive effect of quercetin on cardiovascular system.

**MONITORING OF POTENTIAL EFFECT OF MIDAZOLAM DURING MYOCARDIAL PERFUSION**

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Midazolam is frequently used benzodiazepine in anaesthesiology and intensive care. The aim was to monitor its potential effect during heart perfusion of laboratory rat. The same group of animals (n=10). The 1st treated (midazolam in dose 0.5mg/kg i.p). The 2nd was placebo. After i.p. administration of heparine injection of 500 IU dose, the hearts were excised and perfused (modified Langendorf´s method). Working schedule: stabilization/ischaemia/reperfusion will proceed in intervals 20/30/60 min. Monitored parameters from isolated heart: left ventricle pressure, end-diastolic pressure, contractility (LVP, LVEDP, $dP/dt_{max}$). The treated hearts showed improved postischemic recovery, reaching LVP values of 92 ± 6% at the end of the reperfusion, placebo only 61 ± 7%. In placebo hearts LVEDP rise from 10.0 ± 0.5 mmHg to 43 ± 4 mmHg after, in treated only about 25 mmHg. The treated hearts improved $+dP/dt_{max}$ recovery during reperfusion to 91 ± 8 %. These values were significantly greater than those obtained from the placebo hearts.
Key words: heart ischemia-reperfusion, midazolam

**NEW BIOMARKERS OF METABOLIC SYNDROME IN SERUM AND SUBCUTANEOUS ADIPOSE TISSUE – NON DIABETIC PERSON VERSUS PATIENTS WITH DIABETES TYPE 2 -**

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*Background:* Adipocytes produce a number of cytokines, e.g. leptin, adiponectin, TNFalpha and resistin which modulate tissue insulin sensitivity. This may be involved in the ethiopathogenesis of diabetes mellitus type 2.
*Aims*: *(a)* Investigate the lipid spectrum (cholesterol, HDL-cholesterol, LDL- cholesterol, TAG), markers of insulin resistance (C-peptide, insulin, S-glucose, BMI, HOMA, QUICKI) in serum and subcutaneous adipose tissue in diabetic and non diabetic persons. *(b)* Determine concentration of various cytokines (biomarkers: PPARγ, adiponectin, resistin A,-FABP, E-FABP, leptin, TNFα) in serum and subcutaneous abdominal adipose tissue of type 2 diabetics and non diabetics (males and females). *(c)* Compare their levels in both groups and analyzed their mutual interactions and factors related to insulin resistance
*Method*: We determined concentration of chosen markers using ELISA kits from Bio vendor Czech Republic
*Results and conclusions:* We found differences between the two groups in serum and subcutaneous adipose tissue: with the exception of adiponectin and QUICKI all measured parameters were higher in diabetic patients. We also found a gender difference: most markers were higher in females. Concentration of cytokines was influenced by health status and gender.

**EFFECT OF NEW THERAPEUTIC TRENDS OF DIABETES MELLITUS TYPE 2. - METFORMINE VERSUS METFORMINE PLUS ROSIGLITAZONE – ON LEVELS OF VARIOUS BIOMARKERS OF METABOLIC SYNDROME**

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*Background:* Pharmacological interventions to diminish diabetic and cardiovascular complications of the metabolic syndrome are needed because of the increase of their prevalence in all the word.
*Aims*: *(a)* Investigate the lipid spectrum (levels of cholesterol, HDL-cholesterol, LDL- cholesterol, TAG), markers of IR (C-peptide, insulin, S-glucose, BMI, proinsulin, HbA1C) in patients with 2TDM treated with metformine only and metformine plus rosiglitazone. *(b)* Determine concentration of various cytokines (adiponectin, A,-FABP, leptin) in serum both investigated groups. *(c) C*ompare their levels in both groups and deduct the influence of treatment on measured markers.
*Method*: We determined the concentration of chosen markers using ELISA kits from Bio vendor Czech Republic.
*Results and conclusions:* In patients treated with the combination of metformine with roziglitazone were better ameliorations in markers of metabolic syndrome then in patients treated only with metformine.

| | **MET+ROZI. n=11** | **MET n= 8** |
|---|---|---|
| A-FABP µg/ml | 37,78±14,01* | 56,51±19,51* |
| Adiponectin µg/ml | 11,13±11,50* | 13,04±4,77* |
| LDL mmol/l | 3,02±0,94* | 2,80± 0,99* |
| S-Glucose mmol/l | 7,85±1,27* | 11,08±3,46* |
| HbA1C % | 5,28±0,84* | 6,63±2,11* |
| PAI-1 ng/ml | 94,03±13,80* | 107,16±35,94* |

Tab. 1: Significant difference between both groups. (*p < 0,05)

## INTERRUPTION OF TACHYCARDIC-PRESSOR RESPONSE DURING COLD FACIAL IMMERSION IN YOUNG ATHLETES AT VARIOUS PHYSICAL LOAD

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Present work was undertaken to examine the mechanism of the reversal of sustained or progressively rising tachycardic-pressor response during bicycle ergometry after the application of trigeminal-cardiac reflexes (TCR) elicited by the cold stimuli (CS) to perinasal (NA) or frontal (FA) facial areas combined with-or without breath-holding (+/-B) (20 s). Changes in the heart rate (HR) and arterial blood pressures (BP) evoked by 60 s lasting cold stimulation (gel-filled plastic applicator; <7 °C) to OA and FA separately or FA+OA, respectively, were evaluated in 7 young sportsmen (G 1) and 7 young untrained volunteers (G2) (age 16 - 22y.) during the rest and steady-state or increased load. Both in G1 and G2 under rest conditions (82±9 $c.min^{-1}$, 125±11/78±8 Hg, M±SD) and during steady-state exercise (80W, 10 min, HR≥150 $b.min^{-1}$, mean BP >140 mmHg) CS+B produced more rapid and more intensive fall in HR (5-17s delay, 57-81s duration) and higher rise in BP compared to CS-B. Magnitude of bradycardic response: 1) followed the order NA+FA > FA > NA, 2) was stronger under the exercise than in the rest condition and 3) was more pronounced in G1 compared to G2 in either condition. Stimulation of NA+FA (60s) effectively prevented or reversed (for 56-89s in G1 or 35-55s in G2) sustained rise of HR and caused variable decrease in BP. These effects were observed on any level of the gradually increasing physical load (Δ15W/min) starting from moderate intensity of 85 W up to strenuous exercise at 285-340 W. Intensity and duration of cardiovascular responses to CS showed relation to the rate of rise of tachycardic – pressor responses induced by physical activity and intensity of post-training drop of sympathetic activity. These preliminary results show that TCR might be suitable indicator for the estimation of individual vegetative reactivity and appropriate adjustment of training process.
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## EFFECTS OF BREATHING MANEUVERS AND TRIGEMINAL REFLEXES DURING ARTEFITIAL SUPRAVENTRICULAR TACHY- ARRHYTHMIAS

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Breathing manoeuvres including inspiratory or expiratory breath-holding (IA,EA), Valsalva manoeuvre (VM), likewise the classical carotic sinus massage (CM) and trigeminal-cardiac reflexes (TCR) belong to the spectrum of vagal bradycardic manoeuvres (BM) used with benefit in the acute emergency treatment, or for clinical assessment of the severity and adjustment of effective therapy of paroxysmal supraventricular tachyarrhythmias (SVTs). The aim of the present work was to evaluate the efficiency of various BM in reversal of PSVT into nomotopic rhythm (NR) with the focus on the mechanisms of this conversion. Study was done in 9 patients (18-47 y) diagnosed with AVNRT (7) and AVRT (2) admitted for radiofrequency ablation of arrhythmogenic locus. SVTs were re-established by extra-phasic electrical stimuli (2-4mA, 1ms impulse; 600 ms period) through intracardiac electrodes. In 4 cases SVTs were restored and maintained (177±12 $c.min^{-1}$) by supra-threshold isoprenalin doses (IS) 1-5µg/min. Tachyarrhythmias (196±12 $c.min^{-1}$; M±SD) were treated by BM in 7-10 min intervals in 32 test in the following order a duration: CM (unilaterally, 15 s) → EA (15 s) → IA (20s) → VM (occlusion pressure ~ 35 mm Hg, 20 s) → TCR (cold gel <10 °C) to perinasal and forehead areas, 30-60 s) → TCR+A → TCR+VM. Heart rate was reduced by BM in the all tests in average by 38±4 $c.min^{-1}$. Reversal into NR (decrease of HR by 68±11$c.min^{-1}$) occurred in 14 tests - in AVNRT through blockade of anterograde of fast pathway, or by retrograde blockade of slow pathway or AV-blockade with transient ventricular extrasystoles and/or brief loss of rhythm (3-7s). Conversion occurred most readily after EA (6-19 s), in TCR after 15-32s, in IA after 9-22 s in VM after 8-23s. CM was least effective. TCR showed in repeated test gradual loss of the reactivity, which could be regained and strengthened by combination of TCR+A and TCR+VM.
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## EXPERIMENTAL CLOSED HEAD INJURY IN RATS: WEIGHT-DROP TRAUMA DEVICE

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This report describes an experimental model of closed head injury in rats using a calibrated weight-drop device. Our model produces diffuse brain injury in rodents and reproducible pathological intracranial pressure. In our project we use adult male laboratory Wistar or Sprague-Dawley rats of a conventional breeding colony. All surgeries are carried out under general surgical anesthesia (Thiopental, 50 mg/kg of body weight i.p.). The trauma weight-drop device is composed of two elements. The upper part is a hollow plexiglass column held in place with a ring stand. Weight is constructed as steel segments, each 50 g, to allow stepwise weight variations between 50 and 500 g. A nylon string passes through central holes of steel segments. The second element is a rigid plastic platform located on the foam, 6 cm thick. The animal is placed in a prone position on the plastic horizontal platform and midline incision over the vertex is performed. The bottom opening of the column is positioned in close proximity to the head of the rat between the coronal and the lambdoid sutures. The injury is delivered by dropping the weight from a predetermined height. After the trauma the rats are observed for apnea, convulsions and skull fractures. Subsequently we drill burr holes on the right side of the frontal area with the help of the high-speed drill. Dura mater is perforated by a

needle and the intracranial pressure sensor Codman (Johnson&Johnson) is inserted in the brain tissue to 3 mm depth. Additional physiological parameters are monitored as needed. This simple model of brain injury is capable of creating a graded brain injury and produces predictable and reproducible intracranial hypertension in the rats.

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## THE MECHANISMS INVOLVED IN THE EFFECTS OF PENTOXIFYLLINE ON THE P-GLYCOPROTEIN-MEDIATED MULTIDRUG RESISTANCE IN L1210/VCR CELLS

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Multidrug resistance (MDR) of neoplastic cells represents a specific type of resistance when neoplastic cells become resistant to the diverse groups of chemotherapeutic agents with unrelated chemical structure and mechanisms of pharmacological action. In our study drug sensitive L1210 and multidrug resistant L1210/VCR mouse leukemic cell lines were used as an experimental model. Development of the MDR phenotype in L1210/VCR cells was associated with overexpression and increased transport activity of P-glycoprotein. We tested the ability of several methylxantines to depress the MDR phenotype and found that from tested methylxantines only pentoxifylline (PTX) was able to reverse the drug resistance in L1210/VCR cells. To determine more precise the mechanisms involved in MDR reversal action of PTX we investigated its effects on enzymes involved in modulation of apoptosis. We found that the presence of PTX induced changes in specific phosphorylation of anti-apoptotic Akt kinase in resistant cells. On the other hand, the results suggest that the effects of PTX are not associated with modulation of caspase-3 and caspase-9 pathway.
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## ACTIVATION OF THE AFFERENT NERVES IN THE GUINEA PIG ESOPHAGUS VIA TRPA1 RECEPTOR

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Acid-induced oxidative stress contributes to the pathogenesis of esophageal injury in gastroesophageal reflux disease (GERD). Recent studies revealed that certain endogenous oxidative stress metabolites (such as 4-hydroxy-nonenal, 4-HNE) directly activate nociceptive transient receptor potential A1 (TRPA1) channel. We hypothesized that the esophageal nociceptors (pain nerve fibers) are stimulated via TRPA1. Extracellular recordings of single unit activity were made in the isolated innervated guinea pig esophagus. Calcium imaging was performed on the isolated vagal afferent neurons. In the majority (22/31) of the vagal nodose capsaicin-sensitive (putative nociceptive) neurons the TRPA1 agonists allyl isothiocyanate (AITC, 100μM) induced robust intracellular calcium increase (averaging ~85% of the response to subsequent 0.1μM capsaicin). The nerve terminals of vagal nodose nociceptors in the esophagus were robustly stimulated by the TRPA1 agonists AITC (100μM) and cinnamaldehyde (CA, 300μM) (peak discharge frequency 26±4 Hz, n=7/8 and 20±3 Hz, n=14/17, respectively). 4-HNE (100μM) evoked intracellular calcium increase in the capsaicin-sensitive vagal nodose neurons (n=27/39, averaging ~90% of the subsequent response to 0.3μM capsaicin). 4-HNE (300μM) also evoked action potential discharge in the nerve terminals of CA-sensitive vagal nodose nociceptors in the esophagus (peak discharge 10±2 Hz, n=5/6). We conclude that the TRPA1 agonists effectively stimulate vagal nociceptors in the esophagus. Our data indicate that the vagal nociceptors detect noxious stimuli that are endogenous TRPA1 activators, such as certain oxidative stress products and prostaglandin metabolites. This activation may contribute to the symptoms of esophageal diseases.
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## DIFFERENCES IN OXIDATIVE STATUS AND LUNG FUNCTIONS DURING LONG-TERM INHALATION OF MEDICINAL OXYGEN ($O_2$) IN COMPARISON WITH PARTIALLY IONIZED OXYGEN ($O_2^-$ AND $O_2^-$) IN GUINEA PIGS

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Aim: The aim of the study was to prove the hypothesis that the long-term application of ionized oxygen is save method with less serious impairment of lung functions in comparison with classical oxygen therapy. Material and methods: Experiments were carried out on 40 guinea pigs. Animals were placed in metabolic cage and inhaled 100% medicinal oxygen ($O_2$), or partially negatively ($O_2^-$) or positively ($O_2^+$) ionized oxygen during 17 and 60 hrs. Control animals inhaled atmospheric air. Wet/dry weight (W/D) ratio was determined to evaluate the degree of lung edema. Accumulation of dityrosine and lysine-LPO (lipid peroxidation) products demonstrating oxidative modification of proteins were determined in lung homogenate by fluorescence method. Relative number of cells were evaluated in bronchoalveolar lavage (BAL) fluid and peripheral blood. Results: After 17 hrs the accumulation of dityrosines (in arbitrary units; AU) increased in group with $O_2^-$ (19 ± 0,3) and decreased in group with $O_2^+$(16,1± 0,5) as compared with controls (17,5 ± 0,3) (both $P < 0,01$). In group inhaling non-ionized medicinal oxygen (18,1 ± 0,3) the raise was not statistically significant. After 60 hrs the fluorescence of dityrosines significantly rised after inhalation of $O_2$ as well as after $O_2^-$ as compared with controls (20,2 ± 0,6 and 21,7 ± 1,7 vs.17,5 ± 0,3; both $P < 0,01$), while after inhalation of $O_2^+$ there was no increase (17,3 ± 0,8). After 17 hrs values of lysine conjugates with LPO products significantly increased in comparison with controls (6,7 ± 0,2) after inhalation of $O_2$ (7,1 ± 0,2; $P < 0,05$) as well as $O_2^-$ (8,2 ± 0,2; $P < 0,001$). In the group with $O_2^+$ the fluorescence of lysine conjugates with LPO products (7 ± 0,2; $P > 0,05$) did not rise significantly as compared with controls. After 60 hrs the changes were comparable to those after 17 hrs. After inhalation of $O_2^-$ a $O_2^+$ W/D ratio did not change significantly while after inhalation of $O_2$ it was reduced. Relative number of neutrophils in BAL fluid was elevated in all groups with oxygen therapy, however, in $O_2^+$ group this value was reduced when compared with $O_2$ a $O_2^-$ groups. Conclusion: The results indicate that long-term inhalation of positively ionized oxygen is associated with less adverse effects on lung functions than non-ionized or negatively ionized oxygen.

## SIMULATION OF THE EFFECT OF CHANGED IONIC CONDUCTANCES ON THE PARAMETERS OF HEART MYOCYTE ACTION POTENTIAL

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Changes of the action potential (AP) can be a major symptom of various heart diseases. Action potential duration (APD) differs for different species, for cells from different parts of myocardium, and depends on beating frequency. Moreover, APD changes can result from various pathophysiological states.
To investigate the effect of individual ion channels on the AP we have used the Shannon - Bers model (Biophys. J. 87: 3351, 2004) of rabbit

ventricular myocyte. The myocyte was stimulated by 5-ms current pulses (9.5 μA/μF) at a frequency of 1 Hz. The maximum conductance, related to surface channel density of all ion channels in sarcolemma and sarcoplasmatic reticulum (SR) membrane, was systematically varied in the range of ± 50 %. The amplitude of AP plateau (APP) at t = 50 ms and duration of AP were evaluated.

| | - 50 % conductance change | | | + 50 % conductance change | | |
|---|---|---|---|---|---|---|
| | $\Delta APD_{50}$ | $\Delta APD_{90}$ | ΔAPP | $\Delta APD_{50}$ | $\Delta APD_{90}$ | ΔAPP |
| $I_{ClBk}$ | 21.8 % | 22.1 % | 2.1 mV | -12.8 % | -12.9 % | -2.0 mV |
| $I_{Kr}$ | 18.0 % | 20.6 % | 0.8 mV | -11.5 % | -13.2 % | -0.8 mV |
| $I_{NaK}$ | 13.1 % | 13.9 % | 1.2 mV | -9.5 % | -10.1 % | -1.2 mV |
| $I_{tos}$ | 13.7 % | 13.5 % | 3.7 mV | -10.6 % | -10.2 % | -3.2 mV |
| $I_{SRrel}$ | 8.2 % | 7.5 % | 5.1 mV | -0.3 % | 0.3 % | -1.9 mV |
| $I_{NCX}$ | -17.8 % | -18.4 % | -5.0 mV | 11.6 % | 11.9 % | 3.8 mV |

The changes of APD and APP for majority of channels were less than 5 %. However, in the case of potassium rapid-activating delayed rectifier current ($I_{Kr}$), chloride background current ($I_{ClBk}$), Na-K pump ($I_{NaK}$), slow transient outward potassium current ($I_{tos}$), SR calcium release current ($I_{SRrel}$) and Na-Ca exchanger ($I_{NCX}$), the changes of channels and transporters densities produced significant effects. These results underline the importance of exact balance in ion channel densities on major cardiac characteristics.

## $^1$H MAGNETIC RESONANCE SPECTROSCOPY IN DIAGNOSTIC PROTOCOL OF SOME BRAIN DISEASES

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Magnetic resonance spectroscopy (MRS) allowed to study biochemical changes and metabolic pathways in vitro and in vivo. Proton magnetic resonance spectroscopy ($^1$H MRS) can measure localized levels of cerebral metabolites such as N-acetylaspartate (NAA), choline (Cho), creatine and phosphocreatine (Cre), lactate (Lac) and some others. $^1$H MRS application *in vivo* in the diagnostic protocol of some brain diseases (brain tumors, epilepsy, neurodegenerative diseases and schizophrenia) was the study's focus. *In vivo* magnetic resonance spectra were obtained from the different parts of the brain using clinical scanner Siemens Symphony (1.5 T) and standard protocols. $^1$H MRS provides valuable information about the changes in the concentration of above mentioned metabolites and their ratios, which are typical for each brain diseases. The great advantage of the *in vivo* MRS is that we are allowed to study these biochemical events in real time without any disturbance of the tissue. We may conclude, that *in vitro* $^1$H MRS provides good information about biochemical differences in various type of human brain pathologies and could be used in the standard diagnostic protocol.

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## STUDY OF THE INTERACTION OF PENTOXIFYLLINE DERIVATIVES WITH PROTEINS OBTAINED FROM SENSITIVE L1210 AND RESISTANCE L1210/VCR CELLS

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The resistance of tumor cells to cytotoxic drugs is a problem in cancer chemotherapy. The multidrug resistance (MDR) phenotype associated with the overexpression of membrane P-glycoprotein (P-gp), is defined as a cross-resistance to a wide range of structurally diverse anticancer agents. Several substances (chemosesitizers) can restore the sensitivity of resistant tumor cells against anticancer drugs. This effect was described earlier for pentoxifylline (PTX). To characterize the structural features important for reversal effects of PTX we prepared a set of *N*1-, *N*3-, *N*7- and *C*8-substituted alkylxanthines derived from PTX and tested their effects on Vincristine resistance of MDR cell line L1210/VCR. However, the mechanism, how interact these PTX-derivatives with P-gp is not known. Therefore, we prepared columns with immobilized PTX for affinity chtomatography of cytosolic and particulate fractions of L1210 and L1210/VCR cells lines, respectively. We consequently analyzed eluted fractions by western blot to detect proteins. The results showed that the inhibition of P-gp mediated MDR is increased by: i) prolongation of alkyl chain at the both *N*3 and *N*1 positions, ii) presence of polar substituent on alkyl chain at the position *N*1. The results indicate that the effectivity of xanthines in reversal of MDR resistance is dependent on the longer polar substituent in position *N*1.

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## THE USE OF TRIVALENT CATIONS FOR A MEASUREMENT OF GATING CURRENT FROM THE $CA_V3.1$ CHANNEL

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Positively charged S4 segments of voltage-operated $Ca^{2+}$ channels form putative voltage sensors. Voltage-dependent opening and closing of these ion channels are accompanied by movements of the S4 segments. This charge movement could be detected as a so-called gating current. Gating current is observable when inward ion current is fully blocked. The aim of our study was to evaluate suitability of trivalent cations $Gd^{3+}$, $Y^{3+}$ and $Er^{3+}$ for the measurement of the gating current of the $Ca_V3.1$ calcium channel. $Ca_V3.1$ calcium channel was stably transfected in HEK 293 cells. The bath solution contained (in mM): HEPES 10, $CaCl_2$ 2, $MgCl_2$ 2, NMDG 140; pH 7.4 with HCl. The pipette solution contained (in mM): CsCl 130, Na-ATP 5, TEA-Cl 10, HEPES 10, EGTA 10, $MgCl_2$ 5; pH 7.4 with CsOH. Holding potential was -100 mV. Gating currents were measured by 50 ms long depolarizing pulses to membrane potentials between -90 mV and +70 mV. Three criteria were used to evaluate suitability of each cation: (i) kinetics of current block development; (ii) efficiency of the inward current inhibition; (iii) cation must not influence charge movement itself. All three tested cations inhibited inward calcium current with comparable kinetics. $Er^{3+}$ blocked the $Ca_V3.1$ channel in ten times lower concentration than $Gd^{3+}$ and $Y^{3+}$. Both $Gd^{3+}$ and $Y^{3+}$ in concentration 300 μM and higher, which is necessary for complete current block, inhibited also the gating current of the $Ca_V3.1$ channel. $Er^{3+}$ in concentration of 30 μM was able to inhibit fully calcium inward current without interfering with the gating current. We concluded that $Er^{3+}$ is a suitable calcium current blocker for the analysis of gating currents originating from the $Ca_V3.1$ channels.

## EFFECT OF *Cinnamomum zeylanicum* ON OXIDANT STATUS IN BROILER CHICKENS

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Antioxidant systems are being shown to play an important role in the protection against exogenous oxidative stress. Many authors reported antimicrobial, antifungal and antioxidant properties by spices and essential oils (1, 2, 3). Shahidi et Wanasundara (4) reported that the antioxidant effect of aromatic plants is based on the presence of hydroxyl groups in their phenolic compounds. The purpose of the present study was to examine the effects of different doses of cinnamon intake by feed on antioxidant status of broiler chickens. Thirty two broiler chicks were fed one of four diets containing 0%, 0.1%, 0.05% or

0.025% cinnamon from the day of hatch to 38 d of age.Then all birds were sacrified and blood, liver, kidney and duodenal epithelium for analyses were collected. Dietary addition of 0.1% cinnamon significantly decreased thiobarbituric acid reactive substances (TBARS) levels in plasma and duodenal epithelium. Blood glutathione peroxidase (GPx) activity showed a significant increase in group fed 0.1% concentration of cinnamon. The administration of 0.1% and 0.025% cinnamon significantly reduced alanine aminotransferase (ATL) activity in plasma. Blood superoxide dismutase (SOD), catalase (CAT), aspartate aminotransferase (AST) activities and SH- group levels were not affected by diet. The present study shows that cinnamon exhibits significant antioxidant activity by broiler chicks and it can be used as a source of antioxidants in the dietary supplementation.

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## ADAPTATION TO GENE DISRUPTION AFFECTING CHOLINERGIC TRANSMISSION

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Cholinergic synapses represent connections among broad group of neurons and also between neurons and their effectors, such as interneuronal communication in the central nervous system (CNS), synapses between autonomic nerves and their effector tissues and also neuromuscular junctions. Binding of acetylcholine to its receptors initiates signal transduction cascades. In our study, we wanted to ascertain whether alteration of various elements of the signaling cascades influences both the target receptor types and other receptor systems that affect the function of appropriate tissue. We used lung and heart tissues, different areas of CNS and diaphragm from mice lacking acetylcholinesterase (AchE), butyrylcholinesterase (BuChE), proline rich membrane anchor (PRiMA), a protein anchoring acetylcholinesterase in the cytoplasmic membrane, and $M_2$ muscarinic receptors. We observed changes in the densities of muscarinic receptors, alpha1-adrenoceptors, beta-adrenoceptors, dopamine $D_1$-like and $D_2$-like receptors, $GABA_A$ receptors, NMDA receptors and nicotinic acetylcholine receptors. In addition, intracellular signaling (cAMP levels, G protein coupling, phospholipase C activity) was measured in specific tissues. Disruption of AchE gene led to decrease in number of muscarinic, alpha1-, beta-adrenoceptor binding sites in the lung and also caused decrase of $D_1$-like and $D_2$-like binding sites in the striatum accompanied with increase in $GABA_A$ binding sites and no change in NMDA receptors. Surprisingly, we have found increase in heart muscarinic receptors in AChE knockout that was also the case of PRiMA gene disruption. We also found decrease in the number of nicotinic binding sites in diaphragms of BuChE knockout mice. Disruption of $M_2$ muscarinic receptor gene led to increase in muscarinic receptor binding sites together with decrease in alpha1-adrenoceptors in the lung. In the striatum, there was decrease in dopamine receptor binding sites, whereas these receptors were increased in hippocampus. We can therefore confirm our hypothesis that disruption of different molecules affecting cholinergic transmission also influenced other receptor types that affect the function of appropriate tissue. These changes are tissue specific.

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## FREE RADICALS DOES NOT EXERT SIGNIFICANT INFLUENCE ON FLUIDITY AND TRANSMEMBRANE POTENTIAL OF HEART MITOCHONDRA FROM RATS WITH ACUTE STREPTOZOTOCIN-DIABETES

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Aims: Investigation of changes in biochemical and physical properties of heart mitochondria (MIT) induced in rats by acute diabetes (DIA) and associated with endogenous mechanisms of protection (EMP). Materials and Methods: Male Wistar rats, 220±25g. Experiment: started with a single dose of streptozotocin (STZ, 65 $mg.kg^{-1}$ i.p.), terminated on the day 8 after STZ. MIT isolated with proteases. Estimations: Metabolism; In MIT: functional parameters, $CoQ_{10,}$ NO synthase; conjugated dienes (CD), membrane fluidity (MF), membrane potential (MP). Results: DIA confirmed by metabolism. Decreases: $QO_2$, the rate of oxid. phosp. and MP ($p<0,01$-$0,001$). Increases: MF, $CoQ_{10}$ and in MIT ATPase ($p<0,01$). Unchanged: CD, ADP:O and NO synthase. Conclusions: i) FR may participate in EMP induction positively; ii) A negative correlation between the MF and MP was discovered in the DIA MIT. Grants: VEGA 2/6148/26, 2/7126/27, 2/0173/08, 1/3037/06, APVV: 51-027404.

## ALTERNATIVE PROGRAMMED CELL DEATH IN THE PRIMARY ENAMEL KNOT – A TEM STUDY

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Tooth development comprises coordinated cell proliferation, migration, differentiation and cell death. Apoptosis is involved in all stages of odontogenesis including elimination of the enamel knots. Primary enamel knot (PEK) has been recognised as a signalling centre secreting growth factors crucial for the tooth bud to cap transition. Fgfs produced by PEK stimulate proliferation of surrounding cells; however, not in the enamel knot, since these cells lack FGF receptors. On the other hand, it is likely that Bmps secreted by enamel knot target PEK itself for death. Whether apoptosis only eliminates cells that are not needed anymore or it has any active morphogenic role remains questionable.
Widely used approach to investigate developmental roles of apoptosis is *ex vivo* pharmacological caspase inhibition. However, until now such studies on tooth morphogenesis are not conclusive. Both, data supporting or admitting morphogenic role of apoptosis have been published. Major problem of caspase inhibitors is the fact that apoptosis is blocked in a late stage after permeabilisation of mitochondria that is recently considered as the point of no return. That means that cells of the PEK unable to sense survival signals while exposed to apoptotic signals may ultimately tend forward to death either via caspases or alternatively via a caspase independent cell death (autophagy, necrosis) if the proteolytic function of caspases is blocked. Transmission electron microscopy was used to evaluate samples after general caspase inhibition in tooth explant cultures and to bring more insight into alternative cell death programs.
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## ANALGESIC EFFECT OF mGluRs ANTAGONISTS IN EARLY POSTNATAL PERIOD

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A number of studies on adult animals suggest that modulation of metabotropic glutamate receptors (mGluRs) play a role in pain perception. Group I mGluRs including the mGluR1 and mGluR5 subtypes are excitatory glutamatergic receptors since they induce stimulation of phospholipase C, release of $Ca^{2+}$ from intracellular stores and activation of protein kinase C. The aim of the present study was to investigate the effect of the mGluR5 antagonist, MPEP, and of the mGluR1 antagonist, AIDA, on acute nociception in rats during early postnatal period (postnatal day 16). The nociception was measured as withdrawal thresholds for mechanical and thermal stimulation. The results showed that MPEP does not change the pain thresholds whereas AIDA increased the thermal and mechanical pain threshold on hind limbs. Changes in motor abilities were not observed in any group. It is concluded that Group I mGluRs might play a modulatory role in acute pain during early postnatal period. However, it was shown that this role is different for mGluR1 and mGluR5.
This work was supported by GACR 305/06/1188 and GACR 305/07/0242.

## DIASTOLIC HYPERTENSION CORRELATES WITH SEVERITY OF SLEEP APNOEA/HYPOPNOEA SYNDROME

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The aim of our study was to correlate the results of parallel blood pressure monitoring and polysomnographic recording in patients with arterial hypertension (AH) and sleep apnoea/hypopnoea syndrome (SAHS). Results: From 74 patients investigated in our Sleep laboratory, 9,5% were treated by combination of two, 8% by three, 18% without declared number of drugs and 59% were without detected AH and antihypertensive therapy. According to AHI (indicating the severity of SAHS), there were 3 groups of patients: 1) AH+ mild SAHS 5<AHI≤ 20/h, n=25, 2) AH+ moderate SAHS 20<AHI ≤ 40/h, n=23, 3) AH+ severe SAHS with AHI>40/h, n=26. In all 3 groups AHI, number of obstructive apnoeic episodes (OA), average saturation $O_2$% (Avg. Sat $O_2$%) and duration of saturation under 84% in minutes Sat $O_2$<84%$_{(in\ min.)}$ significantly correlated with changes particularly in nocturnal diastolic blood pressure (DBPn). In our patients with AH+OSAHS nocturnal DBP positively correlated with AHI (p<0,0049), with the number of OA episodes (p<0,0027), and duration in minutes of Sat $O_2$<84% (p<0,05) and negatively correlated with Avg. Sat $O_2$% (p=0,05). Conclusion. The correlation with polysomnographic parameters in DBPn was stronger than in diurnal diastolic blood pressure (DBPd). AHI and number of OA increased DBPn, but number of hypopnoic episodes significantly correlated with DBPd (p=0,0391), suggesting that after several years SAHS could increase DBP also during the day. Correlation Avg. Sat $O_2$% and DBPn demonstrated that the decrease of average oxygen saturation particularly increases the DBP during the night. Longer duration of saturation Sat $O_2$<84% $_{(in\ min.)}$ means more severe hypoxemia and higher DBPn.
Key words: hypertension, blood pressure monitoring, sleep apnoea/hypopnoea syndrome, hypoxemia

## ADIPOSE TISSUE AS A PRODUCER OF ENDOTHELIAL CELL ADHESION MOLECULES IN OBESITY: COMPARISON OF DIFFERENT FAT DEPOTS AND THE INFLUENCE OF VERY LOW CALORIE DIET

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We tested the hypothesis that adipose tissue significantly contributes to increased levels of proatherogenic endothelial cell adhesion molecules (CAM) in obesity and explored the influence of very low calorie diet (VLCD) on this production. 25 obese females (OB) and 14 lean healthy females (C) underwent blood drawing and subcutaneous (s.c.) and visceral (visc.) adipose tissue sampling during gastric banding and elective cholecystectomy surgery, respectively. In VLCD substudy, 20 obese females underwent blood drawing and s.c. fat biopsy before and after 3 weeks of VLCD (550 kcal/day). Serum concentrations of insulin, ICAM-1, VCAM-1 and E-selectin were measured using Lincoplex kits, CAM mRNA expression in fat was measured using RT PCR and protein CAM levels in fat were measured using Lincoplex kits and normalized to protein content. BMI, serum insulin levels, HOMA index, ICAM-1 and E-selectin concentrations of OB were significantly higher relative to C. Serum concentrations of VCAM-1 did not differ. Messenger RNA expression and protein levels of CAM in s.c. fat were not affected by obesity while in visc. fat ICAM and VCAM mRNA expression and its protein levels were significantly increased in OB relative to C. Three weeks of VLCD decreased circulating E-selectin levels while it did not change circulating ICAM-1 and VCAM-1 or its mRNA expression in s.c. fat.
We conclude that obesity increases visceral but not subcutaneous adipose tissue production of CAM. This finding may partially explain the closer relationship of visceral fat accumulation in obesity to cardiovascular complications.

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## EFFECTS OF POLYPHENOLS, CAPTOPRIL, HYDROCHLOTHIAZIDE AND INDAPAMIDE ON MYOCARDIAL FIBROSIS IN L-NAME - INDUCED HYPERTENSION

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We studied the effects of captopril (C), indapamide (I), hydrochlorthiazide (H) and red wine polyphenols (P) on myocardial fibrosis with emphasis on collagens type I. and III. in N(G)-nitro-L-arginine methyl ester (L-NAME) -induced hypertension. L-NAME (40mg/kg) was administered to rats during 7-weeks together with I (1mg/kg), C (10mg/kg), P (40mg/kg), H (10mg/kg) or their combinations. Fibrosis in the hearts was evaluated by picrosirius red staining. L-NAME (compared with control) increased blood pressure and collagens type I. and III. without a change in their proportion. All studied substances decreased blood pressure and proportion of collagens I/III, but did not decrease collagen type III. when compared to the group with L-NAME only. C and I decreased total fibrosis only after their combination, the effect was not significant after separate administration. C (alone or with I) decreased also the abundance of collagen type I. H and P (alone or with I) decreased collagen type I. and total myocardial fibrosis, whereas H was more efficient. Combination of captopril and indapamide has better influence on myocardial fibrosis than their

separate administration, while hydrochlorthiazide and red wine polyphenols have positive effect on myocardium also on their own.
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**PHYSIOLOGY – INTERACTIVE APPROACH FOR INTERNATIONAL EDUCATION**

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The progress in international education due to the European Union platform opens many opportunities and challenges. Recent progress in computer technology allows lecturers to present topics using modern, attractive, and objective methods. Physiology textbooks in English are generally available, practical education, however is usually university or even faculty specific.
Therefore, we aimed to prepare an interactive textbook for practical training in physiology based on the curriculum of the Faculty of Veterinary Medicine, UVPS Brno. The material will be available on 2 CDs and is focused on blood physiology, immunology, cardiovascular and respiratory systems (Physiology I) and neurophysiology, endocrinology, reproduction, metabolism and gastrointestinal tract (Physiology II). Animations and video-sequences demonstrate topics of individual tutorials, more details and further applications are goals of specific seminars and student conferences. Each part involves a protocol pattern giving a chance for result evaluation and conclusions by students themselves.
We hope this material will help students prepare for practical courses, perform experiments, share guidelines for protocol elaboration and open topics in animal physiology for broader discussion.

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**CROSS-FOSTERING EFFECT ON SENSORIMOTOR DEVELOPMENT OF RAT PUPS EXPOSED TO METHAMPHETAMINE**

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Our previous studies demonstrated that methamphetamine (MA) administered during gestation and lactation periods impairs maternal behavior as well as the postnatal development of rat pups. The present study tested the hypothesis that cross-fostering influences the development of rat pups. Mothers were daily exposed to injection of MA (5 mg/kg) or saline (S) for 9 weeks: three weeks prior to impregnation, throughout the entire gestation period and during lactation until the weaning period. As an absolute control (C) females with no injections were used. On postnatal day (PD 1), pups were cross-fostered so that each mother received some of her own and some of the pups of mother with the other two treatments. Sensorimotor coordination was examined between PD 1 and PD 23. Following behavioral tests were used: negative geotaxis (PD 9 and 11), righting reflex on surface (PD 12), righting reflex on mid-air (PD 17) and rotarod (PD 23). Our results showed that the birth weight in prenatally MA-exposed pups was lower than controls or saline-exposed pups regardless of sex. Further, we demonstrated that pre- and postnatal MA exposure impairs sensorimotor functions in these tests: negative geotaxis, righting reflexes and rotarod. On the other hand, postnatal care of control mothers at least partially suppressed the negative effect of prenatal MA exposure. Our hypothesis, that the care of adoptive mother may affect postnatal development of pups, was confirmed.

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**EFFECT OF LARD BASED HIGH FAT DIET ON THE FUNCTIONAL AND BIOCHEMICAL PARAMETERS IN THE RAT HEART**

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The present study was aimed to determine the effect of high fat (HF) diet feeding on the rat heart remodelling. Male Wistar rats were fed with standard pellet diet (SPD) enriched by lard (to 40kcal %) during 48 days (HF group). Some biochemical parameters, such as plasma concentrations of glucose, insulin, NEFA C, TAG and cholesterol were measured by colorimetric or ELISA method. Also insulin tolerance, blood pressure and heart rate were determined. Myocardial fibrosis was evaluated using picrosirius red staining. Total contents and/or activities of MMPs, TIMPs and protein kinases (ERK, Akt) were determined by Western blot analysis or by gelatin zymography. HF diet induced insulin resistance (IR) accompanied by increased levels of plasma insulin. We found also increased protein levels of TIMP-2 in heart tissue, moderate up-regulation of Akt kinase activation in the right ventricle and down-regulation of ERK1/2 in the left ventricle. Zymographic analysis of MMPs revealed strong gelatinolytic activity; however there were no significant differencies between HF and control (SPD without lard) groups. Other tested parameters were also unchanged. In conclusion, 48 day's feeding of rats with HF diet induced IR, however without other complications. It suggests that it is an initial stage of IR and activation of cardioprotective Akt kinase could play the compensatory role in maintenance of metabolic and functional parameters at normal level.
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**THE CHANGES OF SOME HORMONAL, BIOMETRIC PARAMETERS OF EWES OVARIES AND IGF-I DURING ESTROUS INDUCTION**

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Conditions to realize reproduction control in the sheep are formed by the methods of the oestrus induction and synchronization. In our study we targeted the morphological changes in ovaries during folliculogenesis and the determination of progesterone ($P_4$), oestradiol 17β ($E_2$) and insulin-like growth factor I (IGF-I) concentrations in blood serum at different biotechnical influencing the sheep in anestrous period. The blood samples were obtained the animals (n=10) to determine hormone concentrations before performed interventions. Consequently, ewes were induced to oestrus with GnRH (0.5 ml per head). After 5 days, five ewes were synchronised with $PGF_{2\alpha}$ (0.5 ml per head) and to other five ewes ram was introduced. Forty-eight hours after treatment and ram introduction blood samples were taken again to determine of $P_4$, $E_2$ and IGF-I. The ovaries were processed by current histological method after laparotomy and ovariectomy. Histological slides (5–7 μm thickness) were coloured by haematoxylin-eosin and evaluated quantitatively and qualitatively by LUCIA-G analysator. The concentrations of $P_4$ and $E_2$ in blood serum were determined using RIA and IGF-I using ELISA methods. Obtained results were statistically evaluated by t-test and ANOVA. The results of biometry analyse showed no statistically significance similarly as differences of total follicle numbers, follicles < 3 mm, > 3 mm, and also between number of healthy follicles and number of atretic follicles. The comparison between the hormone concentrations in experimental groups showed that at the day 7 after GnRH application and 48 hours after $PGF_{2\alpha}$ the levels of $P_4$ and IGF-I decreased but $E_2$ increased. These changes were not significant. In group 2 not significant increase of the previous hormones were observed. Our results show that the ram pheromones can stimulate secretion of gonadotrophins, ovulation in anestrous ewes and thus positive affect on the folliculogenesis.

**ALTERATIONS IN GLYCAEMIC LEVELS AND MOTOR NERVE CONDUCTION VELOCITY IN RATS IN EXPERIMENTAL MODEL OF DIABETES**

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The study was concerned to determine the effects of Pycnogenol® (P) on the levels of preprandial and postprandial glycaemia, motor nerve conduction velocity (NCV) in streptozotocin-induced (STZ) diabetic rats and to compare the efficiency of P treatment in various doses on studied parameters. Diabetes was evoked by STZ (25mg/kg i.p., 3 days sequential). P treatment (10, 20, 50mg/kg/day p.o.) was administered 2 weeks after diabetes was enveloped. The treatment had lasted for 8 weeks. In diabetic rats the fasting and postprandial plasma levels of glucose were significantly ($p<0.005$) elevated from 5.58 ± 0.25 mM/l to 30.79 ± 1.95 mM/l (fasting) and from 8.9 ± 1.67 mM/l to 35.96 ± 1.45 mM/l (postprandial). Treatment with P dose-dependently and significantly ($p<0.005$) reduced fasting serum glucose levels, and not dose-dependently reduced postprandial glucose levels ($p<0.05$). NCV was measured in halothane anaesthesia on sciatic nerve. P improved the impaired NCV significantly in doses 20mg/kg ($p<0.05$) and 50mg/kg ($p<0.005$) in treated animals in comparison to diabetic rats.
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**BEAT-TO-BEAT VARIABILITY, SPECTRAL ANALYSIS AND REACTIVE CHANGES IN CARDIAC BODY SURFACE POTENTIAL MAPS**

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To our knowledge there is no information on measures and spectral parameters of spontaneous individual variability of the body surface potential maps (BSPMs), or on their dynamic reactive changes in healthy subjects. The aim of this study, using several minutes lasting recordings of BSPMs, was to investigate their beat-to-beat (B-B) fluctuation, to assess its spectral components and baseline drifts due to car-diovascular reactions. Continual records of body surface ECG in 5 healthy men, were taken in supine rest, gradual head up tilting to 60° plus tilted position, gradual head down tilting to supine and sitting. Following parameters were beat-to-beat evaluated: R-R intervals, QRS and QRST characteristics derived from respective integral BSPMs (maximum and minimum values, their localizations, peak to through amplitudes), as well as Karhunen-Loeve eigenvectors related nondipolarity indexes and angle α. A considerable spontaneous B-B variability of the investigated BSPMs parameters, more pronounced in those, related to repolarization was obser-ved. Integrated cardiovascular reactions to tilting or sitting up evoked a significant decline of the QRST BSPM amplitudes with concomitant changes also in other in-vestigated parameters, characterized by transition phenomena and prolonged after-effects. The spectral analysis identified i) significant respiratory oscillations in the high frequency (HF) range 15-20 c/min, diminished in the transition periods of til-ting up and down, present also in the case of absent HF rhythm in the heart rate. Different amplitude in different BSPM parameters, may be influenced by loca- lization of the respective BSPM extremes on the thoracic surface. ii) slower perio-dic variations in the low frequency(LF) band 3-10 c/min, mainly in the QRST inte-gral BSPM and R-R intervals, with an expected LF increase and HF decrease du-ring sympathetic stimulation tests. Detailed analysis of the B-B BSPMs variability allows a more accurate definition of the autonomic modulation of the ventricular activation with a promising possibility to discover subjects with higher risk of vent-ricular dysfunction.

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**EFFICACY OF DIETARY POLYMERIC GLUCOMANNAN TO COUNTERACT MYCOTOXIN TOXICITY IN BROILER CHICKENS**

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Deoxynivalenol (DON) is the most prevalent trichothecene in crops used for food and feed production. Toxic effects concern mainly the immune system and the gastrointestinal tract (1). In order to avoid mycotoxicosis, several strategies have been investigated (2, 3). The most frequently applied method for protecting animals against mycotoxicosis is the utilization of adsorbents mixed with the feed which are supposed to to bind mycotoxins efficiently in the gastrointestinal tract (4). An experiment was conducted to investigate the effects of polymeric glucomannan mycotoxin adsorbent (PG polymer) to counteract toxicity of DON in growing broiler chickens. Fourty two, 1-d old male broiler chicks were fed 1 of 3 diets containing DON for 42 days. The diets included: (1) control (0.2 ppm DON), (2) deoxynivalenol-contaminated (3 ppm DON) and (3) deoxynivalenol – contaminated (3 ppm DON) plus GM polymer (2g/kg diet). All birds were sacrified and blood samples for biochemical analyses were collected. Dietary supplementation of DON significantly altered plasma total protein, calcium, magnesium, triglycerides and free glycerol levels and ALT activity. Inclusion of GM polymer in the diet restored plasma levels of magnesium, triglycerides, free glycerol, total protein and ALT activity induced by dietary deoxynivalenol. Inclusion of polymeric glucomannan mycotoxin adsorbent to DON-contaminated diet, however, did not prevent toxic effect on calcium metabolism.

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**THE EFFECT OF FASTING AND CARBENOXOLONE TREATMENT ON 11β-HYDROXYSTEROID DEHYDROGENASE 1 IN THE LIVER OF THE HYPERTRIGLYCERIDEMIC RAT**

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The increasing number of people in advanced countries suffers from a cluster of associated metabolic disorders that are collectively termed metabolic syndrome and that include hyperinsulinemia, hypertension, glucose intolerance, hypertridyceridemia and obesity. Because not only insulin but also glucocorticoids strongly influence the metabolism of nutrients, we decided to study their local metabolism via 11β-hydroxysteroid dehydrogenase 1 (11HSD1) in liver that represent a glucocorticoid target organ. For our study we used female Prague hederitary hypertriglyceridemic rats (HHTg) and Wistar rats as their normotriglyceridemic counterparts. We previously reported increased expression of 11HSD1 in the liver of female HHTg rats. In this study we describe changes in 11HSD1 mRNA expression upon 24 hour fasting and 14 days of carbenoxolone treatment in female HHTg rat. The levels of mRNA were determined by RT „real-time“ PCR. Fasting lowered 11HSD1 mRNA in both groups (Wistar and HHTg) compared to normally fed animals. Expression of 11HSD1 in HHTg rats after carbenoxolone treatment was significantly lower than in HHTg rats
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## CARDIAC GLUCOCORTICOID METABOLISM DURING CHRONIC HYPOXIA

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Increasing evidence suggests that gluco- and mineralocorticoids modulate cardiovascular homeostasis including hypertrophy and fibrosis. The ability of the cells to respond to these hormones depends on coexpression of their receptors and 11β-hydroxysteroid dehydrogenases that convert glucocorticoids to their biologically inactive 11-oxo derivatives (11HSD2) or vice versa (11HSD1). The aim of this study was to determine the cardiac glucocorticoid metabolism in rats adapted to chronic hypoxia (7000 m, 8 h/day, 5 weeks) that induces right ventricle hypertrophy and fibrosis. Hypoxia decreased the reduction of inactive 11-dehydrocorticosterone (A) to corticosterone (B, 11HSD1 activity) whereas increased the oxidation of B to A (11HSD2 activity). 11HSD2 mRNA level was expressed at a significantly higher level in hypoxic than in control animals but 11HSD1 mRNA expression was not changed in both the myocardium and isolated cardiomyocytes. The results show that cardiac tissue is able to utilize not only B but also A as a source of glucocorticoids and that the use is decreased during chronic hypoxia. These changes emphasize the physiological and pathophysiological role of corticosteroids in cardiac homeostasis.
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## WAVELET ANALYSIS OF ELECTRICAL ACTIVITY FROM RESPIRATORY MUSCLES DURING COUGHING AND SNEEZING IN ANAESTHETIZED RABBITS

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Cough and sneeze are important defensive reflexes of the airways. Despite a very high similarity, some differences in generation of the patterns of these two behaviors were suggested. The main aim of our study was to analyze and compare a frequency composition of electrical activity in the respiratory muscles in coughing and sneezing.
Data were taken from eight adult rabbits under pentobarbital anaesthesia. During inspiratory period, we compared electrical activity of diaphragm in the tracheobronchial cough, sneeze, and quiet breathing. During expiratory period of coughing and sneezing, electromyograms were used from the abdominal muscles. We applied the wavelet analysis to determine a time-frequency distribution of energy during mentioned behaviors due to non-stationary character of EMG signals.
Inspiratory duration of cough, sneeze and quiet inspiration were similar. The maximum of inspiratory power has occured later in sneeze than during quiet inspiration ($p<0.05$). The total inspiratory power during sneeze was higher compared to those found in the cough ($p<0.05$) and quiet inspiration ($p<0.01$). Lower frequencies contributed to an increase of the power even significantly more than in the cough (287.5 Hz up to 575 Hz, $p<0.01$; under 287.5 Hz, $p<0.05$). We found similar distribution of energy in the cough and quiet inspiration. In quiet inspiration the maximum of energy occured at lower frequencies comparing to the sneeze ($p<0.01$). There was similar rate of contribution to the total power (ratio of the power in the frequency band to the total power) for the sneeze and cough, the differences were only found in comparison with quiet inspiration.
The expiratory period of coughing was longer compared to that in sneeze ($p=0.0006$). No significant differences were found in the time – frequency energy distributions.
Our results indicate the possibility that there may exist significant differences in mechanism of generation of the cough, sneeze, and quiet breathing.

## INTERACTIONS OF BIOGENIC AMINES AND ADIPOKINETIC HORMONES IN INSECTS

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Insect neuropeptides belonging to the adipokinetic hormones (AKHs) have diverse functions, but predominantly they are involved in mobilization of energy sources (Gäde et al., 1997, Physiol. Rev. 77, 963-1032) and behave as typical stress hormones. Their central position in operation of stress evoked metabolic processes is supplemented by a role in control of a number of accompanying processes on physiological and behavioural levels. It is generally accepted that certain biogenic amines (octopamine, dopamine, norepinephrine, serotonin, tyramine) are involved in regulation of AKHs production. The relationship between these biogenic amines and AKHs has been studied in the firebug *Pyrrhocoris apterus*, where two AKHs have been identified (Kodrík et al., 2000, Insect Biochem. Mol. Biol. 30, 489-498; Kodrík et al., 2002, Peptides 23, 583-585). An apparent feedback between AKH effects, and dopamine and norepinephrine actions has been found in this insect species. Both of these amines stimulated level of haemolymph lipids, modulated a level of AKHs in CNS and haemolymph, and enhanced walking activity. We cannot exclude that bug's native AKHs are involved in the stimulatory effects of norepinephrine action, while dopamine employs an AKH-independent pathway. Possible mode of action of these amines and their interference with AKHs is a topic of the current study.
This paper was supported by the grant No. 522/07/0788 from the Grant Agency of the Czech Republic (DK).

## CONCENTRATION OF LIPOPROTEIN (a), APOLIPOPROTEIN $B_{100}$ AND LIPIDS IN CHILDREN WITH HEREDITARY CARDIOVASCULAR RISK

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The epidemic character of cardiovascular diseases requires in most states of Europe finding of risk factors of early aterosclerosis already in childhood. The aim of study was to find the serum concentration of lipoproteins and lipids in a group of 41 children with mean age 11 ± 5 years and with increased cardiovascular risk (R). At least one parent overcame an early myocardial infarction before reaching the age of 45 years. Lipoprotein (a) – Lp(a) was determined turbidimetrically and apolipoprotein apo $B_{100}$ – apo $B_{100}$ by the method of electroimmunoassy with antibodies and standards of companies Immuno - Austria and Behringwerke - Germany as well as. Lipids : total cholesterol – TC, triacylglycerols – TG and cholesterol-HDL -- HDL-C were determined using the Czech biochemical sets of Pliva - Lachema company. We found in the group of children with increased cardiovascular risk higher concentrations of Lp(a) in comparison with control group of healthy children without positive family history of early myocardial infarction (C).The concentrations of apo $B_{100}$, TC ($p< 0.001$), TG and non HDL-C ($p< 0.01$) in group R were significantly increased.
We would like to emphasise the significance of prevention including regular control of lipid parameters mainly in children with genetic predisposition, i.e. with increased cardiovascular risk.
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## CHANGES IN PROTEIN METABOLISM OF SEPTIC RAT AFTER β-HYDROXY-β- METHYLBUTYRATE TREATMENT

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β-hydroxy-β-methylbutyrate (HMB) is the leucine metabolite, which could contribute to reversing AIDS- and cancer-related cachexia. The aim of our study was to determine if HMB treatment is able to reverse sepsis-induced changes in protein metabolism in rat.
Male rats weighing 40-60 g were administered with endotoxin (5 mg/kg i.p.) to induce sepsis and implanted with osmotic pump with or without HMB (0.5 g/kg/day). Intact animals were implanted with pump without HMB content ($n \geq 9$ for each group). After 24 hours extensor digitorum longus (EDL) and soleus (SOL) muscles were isolated and used for determination of total and myofibrillar proteolysis (PL), protein synthesis (PS), leucine oxidation (OL), chymotrypsin like activity (CTLA) and expression of α subunits of proteasome. Samples of other tissues were collected for determination of CTLA and lysosomal proteolytic activity. Sepsis induced an increase in total and myofibrillar PL, CTLA and OL in both types of muscles and a decrease in PS in EDL only. In HMB treated septic animals we observed a decrease of OL in both types of muscles, myofibrillar PL and CTLA decreased in SOL and total PL in EDL only. No changes in expression of α subunits of proteasome were observed. Effects of sepsis and HMB treatment on proteolytic activity in other tissues were tissue dependent. The results indicate positive effect of HMB treatment on sepsis-induced changes in protein metabolism in skeletal muscle. This effect is muscle type dependent and is caused by attenuation of CTLA and PL, not by stimulation of PS or changes in proteasome expression.

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## CHARACTERIZATION OF THE FUNCTION OF RAT HEART REMODELED BY ISOPROTERENOL

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Cardiac hypertrophy is a major risk factor for the development of heart failure and sudden cardiac death. Myocardial remodeling in rats was induced by repeated injections of isoproterenol (5mg/kg s.c. for 7 days, Iso5, n=7). In anesthetized animals (tiopenthal 45 mg/kg) the electrocardiograms were recorded. Iso5 rats showed higher Sokolow-Lyon index or Cornell voltage than the baseline criteria found in control (n=7) group. Electrical activity of perfused hearts isolated from Iso5 animals was characterized by a longer QT interval and higher R amplitude compared to control ones. Moreover, the susceptibility to episodes of spontaneously terminated ventricular arrhythmias was higher in Iso5 than in controls. Hemodynamic measurements of Iso5 hearts revealed weaker contraction (55±33 vs 106±15mmHg) and increased left ventricular diastolic pressure of spontaneously beating hearts compared to control hearts. The Iso5 hearts were characterized by significantly ($p \leq 0.05$) increased heart (1.50±0.03 vs 1.34±0.02g), and left ventricular (LV) (1.00±0.20 vs 0.79±0.19g) wet weight as well as by thicker LV free wall (4.3±0.5 vs 3.5±0.3 mm). It can be concluded that repeated doses of isoproterenol induce myocardial remodeling associated with hypertrophy as well as with the changes in ECG and contractile and diastolic dysfunction. Sokolow-Lyon index is a good predictor of increased left ventricular mass associated with catecholamine-induced cardiac remodeling in rats.
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## EFFECT OF TEMPERATURE ON GILL MORPHOLOGY AND ION TRANS- PORTER DISTRIBUTION IN THE GILLS OF KOI CARP (*C. CARPIO*, L.)

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This paper offers some new data of a quite common fresh- water teleost, the Koi carp (*Cyprinus carpio*, L.). This fish is usually kept at room temperature and has the ability to survive shorter periods of hypoxia. Yet, little is known about the influence of temperature on the ion transporter expression within the gill epithelial cells. The branchial epithelium serves the fish as the main osmoregulatory organ; in freshwater fish, the plasma is hyperosmotic in comparison to the external environment, leading to passive water uptake and ion loss over the gill epithelial surface. The excess water gained is secreted through the production of high amounts of diluted urine, but the lost ions must be replaced by active transport via ion transporter proteins present in the gill epithelia. With the means of immuno- histochemical techniques, we found that the expression level of some of these ion transporters changes in relation to the temperature, and that the increasing temperature may result in mild hypoxia in the water environment. $Na^+/K^+$ ATPase decreases its immunoreactivity with increasing temperature. $Na^+/H^+$ exchanger 3 shows the highest expres- sion at room temperature (22°C), but also, the lowest re- activity was observed at the highest temperature (28°C). Vacuolar type $H^+$ ATPase does not change the expression level significantly between the lowest experimental tem- perature (4°C) and room temperature, but it is again decreased in 28°C. We hypothesized that this general decrease was caused by a "channel arrest", a phenomenon that may occur in hypoxia adapted animals.

## THE EFFECTS OF TRIMECAINE ON BUPIVACAINE-INDUCED CARDIOTOXICITY IN THE ISOLATED RAT HEART: A PILOT STUDY

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Introduction: trimecaine is methylated derivative of lidocaine and displaces bupivacaine from its receptor on the sodium channel. Animal trials showed increased threshold for bupivacaine-induced ventricular fibrillation in combination of lidocaine and bupivacaine. However, trials in isolated animal hearts did not confirm this positive effect of lidocaine and thus the role of local anesthetics in affecting bupivacaine-induced cardiotoxicity remains unclear. Methods: An isolated rat heart perfused at constant pressure according to Langendorff was used. Heart rate, coronary flow, PQ and QRS intervals were recorded. After 30 min of stabilization, the hearts were exposed to either bupivacaine or bupivaciane-trimecaine mixture for 30 min and then a period of washout for 30 min followed.
Results: In spontaneously beating hearts, bupivacaine and bupivacaine-trimecaine mixture led to a significant decrease in heart rate, coronary flow and to an increase in PQ and QRS intervals. Due to small number of animals in this pilot study we can not demonstrate the significant difference between both groups. Conclusion: We followed the effects of different concentrations of bupivacaine and trimecaine in order to get starting information for future study with bigger number of animals to demonstrate the effect of lidocaine and trimecaine on bupivacaine-induced cardiotoxicity.

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**BAROREFLEX SENSITIVITY IN YOUNG OBESE PEOPLE**

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Obese people suffer from many chronical disorders including cardiovascular ones. Obesity can be accompanied by the change of sympathetic and parasympathetic nervous activities. Therefore, the number of studies focused on obesity and its complications even in children and adolescents increases.
The aim of this study was to find out differences in baroreflex sensitivity between obese and non-obese young people. Fourty subjects were examined – 20 obese (12 girls, 8 boys age 12 -18 years, mean BMI = 32.5 ± 0.9 kg/m$^2$) and 20 non-obese (age and gender matched, mean BMI = 20.7 ± 0.3 kg/m$^2$) children and adolescents. Subjests were examined in supine position in a quiet room during 50 min. Systolic blood pressure (SBP) was monitored continuously by Finapres (Ohmeda, USA). R-R intervals were obtained from ECG recording (NihonKoden Cardiofax 9620, Japan). Baroreflex sensitivity (BRS) was determined in three 3-minutes lasting time intervals: T1 (from 15$^{th}$ min), T2 (from 30$^{th}$ min) and T3 (from 45$^{th}$ min). BRS was assessed using cross-spectral analysis of SBP and R-R intervals oscilations (BRS index) or SBP and heart rate oscilations (BRSf index). BRS and BRSf indices were calculated from transfer function in low frequency range (0.04 – 0.15 Hz) as a weighted mean value of gain (coherence served as a weight values). BRS was significantly reduced in young obese people compared to healthy controls (ANOVA: factor group: p = 0.002). There were found significant differences in time during supine rest (ANOVA: factor time: p = 0.004). In the non-obese subjects, BRS index tended to increase whereas there was no significant change in this parameter in obese group (ANOVA: interaction group x time: p = 0.042). Significant increase in BRSf index was found during supine rest in both group (ANOVA: factor time: p = 0.011), however, no significant differences in BRSf index value and changes between the groups were found (ANOVA: factor group: p = 0.125; interaction group x time: p = 0.736).
In young obese people, decreased baroreflex sensitivity and significant changes of BRS and BRSf indices were found during supine rest. BRS and BRSf indices were shown to be partially independent parameters.

**CYTOKINES AND ENDOTHELIAL FUNCTION IN PATIENTS WITH DIABETES MELLITUS TYPE 2 - MALES VERSUS FEMALES**

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*Background:* Metabolic syndrome may act as link between venous thrombosis and atherosclerosis. It is associated with endothelial damage/dysfunction, which triggers atherogenic lesions and hypercoagulability.
*Aims*: *(a)* Investigate markers of metabolic syndrome (BMI, HbA1C, adiponectin, A-FABP, leptin) in males and females with DM type 2. *(b)* Determine concentration of plasma's coagulation factors (PAI-1, factors VII, von Willebrand factor, trombomudulin, t-PA). *(c)* Compare their levels in both groups and assess correlations between them.
*Results and Conclusions:* In males positively correlated t-PA with A-FABP, PIA-1 with E-FABP and negatively correlated leptin with adiponectin and PAI-1 with factor VII. In females correlate HbA1C with A-FABP positively. Concentration of investigated parameters was influenced by gender.

| | Males | Females |
|---|---|---|
| BMI ( kg/m$^2$) | 29,31 ± 5,52 | 28,56 ± 3,85 |
| HbA1C (%) | 6,08 ± 1,71 | 5,78 ± 1,00 |
| Leptin (ng/ml 0) | 12,48 ± 9,19 | 25,15 ± 13,46 |
| Adiponectin (µg/ml) | 8,11 ± 3,30 | 13,53 ± 7,50 |
| A-FABP (ng/ml) | 30,83 ± 9,03 | 49,14 ± 13,89 |
| E-FABP (ng/ml) | 1,08 ± 0,32 | 1,11 ± 0,55 |
| t-PA (ng/ml) | 9,92 ± 6,02 | 8,83 ± 5,15 |
| PAI-1 (ng/ml) | 315,75 ± 168,24 | 238,94 ± 96,18 |
| Trombomodulin | 49,08 ± 25,13 | 47,50 ± 24,67 |
| vW activ (%) | 87,17 ± 45,79 | 90,56 ± 36,75 |
| F Factor VII (%) | 234,67 ± 69,75 | 289,67 ± 49,98 |

Tab. 1: Significant difference between both groups. (*p < 0,05)

**THE EFFECT OF GAMMA IRRADIATION ON PLASMA TOTAL ANTIOXIDANT STATUS**

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The total antioxidant status (TAS) was investigated in the blood serum of 48 chickens (age 28 days) after gamma irradiation at doses of 2 and 5 Gy ($^{60}$Co source, Chisostat, Chirana, dose rate 0.3 Gy/min., whole body irradiation). Serum total antioxidant status was assessed by a spectrophotometric method with a RANDOX-Kit (Total antioxidant status, RANDOX laboratories, UK). The TAS was determined 6, 24, 48 and 72 hours after irradiation. The results show that TAS was nonsignificantly elevated 6 hours after irradiation with both doses of gamma rays (C = 1.30 ± 0.34 mmol/l; 2Gy = 1.35 ±0.22 mmol/l; 5Gy = 1.44 ±0.22 mmol/l). Twenty-four (2Gy = 1.27 ±0.21 mmol/l; 5Gy = 1.14 ±0.27 mmol/l) and 48 hours after irradiation (2Gy = 1.09 ± 0.30 mmol/l; 5Gy = 0.84 ±0.30 mmol/l), TAS had decreased. The TAS increased again 72 hours after irradiation with both doses of gamma rays (2Gy = 1.24 ±0.21 mmol/l; 5Gy = 0.96 ±0.13 mmol/l). Significant differences were found only in chickens irradiated with 5 Gy of gamma rays. There was a significant difference between the groups 48 hour after irradiation and the control group ($p < 0.05$), 72 hours after irradiation and the control group ($p < 0.05$), 6 hours after irradiation and 48 hours after irradiation ($p < 0.01$) and 6 hours after irradiation and 72 hours after irradiation ($p < 0.001$). Our investigations revealed that 6 hours after irradiation the TAS increased depending on the dose of gamma rays. We considered that this elevation was caused by escape of intracellular antioxidants from damaged cells. 24 and 48 hours after irradiation TAS decreased in both experimental groups. This decrease might be a result of exhaustion of plasma antioxidants after pervious elevation. 72 hours after irradiation the TAS increased again in both doses of gamma rays, in group irradiated with a dose of 2 Gy the TAS almost recovered to control values.
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**REPROTOXICITY OF CHRONIC INTOXICATION WITH HEAVY METALS DURING THREE GENERATIONS IN RATS**

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The aim of the present investigation was to evaluate the effects of multigeneration (P, F1 and F2) intoxication with low doses of lead, mercury and cadmium dissolved in drinking water (200-times above maximal permissible dosage) on reproductive potency of 80 Wistar rats (40 males; 40 females in each generations) and the physical health of

their progeny. The animals were divided into 4 groups - control (C) and 3 groups intoxicated by metals (Pb, 100 µmol/l; Hg, 1 µmol/l, Cd, 20 µmol/l, respectively). Females gave births from 13th to 78th week of experiment. Parameters of reprotoxicity such as number of litters, total number of newborns (assigned in the birth day), number of newborns per litter and number of weanlings (raised youngs that reached 28th day of life) were measured in 13-week intervals. Our data show decrease in most reproduction parameters in intoxicated animals of F1 and F2 generations (Tab. 1).

Tab. 1 – Selected parameters of reprotoxicity (number of litters/number of neonates/number of sucklings)

| Group | P | F1 | F2 |
|---|---|---|---|
| C | 98/754/686 | 99/766/698 | 96/751676 |
| Pb | 106/853/599 | 82/725/530 | 40/357/272 |
| Cd | 90/706/606 | 95/752/677 | 97/755/695 |
| Hg | 128/1015/574 | 101/772/471 | 54/321/205 |

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## *TETRAHYMENA PYRIFORMIS* – A MODEL ORGANISM FOR THE SCREENING OF MYCOTOXINS

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The toxic action of selected mycotoxins on a biological subject was tested in the protozoan *Tetrahymena pyriformis*. The following toxins were tested: toxin T-2 at doses from 1.52 µg to 100.0 mg per litre of medium, ochratoxin A at doses from 12.21 µg to 800.0 mg per litre of medium, and rubratoxin B at doses from 12.21 µg to 800.0 mg per litre of medium. In toxin T-2 the $LD_{100}$ was found to be about 390.63 µg and $LD_{50}$ about 48.83 µg per litre of medium. In ochratoxin A the $LD_{100}$ was about 25.0 mg and $LD_{50}$ 3.13 mg per litre of medium. In rubratoxin B the $LD_{100}$ was about 200.0 mg and $LD_{50}$ was 25.0 mg per litre of medium. Thi findings suggest that *Tetrahymena pyriformis* is much higher sensitive to mycotoxins than most cell cultures. Therefore this model is suitable test-organism for the screening of mycotoxins in various sources.

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## ALTERNATIVE (NON-ANIMAL) METHODS IN EDUCATION AND RESEARCH

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There are few areas of animal use that arise so many emotions and questions as that of their use in education. The physical presence of an animal (whether alive or dead) is a dramatic event experience for most students, and their effects it has will depend heavily on their previous experience with that species, their moral values and the perceived necessity of the practical. Most of the current literature on this subject is highly emotive, based on relative little data and provide few conclusions. This paper attempts to clarify the issues raised and present an overview of the alternatives available with their strengths and weakness. Over the past decades fresh-water-living ciliated protozoan *Tetrahymena pyriformis* became undoubtedly the species of choice throughout the fields of functional biology, ecology, veterinary and human toxicology and radiobiology. The plentitude of *Tetrahymena*'s biology, reactivity to environmental changes and recent availability of its full - genome sequence made from *Tetrahymena* an optimal unicellular model organism with major benefits in molecular bioscience, biotechnology, *in vivo* functional genetics and in biomedical research from finding the function of predicted human genes up to studying the pathogens of major medical or agricultural significance. Finally, it offers guidelines for humane education that take into consideration both the practical issues and the feelings of all those involved.

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## CELL DEATH IN ENAMEL KNOTS

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Enamel knots represent signalling centres of developing tooth germs. These specific clusters of cells participate in transition of the tooth bud in the tooth cap (primary enamel knot) and seem to be relevant to cusp formation in multicuspid teeth (secondary enamel knots). After fulfilling their signalling mission, enamel knot cells are eliminated by programmed cell death. Primary enamel knots of mouse molars were investigated at the stage E13.5 – 15.5 of embryonic development. Approaches of molecular histology were used to study activation of pro-apoptotic molecules (caspase – 3), apoptotic DNA cleavage (TUNEL test) and morphological signs of apoptosis (apoptotic bodies). Additionally, caspase – 3 knock-out phenotype was evaluated. Techniques of explant cultures were applied for general caspase inhibition. Caspase – 3 becomes activated in primary enamel knot areas corresponding with TUNEL positive cells and appearance of apoptotic bodies in the primary enamel knot. Almost no TUNEL positivity was observed in caspase – 3 knock-outs and no apoptotic bodies were present in the tooth germs. Additionally, no signs of apoptosis can be observed after general caspase inhibition. However, cells from the primary enamel knot seem to disappear even after caspase – dependent apoptosis inhibition. Therefore, other mechanisms must be considered. Autophagy seems to be a hot candidate for the further study.

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## CHANGES IN PAIN PERCEPTION AFTER EPILEPTIC SEIZURE

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Epileptic seizures are usually followed by postictal inhibition in early postictal period. This relatively short period is also characterized by postictal analgesia. We studied this phenomenon over 96 hours following (flurothyl gas) induced seizures in adult male Wistar rats. Nociception of control (no seizure) and seizured groups were tested using the plantar and von Frey hair tests. We determined latency of forepaw and hind paw reactions using plantar tests and the number of von Frey hairs reactions. Shortly after seizures, longer plantar test latencies were seen relative to the control group. Plantar test reaction times of forepaws were significantly shorter than in hind paws before the seizures. The effect disappeared post-seizure and surprisingly, it also disappeared at the corresponding time in controls; it reappeared after 48 h in the seizure group and after 24 h in controls. Differences in the von Frey hairs test occurred at 5 and 60 minutes post-seizure, however, these differences could not be explained by limb anatomy; although, different thermal and mechanical nociception mechanisms could be

significant. The unexpected reactions in controls could be related to brief social and sensory interactions between the two groups.
This work was supported by MSM RG 0021620816

**DETERMINATION OF ERYTHROCYTE MEMBRANE DEFORMABILITY BY MEANS OF COLOID –HAEMOLYTIC ACTION OF MERCURY IONS IN WOMEN ATHLETS AND NON ATHLETS WOMEN WITHOUT AND AFTER EXERCISE**

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Red blood cell were compared for membrane deformability determination by colloid-haemolytic action of mercury ions (Mojzis J, et al. 1999) used at 15 group women athelts aged 15-17 years old and one group 15 women non athlets group 17- 25years old. Red blood cell membrane deformability were compared in women athlets and non athlets women without any exercise.Blood sample were removed from a finger puncture and 0.15ml were ad to the diferent level ionic strenght isotonic solution depended on concentration of 5% glucose and 0.9% NaCl physiological solution. Heparin was used as anticoagulans. Exercise was carried out on bicycle ergometer and procedure comparison red blod cell membrane deformability was done again. Statistical Student t-test was used and the lowest level statistical significance was chosen $p \leq 0.05$ with SM ± SD. Women athlets before execise reveal significantly higher haemolytic action and better red blood cell memrane deformability as non women athlets. Repeted situation was seen after exercise, too. We concluded higher cell membrane deformability is a consequence of better antioxidant activity in women athlets in both given experimental groups.

Literature
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**ACTIVATION OF PI3K/AKT IS NOT INVOLVED IN THE MECHANISM OF REDUCED ARRHYTHMOGENESIS IN THE DIABETIC AND PRECONDITIONED RAT HEARTS**

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PI3K/Akt activation is involved in protection (infarct size limitation) against ischemia/reperfusion injury (I/R) induced by ischemic preconditioning (IP) in the diabetic (D) and non-diabetic (ND) hearts. We explored the role of PI3K/Akt in susceptibility to ischemic arrhythmias in Langendorff-perfused hearts of 1-wk D rats (STZ 65 mg/kg, i.p.) and in ND hearts preconditioned (ND-IP) by 1 cycle of I/R (5 min each) before 30-min LAD occlusion with or without prior 15-min perfusion with PI3K/Akt inhibitor LY294002 (LY; 5 μM). Total number of ventricular premature beats (VPB) was significantly lower in D and ND-IP groups (224 ± 53 and 195 ± 40, resp.) as compared with the controls (C; 538 ± 58; $P<0.05$). The incidence of ventricular tachycardia (VT) that occurred in all C hearts was reduced to 22% and 20%, and its duration was shorter (19 ± 13.5 s and 0.7 ± 0.3 s vs. 43.6 ± 8.6 s in C; $P<0.05$). LY reversed reduced arrhythmogenesis neither in the D group (VPB 251 ± 73; VT 10.8 ± 5.2 s) nor in the ND-IP hearts (VPB 77 ± 19; VT 0.43 ± 0.43 s). To discern PI3K-independent effects of LY, wortmannin (100 nM) was applied and showed similar effects as LY in a setting of ND-IP (VPB 88 ± 25, no VT). In conclusion, activation of PI3K/Akt pathway is required for antiarrhythmic protection neither in the D myocardium nor in the preconditioned ND heart. *VEGA SR 2/0173/08, APVT 51-027404, APVV SK-CZ-0049-07, GACR 305/05/0875.*

**FLUOROMETRY OF CREATINE KINASE DURING SUBSTRATES-INDUCED STRUCTURAL CHANGES OF ITS MOLECULE**

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Myofibrillar creatine kinase (CK) (E.C. 2.7.3.2) that buffers ATP during fluctuating muscle energy metabolism has been selected for studies of conformational changes underlying the cellular control of enzyme activity. Computed data revealed the substrates-dependent three conformations of CK [1]. The intrinsic tryptophans´ fluorescence lifetimes of CK, CK-ATP and CK-ATP+creatine brought forward an experimental agreement with the computed data and their comparison with the lifetimes of dansylated CK molecules indicated presence of an efficient fluorescence resonance energy transfer (FRET) [2]. In this study, quenching of tryptophans´ fluorescence by acrylamide for the three tested substrate combinations (CK,CK-ATP,CK-ATP+creatine) indicates the different accessibility of tryptophan residues to quenching due to respective conformational changes. The time-resolved measurements of CK labelled with FITC (fluorescein 5-isothiocyanate) and CK labelled with IAF(5-iodoacetamid-fluorescein) revealed different anisotropy decays with respect to the tested substrate combinations. The rotational correlation times of the CK molecule double-labelled with FITC and ErITC (erythrosin 5'-isothiocyanate) equaled 34, 27 and 30 ns, for the tested substrate combinations, respectively. In all experiments a reversible protection of cysteines with tetrathionate and dithiothreitol saved the specific enzyme aktivity (affected by pH), ranging from 13 to 80 $\mu mol.min^{-1}.mg^{-1}$ . This study brings further experimental data from which three conformations with respect to substrates may be drawn for the proposed concept.
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**CARDIOPROTECTIVE EFFECTS OF *HEMIDESMUS INDICUS* AND *HIBISCUS ROSA-SINENSIS* ON ISCHEMIA/ REPERFUSION INJURY IN ISOLATED RAT HEART**

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*Hemidesmus indicus* (HI) and *Hibiscus rosa-sinensis* (HRS) are widely used ayurvedic medicine. We investigated cardioprotective effects of these plants applied for 15 min at concentrations of 90, 180 and 360 mcg/ml in Langendorff-perfused rat hearts prior to 25-min global ischemia/120-min reperfusion (I/R). Functional recovery (left ventricular developed pressure, LVDP), reperfusion arrhythmias and infarct size (TTC staining) served as the end-points. A transient increase in LVDP (32%-75%) occurred at all concentration of HI, while coronary flow (CF) was significantly increased after HI 180 and 360. Only a moderate increase in LVDP (21% and 55%) and a tendency to increase CF was observed at HRS 180 and 360. Both, HI and HRS at 180 and 360 significantly improved postischemic recovery of LVDP. Both drugs dose-dependently reduced the numbers of ectopic beats and duration of ventricular tachycardia. The size of infarction was

significantly decreased by HI 180 and 360 (30.1 ± 4.9% and 20.3 ± 1.4% vs. 43.2 ± 2.4% in C; P<0.05-0.01), while HRS significantly reduced the infarct size in a dose-dependent manner (29.3 ± 4.7%, 24.8 ± 3.6% and 22.5 ± 2.4%, resp.). In conclusion, both drugs might cause vasodilation, positive inotropic effects and cardioprotection in a setting of I/R, however, further study is needed to elucidate the exact mechanism of their action. *Supported by VEGA SR 2/0173/08, APVT 51-027404 and the National Scholarship Programme of the SR.*

## ULTRASTRUCTURAL CHANGES OF MYOCARDIUM REMODELED BY ISOPROTERENOL

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Elevated catecholamines have long been known to induce small necrotic foci in the heart. Adaptive responses of the heart due to increased circulatory demands of the body in isoproterenol (Iso)-treated rats (5mg/kg s.c. for 7 days) were characterized with an increased left ventricular mass, QT interval prolongation, increased values of voltage criteria as well as changes in cardiac contractile force. In association with described global effects of isoproterenol on the heart we investigated ultrastructural alterations in the left ventricular myocardium. Tissue samples from the left ventricle of 5 adult male rats were processed for electron microscopy. Ultrastructural analysis showed enormous incidence of caveolae in plasma membrane and in the mouth of T- tubules. Membranes of the T-system showed earlier stages of vesiculation with profound vesiculation of T-system in the dyads. In the places with increased volume of cytosol, degenerated mitochondria with elongated shape and small diameter were seen. In several places branching of the myofibrils and zig-zag Z-lines were found. At the periphery of the myocytes transversally oriented myofibrills were present. Occasionally autophagic vacuoles with included mitochondria were detected. Morphological alterations of cardiomyocyte plasma membrane and related structures as well as ultrastructural abnormalities in dyads, mitochondria, and myofibrils may contribute to the electrical remodeling and LV dysfunction, pointing to complex interactions between cardiac excitability and contractility. The presence of autophagosomes supports the procces of myocardial tissue remodeling induced by Iso.

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## MODULATION OF CASPASE 3 ACTIVITY IN MOUSE LIMB DEVELOPMENT

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The regression of interdigital zones in vertebrate limbs results in the separation of the digits and represents one of the best known examples illustrating the morphogenetic role of apoptosis in development. However, investigation of the signalling pathways lying behind this process is still in process. We used a novel explant culture approach to investigate and modulate apoptotic signalling pathways in mouse front limbs. The distribution of proliferation, apoptosis and caspase 3 was evaluated. These data were compared to those obtained in experiments where the pharmaceutical inhibitors of caspase machinery were engaged. The comparison showed the involvement of caspase 3 in all phases of interdigital webbing regression and engagement of caspase – 3 in interdigital apoptotic process. Nevertheless, the data from *ex vivo* explant culture experiments comply with general opinion that the apoptotic machinery in limbs has potent compensatory mechanisms which ensure the elimination of interdigital mesenchyme even in the case of apoptosis inhibition. Caspase – 3 inhibition delays, however, cannot completely prevent the onset of interdigital mesenchyme elimination. Alternative forms of cell death, compensatory mechanisms and role of other caspases in embryonic development are recently under study.

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## RAPID EFFECTS OF DEXAMETHASONE ON CARDIOVASCULAR PARAMETERS IN RABBITS WITH EXPERIMENTAL MECONIUM ASPIRATION SYNDROME (MAS)

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Introduction: Glucocorticoids may improve lung functions in MAS, but may have adverse effects on cardiovascular system. This study evaluated if and to what extent may intravenous dexamethasone affect cardiovascular functions in meconium-instilled animals. Methods: Rabbits were intratracheally instilled 4 ml/kg of meconium suspension (25 mg/ml) or saline. Meconium-instilled animals intravenously received two doses of dexamethasone (each of 0.5 mg/kg; Mec+Dex) or saline (Mec) 0.5 and 2.5 h after meconium instillation. Saline-instilled animals received two doses of dexamethasone (each of 0.5 mg/kg; Sal+Dex) or saline (Sal) 0.5 and 2.5 h after meconium instillation. Animals were ventilated for additional 5 h after the first dose of treatment. Blood pressure, heart rate, and short-term heart rate variability (HRV) were evaluated during and immediately after treatment administration, as well as within 5 h after administration of the first dose of treatment. Results: In meconium-instilled animals, dexamethasone significantly increased systolic blood pressure, decreased heart rate, increased HRV parameters (MSSD, spectral powers in HF and LF band, and total power), and caused cardiac arrhythmia during and immediately after the administration. In saline-instilled animals, dexamethasone had no significant immediate effects on evaluated parameters. Although acute influence of dexamethasone on blood pressure and cardiac rhythm almost disappeared within 30 min, in both dexamethasone-treated groups heart rate was significantly lower at 5 h of the treatment and HRV parameters higher till the end of experiments, especially in meconium-instilled animals. Conclusion: Systemic administration of glucocorticoids showed adverse effects on cardiovascular functions in rabbits with MAS.

The study was supported by Grant VEGA No. 1/2306/05.

## EFFECTS OF ONE AND TWO DOSES OF DEXAMETHASONE ON LUNG FUNCTIONS IN EXPERIMENTAL ANIMALS WITH MECONIUM ASPIRATION

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Introduction: Administration of anti-inflammatory drugs may alleviate lung inflammation due to meconium aspiration. This study compared effects of one and two doses of corticosteroid dexamethasone on the lung functions of experimental animals with meconium aspiration. Methods: Air-ventilated adult rabbits intratracheally received 4 ml/kg of saline (Sal, n=5) or human meconium (25 mg/ml). From this moment, all animals were oxygen-ventilated. When respiratory failure developed, meconium-instilled rabbits received one dose of dexamethasone (0.5

mg/kg i.v.) 0.5 h after meconium instillation (Mec+Dex1, n=7), two doses of dexamethasone (each of 0.5 mg/kg i.v.) 0.5 h and 2.5 h after meconium instillation (Mec+Dex2, n=8), or were left without treatment (Mec, n=8). All animals were oxygen-ventilated for additional 5 h after the first dose of treatment. Ventilatory parameters, blood gases, right-to-left pulmonary shunts and total and differential white blood cell (WBC) counts were evaluated regularly. At the end of experiments, animals were killed by an overdose of anesthetics. Right lungs were used to determine in vitro airway reactivity together with strips from trachea, as well as for determination of lung edema by wet/dry weight ratio and oxidative damage to lipids and proteins by estimation of thiobarbituric acid-reactive substances, tyrosine and lysine-lipid peroxidation products in the lung homogenate. Left lungs were saline-lavaged and differential WBC was estimated in the lavage fluid sediment. Results: Two doses of dexamethasone effectively reduced right-to-left pulmonary shunts, improved oxygenation, reduced ventilatory pressures, diminished meconium-induced lung edema, tracheal hyperreactivity to histamine, decreased neutrophil count in BAL associated with higher WBC and neutrophil counts in the blood and reduced oxidative modifications of proteins and lipids in lung homogenate compared to Mec group. Single-dose dexamethasone improved lung functions, reduced lung edema, BAL neutrophils and tracheal hyperreactivity to histamine, but to lower extent than two-dose dexamethasone, and failed to prevent oxidative lung injury. Conclusion: Two-dose dexamethasone improved lung functions and gas exchange, reduced lung edema, inflammation and airway hyperreactivity in meconium-instilled rabbits more effectively than single-dose treatment.

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## PRELIMINARY EXPERIENCE WITH CONTINUOUS GLUCOSE MONITORING IN THE ICU: A FEASIBILITY STUDY

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BACKGROUND and AIMS: Continuous glucose monitoring might be a promising approach in optimizing insulin therapy in critically ill patients. Therefore, we evaluated the performance of an on-line continuous glucose monitoring system (CGMS) in the conditions of cardiosurgical intensive care unit (ICU). METHODS: 7 adults (4 women, 3 men, average age 62.6±17.4 years) undergoing elective cardiosurgical operation were enrolled. Continuous glucose monitoring in the postoperative period was performed using a subcutaneous on-line glucose monitoring system (Guardian Real-Time) and compared with arterial glycaemia. The results were evaluated using Clarke error-grid analysis (C-EGA) and Spearman's rank correlation test. RESULTS: During the monitoring, lasting in average 3.8 days, a total number of 246 paired glycaemic values were obtained. Of these values, 70% were found in the A-zone and 23% in the B-zone of C-EGA and only 7% were in the clinically unacceptable zone. There was a strong positive correlation between Guardian values and arterial glycaemia (r 0.758, $p<0.001$). CONCLUSION: In our pilot trial the performance of Guardian Real-Time CGMS appeared to be suitable for clinical use in the cardiosurgical ICU. Larger studies are required to confirm this finding and to test whether this approach can improve glucose control in critically ill patients.

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## STREPTOZOTOCIN DIABETES-INDUCED CHANGES IN MEMBRANE FLUIDITY AND $Mg^{2+}$-ATPase ACTIVITY OF RAT HEART MITOCHONDRIA EXHIBIT SEASONAL DIFFERENCES

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Background: Investigation of the effect of acute streptozotocin (STZ)-diabetes (DIA) on $Mg^{2+}$-ATPase activity (ATPase), the content of conjugated dienes (CD) and membrane fluidity (MF) of rat heart (H) mitochondria (MIT), realized in different parts of the year, revealed considerable seasonal differences. Aim: Verify the regularity of season-linked quantitative and qualitative differences in parameters investigated. Materials and Methods: Male Wistar rats, 220±25g. Experiment started with a single dose of STZ (65 $mg.kg^{-1}$ i.p.), terminated on the day 8 after STZ. MIT isolated with protease. Estimations: Metabolism; In MIT: CD, MF and ATPase. Results: Values of ATPase in the November-April period (N/A) always exceeded those in the May-October period (M/O, healthy-DIA, $p<0.05$-$0.001$) whereby the results in DIA H were always higher than in the healthy H (N/A-M/O, $p<0.001$-$0.05$). Excluding M/O the changes in CD content followed the changes in the ATPase and MF. Conclusion: ATPase and MF values in N/A always exceed those in M/O both in DIA and healthy H. Granty: VEGA 2/0173/08, 1/3037/06, 2/7126/27, APVV 51-027404.

## DOES PERIODIC REOXYGENATION PLAY A ROLE IN CARDIOPROTECTION AFFORDED BY CHRONIC INTERMITTENT HYPOXIA?

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Chronic hypoxia, particularly that of intermittent nature, is associated with increased oxidative stress which play an important role in the induction of improved cardiac ischemic tolerance (1). To find out whether chronic hypoxia without periodic reoxygenation also affords cardioprotection, we compared rats adapted to either continuous normobaric hypoxia ($FIO_2$ = 0.1, 24 h/day) or intermittent hypobaric hypoxia (5500 m, 8h/day) for a period of 1-90 days. The susceptibility to regional ischemia-induced ventricular arrhythmias was assessed in anesthetized open-chest rats subjected to 20-min LAD coronary artery occlusion; infarct size was determined after 3-h reperfusion (TTC staining). In both models, the most pronounced antiarrhythmic protection was observed after 1-5 days of hypoxia. On the other hand, the strongest effect on infarct size reduction occurred after 30 days when continuous hypoxia was more effective (41.5 ± 3.0 % of the area at risk) than intermittent hypoxia (49.4 ± 3.4 %) compared to normoxic controls (60.6 ± 2.2 %). Our results demonstrate that both chronic continuous and intermittent hypoxia are cardioprotective and suggest that periodic reoxygenation does not play a major role in the protection afforded by intermittent hypoxia.

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## CARDIOPROTECTIVE EFFECT OF CONTENT SUBSTANCES FROM *CLEOME DROSERIFOLIA*

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*Cleome droserifolia* is traditional Egyptian herbal medicines. A total of 10 isolated flavonoids were tested in vitro by Peroxynitrite and DPPH assays. The Quercetin 3-glucoside-7-rhamnoside was used for the cardio-protective effect testing. Two groups of animals (n=10). The hearts were excised and perfused (modified Langendorf´s method). Working schedule: stabilization/ischemia/reperfusion (20/30/60min). The tested substance was added to perfusate in dose 50 mg/1000ml all along time the experiment only the treated hearts. In control hearts left ventricular pressure (LVP) recovered until 64 ± 4% of preischemic values at the end of the reperfusion. The treated hearts showed improved postischemic recovery reaching LVP values of 88 ± 5%. Ventricular end-diastolic pressure (LVEDP) in control group rise from 11.2 ± 0.4 mmHg to 42 ± 2 mmHg, in treated group was LVEDP 28 ± 3 mmHg after reperfusion. Contractility ($+dP/dt_{max}$) recovery during reperfusion in treated group to 88 ± 6 %, there values were significantly higher than control hearts.

## ACUTE EFFECTS OF SIGMA RECEPTOR LIGAND HALOPERIDOL ON ELECTROGRAM AND CORONARY FLOW IN RAT ISOLATED HEART

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Mechanisms of life-threatening cardiovascular side effects of sigma receptor ligands (cardiac arrhythmias such as torsade de pointes, ventricular fibrillation or even cardiac arrest) are not fully elucidated yet. Therefore we examined the effects of their representative haloperidol on 3-D electrogram and mean coronary flow in isolated rat hearts.
Eight adult male rats (body mass 254±27gr) were sacrificed under deep ether anesthesia. The hearts were perfused according to Langendorff with Krebs-Henseleit solution (K-H) at constant pressure (85mmHg) and 37°C ($CaCl_2$, 1.2 mM). The experiment consists of four 30min periods: control, 10nM haloperidol, washout, 10nM haloperidol. Ten successive RR intervals were averaged at the end of control (steady state heart rate). This value was used for normalization of heart rate during the rest of experiment. In the same way, QT intervals were examined in order to determine L-QT. The incidence of arrhythmias was assessed according to Lambeth Conventions. Coronary flow was measured every 5$^{th}$ minute. Normalized spontaneous heart rate showed a clear tendency to decrease during both haloperidol applications and this effect was partially reversible. In all hearts, the QT intervals lengthened in the first haloperidol period, partially restored in washout and in the second haloperidol administration QT interval remained unchanged. No significant incidence of life-threatening arrhythmias was observed, except of premature ventricular complexes (occurring as singles, salvos or tachycardia). Three hearts were classified by number 3. The changes of coronary flow were inconsistent and insignificant. In conclusion, QT prolongation observed in our experimental model can explain the occurrence of arrhythmias. No change of QT interval in the second haloperidol application can be reasoned by down-regulation of cardiac sigma receptor.
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## EXPRESSION OF CALSEQUESTRIN IN SKELETAL AND HEART MUSCLES OF RATS WITH ALTERED THYROID STATUS

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Calsequestrin (CSQ) is a low-affinity and high-capacity $Ca^{2+}$-binding protein. It is located within the terminal cisternae of the sarcoplasmic reticulum, close to the luminal site of the junctional membrane. Its skeletal form (CASQ1) is expressed in fast-twitch and slow-twitch muscles, its cardiac form (CASQ2) is present in cardiac and slow-twitch muscles. CSQ controls the ryanodine receptor channel to which it may be anchored by triadin and junction (1). Many hormones exert a strong systematic influence on skeletal muscle during development as well as in the adult stage. For the thyroid hormones, it has been shown in rats that both hypothyroid (HY) and hyperthyroid (TH) states were paralleled by modifications in the MyHC isoform content (2). We have investigated whether the effect of the thyroid hormones might alter calsequestrin expression in the slow soleus (SOL) and the fast extensor digitorum longus (EDL) hind limb muscles and in the heart of adult female inbred Lewis strain rats. HY rats were treated with 0.05 % solution of methimazole (2-mercapto-1- methylimidazole, Sigma) in drinking water, the TH status was induced by intraperitoneal injections of 3, 3',5-triiodo-L-thyronine (Sigma, sodium salt, $T_3$, 150 μg/kg body weight) 3 times a week. CSQ was determined by SDS-PAGE followed by western blot analysis. Gene expression was assessed using reverse transcription and subsequent real time polymerase chain reaction (RT-PCR). Our results suggest that the protein and mRNA transcript levels for CASQ1 are the highest in the EDL, medium in the SOL and hardly detectable in the heart. Alteration of the thyroid status did not change significantly the relation of CSQ1 to the GAPDH at protein level. At mRNA level, the HY status decreased and the TH status increased the CASQ1 expression in the EDL, while it resulted in opposite changes in the SOL. The mRNA transcript levels for CASQ2 were the highest in the heart, medium in the SOL and the lowest in the EDL muscle. The HY status slightly increased the CASQ2 mRNA expression in the EDL compared to EU and TH statuses. It remains an open question whether thyroid hormones alter not only the muscle phenotype, but also the calcium homeostasis and therefore the excitation-contraction coupling.
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## THE EFFECTS OF DIFFERENT STRESSORS ON CARDIAC $\beta_3$-ADRENOCEPTORS

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The presence of $\beta_3$-adrenoceptors ($\beta_3$-AR) has been already documented on the gene and as well on the protein levels in the heart. However the role of $\beta_3$-AR in cardiac physiology has not been clarified till yet and there are no results demonstrating the $\beta_3$-AR ligand binding in the heart. We wanted to find out the prospective effects of the cold and immobilization stress treatment on the amount of the $\beta_3$-AR in the rat heart. Our effort was to identify the amount of $\beta_3$-AR binding sites in the rat heart, to compare these results with the mRNA levels and protein concentrations in proper heart regions and to measure the basal and stimulated adenylyl cyclase activity. The specific radioligand for $\beta_3$-adrenoceptors, $^3$H-SB206606, was used in the binding experiments with membrane preparations from the rat left (LV) and right (RV) heart ventricles and from the left atrium (LA) using SR59230A as a selective $\beta_3$-AR antagonist. Gene expression of $\beta_3$-AR was significantly affected by immobilization stress in LV, RV and as well in LA. 7 IMO caused the increase in the amount of $\beta_3$-AR mRNA in LV and in LA and the same trend we could observe on protein levels and in the density of $\beta_3$-AR binding sites. In RV there was an increase in the amount of mRNA for $\beta_3$-AR, but there were no significant changes in the protein concentration and in the number of receptor binding sites. On the other hand, 1 IMO induced the increase in the amount of the mRNA for $\beta_3$-AR in LV, but there was decrease in the quantity of $\beta_3$-AR binding sites. Single and repeated cold treatment caused the analogical effects. The activity of adenylyl cyclase was affected by stress as well.
On the basis of these results we can suppose the importance of $\beta_3$-AR in the response to different stressors in the heart.

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**EFFECT OF ISCHEMIC PRECONDITIONING ON MRNA AND PROTEIN LEVELS OF SECRETORY PATHWAYS CA$^{2+}$ ATPASE (SPCA) AFTER GLOBAL CEREBRAL ISCHEMIA/REPERFUSION IN RATS**

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Ischemic preconditioning (IPC) represents an important phenomenon of adaptation of CNS to sub-lethal short-term ischemia, which results in increased tolerance of CNS to the lethal ischemia. The Golgi apparatus, as a part of secretory pathways (SP), is a newly recognized $Ca^{2+}$ store, which regulates secretion of neurotransmitters and secretory proteins for growth/reorganization of neuronal circuit by secretory $Ca^{2+}$ATPases (SPCA1). In addition, SP are involved in stress sensing and transduction of apoptotic signals and remodeling of dendrites. Forebrain ischemia/reperfusion initiates cellular catastrophic cascade in which many subcellular organelles play an important role. In this study we have determined the effect of ICP on ischemia/reperfusion-associated alterations of mRNA and protein levels of SPCA1 in the hippocampus of rats. Global brain ischemia was induced by 4-vessel occlusion in duration of 15 min. Rats were preconditioned by 5 min of sub-lethal ischemia and 2 days later, 15 min of lethal ischemia was induced. RT-PCR and Western blot analysis clearly detected expression of SPCA1 gene in injured area after ischemic/reperfusion insult. In addition, injured tissue responded on the level of mRNA by the increase of gene expression. In the both areas, (hippocampus and cortex) an increase of mRNA was maximal in the reperfusion period. IPC significantly elevated tissue response by the elevation of expression profile. Similar pattern was observed on the translational level by Western blot analysis. Protein level of SPCA1 was highest in the reperfusion time and IPC initiated elevation of tissue response. Our results showed that IPC affects ischemia-induced alterations of hippocampal and cortical response. This suggests for a potential role of secretory pathways involved in neuronal damage and/or remodelation of neuronal circuits as response to preischemic challenge.

**THE EFFECT OF HORMONAL STIMULATION ON CATECHOLAMINE LEVELS IN THE PITUITARY GLAND**

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The effect of hormonal stimulation on catecholamine levels and activity of its degradation enzyme monoaminooxidase (MAO) in the hypophysis of ewes in the oestric period was studied by the radioenzymatic method. Monoaminoxidase activity was determined radiochemically. The oestrus of ewes was synchronized with chlorsuperlutin. After completed synchronization we induced superovulation in the experimental group by means of 1500 IU serum gonadotrophin (SG). The extrahypophyseal hormone SG which show LH and FSH activity has a long half-life of biological degradation in the organism and its application is associated with hyperestrogenization. High oestrogen levels have a specific impact on hypothalamic adrenergic receptors and influence catecholamine levels and function. The results indicate that hormonal serum gonadotrophin stimulation increase ($P < 0.01$) the pituitary dopamine and epinephrine levels in ewes significantly. In comparison with the control group norepinephrine concentration did not change in this tissue. MAO activity in the hypophysis decreased significantly to almost one half in comparison with control values ($P < 0.001$). According to our results the serum gonadotrophin in combination with hyperestrogenization (1,2) influences dopamine and norepinephrine metabolism in the hypophysis of hormonally stimulated ewes and reduces monoaminooxidase activity selectively.

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**ADIPONECTIN AND METABOLIC SYNDROME**

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The metabolic syndrome (MS) represents a permanent discussion about the possibilities of its influencing. The first therapeutic goal is obesity and distribution of body fat, 2nd insulin resistance, and 3rd specific therapy of cardio-metabolic risk factors (RF). Its significant components are atherogenic dyslipidaemia, hypertension, prothrombotic state, pro-inflammatory state, and hyperglycaemia. The latest studies have changed the view on the fatty tissue demonstrating its metabolic activity. By mean of adipocytokines it participates in regulation of physiology and tissue patho-physiology, and in the genesis and development of the diseases associated with obesity. Adiponectin-insulin-sensitising hormone with antiatherogenic and anti-inflammatory effects is produced by adipocytes. It influences the metabolism of glucose, lipids, and resistance to insulin. A decrease in the adiponectin concentration is associated with a higher occurrence of insulin resistance, risk of more frequent origin of diabetes mellitus of type 2 as well as with the development of microvascular and macrovascular complications. Within our project we examined 50 patients meeting the criteria of MS of the mean age 48.97 years (32 women,18 men). The control group consisted of the probands (13 women, 10 men) of the mean age of 53.32 years without any serious RF MS. Sampling was performed at the same time intervals, fasting. The serum was stored at the temperature of -20 $^0$C until its analysing. Selected immunological and biochemical parameters (PAF, pro-inflammatory cytokines-TNFα, adiponectin) were detected. The values of BMI in the patients examined ranged from 26.7-35.54 kg/m$^2$. The mean values of TNFα in the control and observed groups were within the range of 5–20 pg/ml and 4.2-52.97 pg/ml, respectively. PAF values exceeded the physiological limit. Adiponectin concentrations in the serum ranged from 1.06–40.46 ng/ml, and in a relationship to BMI association to lower values was recorded. Regardless, what diagnostic criteria are used for MS, there have been made efforts to find suitable bio-markers that will be intended for cardio-metabolic RF for decreasing premature cardio-vascular mortality. One of them should be also adiponectin as an early marker of inhibition of progression of the glucose metabolic disorders as well as cardio-vascular diseases.
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**CHANGES OF DOPAMINE RECEPTOR SYSTEM IN LURCHER MICE**

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The Lurcher mutants are mice with functional mutation in δ2 glutamate receptors which are predominantly expressed in the Purkinje cells and thus are crucial for proper cerebellar function. These mice display ataxia and reduced ability of motor-related learning. The objective of this work was to elucidate the changes in dopamine receptor system caused by this mutation in C57Bl and C3H Lurcher mutants. Hence the behavioral effects (spatial learning in the Morris water maze) of activation and inhibition of $D_1$-like dopamine receptors, the changes of $D_1$-like (D1R) and $D_2$-like (D2R) dopamine receptor densities in striatum, cerebellum and hippocampus (using receptor binding studies with $^3$H-SCH 23390

(D1R specific ligand) and $^{3}$H-spiperone (D2R specific ligand) and the adenylyl cyclase activity in hippocampus in the C57Bl mutants (using competitive EIA) were studied. In addition, the number of NMDA receptor binding sites (using specific antagonist $^{3}$H-CGP39653) in hippocampus of C57Bl strain was measured. We found that the Lurcher mutants manifest a deficit in spatial learning but mice of both types reacted similarly to D1R agonist (no effect) and antagonist (worsening). The C57Bl strain revealed a substantial increase in the receptor densities of both types (to 388% of control in D1R and to 511% of control in D2R) in hippocampus while in the C3H strain only D1R were increased (to 243%). Moreover, in the C57Bl strain, D1R were decreased in cerebellum (to 66% of control) while in the C3H strain they remained unaffected. No changes were found in striatum. Therefore, we focused receptor/signalling in hippocampus of C57Bl Lurcher mutants. We found an increase in the number of NMDA receptor binding sites as well as in the basal concentration of cAMP. However, unlike in the wild types, the activity of adenylyl cyclase did not further rise in the mutant mice when stimulated by D1R agonist (SKF-38393). Our results suggest specific participation of dopamine receptor system in coping with olivocerebellar degeneration.

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## COUGH, ASPIRATION, AND EXPIRATION REFLEXES INDUCE WIDELY SPREADING FOS LABELING IN THE CAT BRAINSTEM

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Fos-like immunoreactivity (FLI) caused by expression of immediate-early gene *c-fos*, a marker of neuronal activation was employed to localize brainstem neuronal populations functionally related to the tracheal-bronchial cough (TBc), aspiration (AR) and expiration reflex (ER). Spontaneously breathing, non-decerebrate, pentobarbital anesthetized cats were used, 6 animals in each stimulated group and 7 control cats. In the medulla all 3 reflexes enhanced FLI in the region of *solitary tract nucleus* rostral to the obex (AR-related FLI also higher than FLI in ER and TBc) as well as in an *intermediate ventral respiratory group*. Increased FLI was found within the region of *retroambigual nucleus* in TBc and AR. FLI within the *most rostral ventrolateral medulla* was higher in TBc and ER (ER-related labeling is also higher compared to that in AR). AR-related FLI was found in the area of *lateral tegmental field*, *caudal solitary tract nucleus* (within both areas FLI is higher also compared to findings in ER), and within the *raphe*. ER-related FLI was also found within the *medullary vestibular area*. In the pons FLI was higher within the *area of rostral dorsolateral pons* both in TBc and AR compared to that found in control and ER. FLI in TBc was found in the *posteroventral cochlear nucleus* (higher also compared to FLI in AR). In the caudal midbrain FLI was enhanced within the *ventral and lateral periaqueductal gray* in AR (higher also conpared to that in TBc). FLI diminished within the *lateral periaqueductal gray* in ER and TBc (also in comparison with AR-related FLI). Within the *central tegmental field* higher FLI was found in AR and TBc. In the area of the *rostral mesencephalic midline* FLI was increased in ER and TBc compared to that in control and AR. Our results indicate that: 1) a complex multilevel neuronal network is involved in production of defensive airway reflexes, 2) the areas of rostral ventrolateral medulla and rostral mesencephalon may contribute to forceful expirations, whereas the medullary tegmental fields, the rostral dorsolateral pons, and the caudal mesencephalon might be involved in strong inspiratory efforts, 3) some of brainstem regions may contain neurons specifically stimulated during particular behavior.

## NORMOBARIC INTERMITTENT HYPOXIA HAS PRECONDITIONING EFFECT ON LEARNING IMPAIRMENT CAUSED BY EPILEPTIC SEIZURE

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Epileptic seizures are common disabling incidents which have cognitive consequences. Flurothyl vapors evoke tonic-clonic epileptic seizures in rats causing deterioration of spatial learning and memory in the Morris water maze. We described preconditioning effect of hypobaric hypoxia to flurothyl epileptic seizures (FS). Intermittent normobaric hypoxia (INH) causes cognitive changes. Present study was aimed to investigate whether INH would exhibit similar to hypobaric hypoxia cross-preconditioning on FS in Wistar rats. Behavioral changes were tested in Morris water maze (MWM). Three sets of experiments were performed. First group was exposed to INH and to flurothyl 3 days after; second – to hypoxia alone and third – to flurothyl alone. Results were compared with naïve control animals. FS alone, in combination with INH and in lesser extent INH alone caused worsening of performance in WMW, in comparison to controls. There was significant improvement of performance of animals preconditioned by INH in contrast to FS group. These findings confirm our hypothesis that short-term (1 hour) INH has protective preconditioning effect. Despite this effect it still causes some worsening of learning and memory comparing to our previous results with hypobaric hypoxia. In the other words, INH improves animals' performance but to the level of its own damage.

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## DIFFERENTIATION OF MUSCLE FIBER TYPES AND EXPRESSION OF MYOSIN HEAVY CHAIN ISOFORMS IN SLOW AND FAST RAT SKELETAL MUSCLES RE-EVALUATED

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Contractile properties of skeletal muscle phenotype are best reflected, from the slowest to the fastest, by mATPase activity or by immunohistochemical determination of type 1, 2A, 2X/D and 2B skeletal muscle fibers, the latter one based on staining myosin heavy chain (MyHC) 1, 2a, 2x/d and 2b isoforms using specific monoclonal antibodies. These methods define the fiber type composition of all individual fibers. The total content of MyHC isoforms in the whole muscle can be determined by SDS-PAGE method. It is well known that the fiber type composition and MyHC content undergo changes during postnatal development and that their development can be affected by an altered thyroid status (1-3). We have therefore compared the postnatal phenotype changes of slow antigravity soleus (SOL) and fast extensor digitorum longus (EDL) muscle in euthyroid, as well as in experimental hypothyroid and hyperthyroid (i.e. after chronic treatment with methimazole and $T_3$, respectively) 2-week- to 17-month-old inbred Lewis rats. This comparison has shown that *firstly*, the phenotype of the fast EDL, which is the physiological ankle flexor, did not undergo significant changes after the 1$^{st}$ month. On the other hand, the SOL still underwent significant changes up to 4 months of age, apparently induced by its tonic antigravity function. *Secondly,* comparison between slow and fast fibers has revealed no difference between the mATPase and immunohistochemical MyHC detection. In contrast, especially the SOL muscle contained a higher content of the faster 2a MyHC isoform determined by SDS-PAGE than the percentage of fast type 2A fibers determined by histo- or immunohistochemistry was. This indicates that probably many phenotypically slow fibers co-express 2a MyHC isoform, but in the amount that is not high enough to affect their phenotype. *Thirdly,* our results demonstrate that an alteration of the thyroid status leads to typical changes in the expression of MyHC

protein isoforms in both the EDL and SOL muscles, the hypothyroid status favors slower fiber types and MyHC isoforms, while the opposite is true about the hyperthyroid status.

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**NITRIC OXIDE AS A STRESS-LIMITING MOLECULE IN VASCULAR SYSTEM OF WISTAR-KYOTO RATS**

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The aim of this study was to investigate the effect of chronic social stress produced by crowding on blood pressure (BP) and vascular function in Wistar-Kyoto (WKY) rats. Adult, male rats were divided into control (480 $cm^2$/rat) or stressed (200 $cm^2$/rat) group for 8 weeks. BP (determined by tail-cuff) was not influenced by chronic stress. Nitric oxide (NO) synthase activity in the aorta (determined by [$^3$H]-L-arginine conversion) was elevated in crowded rats ($p<0.001$). L-NAME-sensitive component of endothelium-dependent vasorelaxation was investigated in the preconstricted branches of the superior mesenteric arteries (MA, mean normalized internal diameter 287±12 μm) using the wire myograph as a difference between acetylcholine-induced vasorelaxation before and after acute NO synthase inhibitor ($N^G$-nitro-L-arginine methyl ester, L-NAME, 300 μmol/l) pre-treatment. Chronic stress increased average value of vasorelaxation of the MA compared to controls ($p<0.007$). L-NAME-sensitive component of vasorelaxation was increased in stressed rats vs. controls ($p<0.001$). Vasoconstriction to noradrenaline was reduced in stressed rats ($p<0.001$). Attenuating effect of NO in phenylephrine-induced vasoconstriction was higher in stressed rats ($p<0.007$). The observations showed that stress-exposed WKY rats were able to cope with chronic stress by improvement of vasorelaxation associated with elevated NO synthesis. Increased production of NO in the vascular system of rats may attenuate the vasoconstrictor effects of stress mediators and this compensatory mechanism may protect WKY rats from developing of stress-induced hypertension. Data suggest that NO plays an important role in stress and adaptive responses in the vascular system as a stress-limiting molecule.

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**ISCHEMIC PRECONDITIONING INHIBITS INITIATION OF MITOCHONDRIAL APOPTOSIS AFTER GLOBAL BRAIN ISCHEMIA.**

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Mitochondria are pivotal regulators of cell life and death through their role in energy production, intracellular calcium homeostasis, and their involvement in apoptosis. Ischemia-induced mitochondrial dysfunction is considered to be an important event coupling cerebral blood flow arrest to neuronal cell death. Ischemic preconditioning (IPC) represents an important adaptation of CNS to sub-lethal ischemia, which results in increased tolerance of CNS to the lethal ischemia. In presented study, we have determined the effect of IPC on ischemia/reperfusion-induced mitochondrial dysfunction and apoptosis. Global brain ischemia was induced by permanent occlusion of vertebral arteries and temporal occlusion of carotid arteries for 15 minutes. Rats were preconditioned by 5 minutes of sub-lethal ischemia and 2 days later 15 minutes of lethal ischemia was induced. Our results showed that IPC affects ischemia-induced mitochondrial dysfunction in two different ways. Ischemia-induced repression of mitochondrial translation was moderately attenuated by IPC. Slight protective effect of IPC was documented for complex IV, but not for complex I. With respect to mitochondrial apoptosis, IPC abolished completely ischemia-induced translocation of p53 to mitochondria and led to significant inhibition of ischemia-induced activation of Caspase-9 activity. In addition, significant protective effect of IPC on ischemia-induced DNA fragmentation was observed as well. Our results indicate that although ischemia-induced mitochondrial dysfunction is not significantly affected by IPC, processes involved in mitochondrial apoptosis are almost completely abolished by IPC.

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**P53-DEPENDENT APOPTOSIS IN ACUTE LEUKAEMIA**

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Tumour suppressor protein p53 prevents cancer development through various mechanisms, including induction of cancer cell apoptosis. The aim of our work was to study transcription of some genes related to p53-dependent apoptosis. We have focused our interest to genes of pro-apoptotic proteins p53 and bax as well as anti-apoptotic proteins bcl-xl, bcl-2, myeloid cell leukemia-1 (MCL1) and heat shock protein 70.1 (hsp70.1). In addition, transcription of *glyceraldehyde-3-phosphate dehydrogenase* (*gapdh*) was investigated since dysfunction of mitochondrial apoptosis, including that of p53-dependent, are often associated with increased transcription of genes coding for enzymes of glycolysis. We have demonstrated that acute leukaemia, both myeloblastic (AML) and lymphoblastic (ALL), is associated with significantly elevated levels of p53 and bax mRNA in leukaemic cells. With respect to ALL, significantly elevated levels of bcl-xl mRNA could explain for relative resistance of ALL cells to p53-dependent apoptosis. Although level of bcl-xl mRNA in AML cells was not significantly different from that in normal cells, it was elevated above normal level in some individual cases. Interestingly, the AML cells expressing higher amounts of bcl-xl mRNA were significantly less sensitive to cytarabine. Altered alternative processing of MCL1 primary transcripts leading to decrease of pro-apoptotic short variant of MCL1, MCL1s, was observed in the case of both AML and ALL. Finally, transcription of *gapdh* was increased in the case of AML and transcription of *hsp70.1* and *bcl-2* producing anti-apoptotic proteins were not affected in acute leukaemia.
Our results indicate important role of dysregulation of p53-dependent apoptosis in development of acute leukaemia and modulation of leukaemic cells sensitivity to cytostatics.

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**THE EFFECTS OF SIMVASTATIN ON THE SIZE OF MYOCARDIAL INFARCTION AND VENTRICULAR ARRHYTHMIAS IN THE RAT HEART**

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Statins are known to have additional benefits in a setting of acute ischemia/reperfusion (I/R) beyond their cholesterol (CH)-lowering effects related to PPAR signaling. We have previously shown protective

effects of simvastatin (S) on I/R in rats with high levels of CH. In the present study we characterized the effect of S (10 mg/kg/day, p.o.) on susceptibility to ischaemic arrhythmias and myocardial infarction in the normal rats. After 5 days of treatment, plasma levels of CH did not differ between S and control (C)group. In Langendorff-perfused hearts, I/R (30-min LAD occlusion/120-min R) down-regulated PPAR gene expression (mRNA levels of PPAR isoforms reduced by 40-50%). In the S group, total number of ventricular premature beats (VPB) was reduced to 162 ± 62 (vs. 551 ± 61 in C; $P<0.05$). Duration of ventricular tachycardia (VT) was significantly shorter (9.5 ± 4.1 s vs. 43.6 ± 8.6 s in C; $P<0.05$). The infarct size normalized to area at risk size was smaller in the S group (11.5 ± 0.4% vs. 33.7 ± 4% in C; $P<0.05$). In conclusion, treatment with S may confer an efficient protection against I/R, independent of its lipid-lowering activity. Activation of PPARs may potentially account for the protective effects of statins in the heart of normocholesterolemic rats. *Grants VEGA SR 2/0173/08, APVT 51-027404 and National Scholarship Programme of the SR.*

## QUANTITATIVE ANALYSIS OF BODY SURFACE INTEGRAL MAPS IN OBESE BOYS

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The aim of the study was to characterize the qualitative and quantitative effects of obesity on the cardiac electric field in boys and young men - by investigation of the isointegral body surface potential maps (IBSPM) of depolarization (QRS), repolarization (STT) and of the whole ventricular complex (QRST). Enrolled in this study were healthy boys and men, aged 16 – 22 years. There were 23 obese with body fat percentage > 28% (age 17,9 ± 0,9 y.) and 52 controls with normal body weight (age 15,2 ± 1,3 y.) The PC based ECG computer system was used to record the electrical potential values (EP) from the thoracic surface, in midresipratory position in seated subjects. Derived from IBSPM the respective maximal and minimal EP values, their distribution and peak-to through amplitudes were evaluated. The bootstrap Yuen-Welch ANOVA method and post hoc bootstrap Yuen-Welch test were used for testing the significance of differences of IBSPM parameters between the groups. Significantly lower positive values ($p<0.01$) of the repolarization IBSPM were observed in the obese subjects, namely in the sternal and right precordial region. In addition, the negative values on the upper and dorsal part of the chest tended to be “less negative” by 30-150% (in relation to their localization), as compared to the values in the control group. Consequently the full relief of the mean IBSPM in the obese group was more flat. On the other hand the depolarization IBSPM parameters did not show any significant differences in amplitudes or distribution, except significantly lower values of minima ($p<0.01$) in normal controls. These results in relation to our previous findings of analogical repolarization ISBPM changes - due to an increased sympathetic activation of the heart - evoke a hypothesis about a possible participation of this factor in the observed obesity related differences.

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## THE DIRECT MEASUREMENT OF FREE RADICALS BY USING THE EPR METHOD FOR THE EVALUATION OF PAIN INTENSITY

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It is extremely difficult to evaluate objectively the intensity of pain. It is possible to use the imaging methods like fMRI or PET, which are technically complicated and expensive. Therefore we decided several years ago to use biochemical methods like the measurement of metabolites of lipids, saccharides and proteins. As a specific determination the ROS (reactive oxygen species) were used, for example MDA, TBARS and enzymes SOD and GSP. Nearly all free radicals and their metabolites were elevated after nociceptive stimulation both in experimental animals and men with pain deceases or syndromes (both in the blood and in brain tissue). They were also decreased after the application of antioxidative cocktail (vitamins A, E, C and selenium). In this paper we used the direct measurement of free radicals by means of EPR (electron paramagnetique resonance) method. We measured free radicals hydroxyle and nitroxide and also singlet oxygen. In adult Wistar rats we used the mechanical nociceptive stimulation by clamping the both hind limbs or the postsurgical pain after the laparotomy. The free radicals were measured before and after nociceptive procedures in the tails of anaesthetised animals. Results: After mechanical and surgical pain, the hydroxyl and nitroxide radicals as well as singlet oxygen are increased. After the application of antioxidants all these increased values are normalised. Conclusion: EPR method was firstly used in living animals and the results are very promising for future research

## COMMON PATTERN OF THE NOCICEPTIVE INNERVATION OF THE LUNGS AND ESOPHAGUS IN THE GUINEA PIG

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The lungs and esophagus are innervated by afferent neurons located in vagal nodose and jugular ganglia, and spinal dorsal root ganglia (DRG). Embryonically, the vagal nodose neurons are derived from embryonic placodes, while vagal jugular and spinal DRG neurons are derived from neural crest. We addressed the overall hypothesis that the phenotype of nociceptive afferent nerve fibers in the lungs and esophagus is dictated by the embryonic origin (placodes vs. neural crest) but is independent on the innervated tissue (lungs vs. esophagus). The afferent nerves were investigated by using combination of retrograde neural tracing, immunohistochemistry, single cell RT-PCR, and extracellular and patch clamp recordings of the afferent nerve activity in the guinea pig. Based on neurophysiological characterization of the afferent nerve terminals the nociceptive afferent neurons were defined by sensitivity to the TRPV1 agonist capsaicin. In the lungs and esophagus, nodose, but not jugular and DRG nociceptors are effectively stimulated via the serotonin 5-HT3 receptor and purinergic P2X receptors. The difference in purinergic activation correlates with the expression of the P2X2 receptor detected in nodose, but not in jugular and DRG nociceptors. Nodose, but not jugular nociceptors are effectively activated via the adenosine A1 receptor. The vast majority of jugular, but not nodose nociceptive neurons express neurokinins. We conclude that the phenotype of neural crest-derived (vagal jugular and spinal DRG) nociceptors is distinct from the placodes-derived (vagal nodose) nociceptors. The phenotypes of nociceptors innervating the lungs and esophagus are, in large part, determined more by their embryonic source than by the environment of the tissue they ultimately innervate. Funded by NIH DK074480 (M.K.) and HL062296 (B.J.U.)

**EFFECT OF C-547 (A NOVEL ACETYLCHOLINESTERASE INHIBITOR) ON ACHE ACTIVITY IN STRIATED AND HEART MUSCLES**

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In humane medicine, acetylcholinesterase (AChE) inhibitors are employed for treatment of myasthenia gravis, Alzheimer disease or cardiac diseases. Adverse side-effects of AChE inhibitors including nausea, vomiting, diarrhea and abdominal pain may be partly avoided by selection of inhibitors with desired characteristics (e.g. long-lasting effect, higher selectivity for CNS in Alzheimer patients or for skeletal muscle in myasthenia gravis). A novel AChE inhibitor, 1,3-bis[5(diethyl-o-nitrobenzylammonio)pentyl]-6-methyluracil (laboratory code C-547; different from classical carbamates, organophosphates, or onium salts) showed „in vivo“ prolonged relaxation of the skeletal muscles of tested animals running on the treadmill, while the effects on respiration were minimal (1). The different degree of relaxation in animals was confirmed by electrophysiological measurements on soleus, extensor digitorum longus (EDL) and diaphragm preparations (2). We have evaluated effect of C-547 on the activity of various molecular forms of AChE (prepared employing differential solubility in low-salt, high-salt and detergent solutions according to (3) in the rat (Wistar) brain, heart, soleus, EDL and diaphragm muscles. Analysis of enzyme kinetics of C-547 inhibition of rat AChE did not show any significant difference among individual molecular forms of AChE isolated from the soleus, EDL and diaphragm. AChE isolated from the heart was substantially more resistant to C-547 inhibition than that isolated from muscles, however this effect was due to high proportion of butyrylcholinesterase. Our results suggest that AChE inhibitors based on 9-methyluracil derivatives might possess characteristics desirable for potential therapeutic use.

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**DOES ACUTE METHAMPHETAMINE TREATMENT AFFECT LEARNING AND MEMORY IN ADULT MALE RATS PRENATALLY EXPOSED TO THE SAME DRUG?**

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Studies showed that stimulant drugs alter both behavioral and cognitive processes. Little is, however, known about the long-term consequences of prenatal methamphetamine (MA) exposure. The aim of the present study was to examine the effect of acute MA treatment on learning and memory in adult male rats prenatally exposed to the same drug. Adult male rats prenatally exposed to MA (5 mg/kg), saline or no injection were tested for learning in Morris water maze. Half of the animals were injected daily with MA (1 mg/kg) after finishing the testing. Three types of tests were used: (1) test of learning was performed for 5 consecutive days (“Place navigation test”), (2) “Probe test” in 6$^{th}$ day of experiment and (3) test of memory in day 12 of the experiment (“Retention memory test”). Our results demonstrate that prenatal MA exposure did not affect the test of learning and the “Probe test”. In the test of memory prenatally MA-exposed rats showed better results when compared to animals prenatally exposed to saline. Further, acute MA administration increased the speed of swimming in all rats regardless of prenatal drug exposure and the type of test. However, acute MA administration increased the speed of swimming in rats prenatally exposed to MA significantly more than in rats without any prenatal exposure. In addition, all rats with acute MA application had longer trajectories than the rats without acute drug administration in the test of learning. The present study demonstrates that: (1) Prenatal MA exposure does not affect learning in Place navigation test and Probe test. (2) Rats prenatally exposed to MA have better memory than rats prenatally exposed to stress. (3) Prenatal MA exposure increases the sensitivity to acute drug injection in Place navigation test. (4) Acute MA application impairs learning in Place navigation test.

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**MARKERS OF OXIDATIVE STRESS IN ACUTE MYOCARDIAL INFARCTION TREATED BY PERCUTANEOUS CORONARY INTERVENTION**

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Aim: Evaluation of the dynamics of oxidative stress markers in patients with acute myocardial infarction (AMI) treated by primary percutaneous coronary intervention (PCI) is the objective of this study.
Methods: Thirty consecutive patients with AMI with ST elevation were enrolled. The plasma lipid peroxidation end-product malondialdehyde (MDA) and total antioxidant capacity (TAC) of blood plasma were evaluated. Peripheral venous blood samples were obtained in 6 intervals (prior to reperfusion- 0 min., 1 min., 5 min., 1 hour, 3 hours and 3 days after reperfusion).
Results: Total antioxidant capacity values (1.26 ± 0.32 mmol/l) were slightly below the reference range (1.30 – 1.77 mmol/l) at admission. Within one hour after reperfusion values in most cases significantly declined (1 min, 1.10 ± 0.33 mmol/l; 5 min, 1.08 ± 0.18 mmol/l; 1 h, 1.06 ± 0.21 mmol/l, p= 0.03). From the 3$^{rd}$ hour a reversal of values started (1.14 ± 0.29 mmol/l) with return to normal values 3 days later (1.29 ± 0.24 mmol/l). Malondialdehyde levels showed sustained decrease over the 3 hours after reperfusion of occluded artery (1$^{st}$ min 1.57 ± 0.37 μmol/l, 5$^{th}$ min, 1.56 ± 0.54 μmol/l, 1$^{st}$ h 1.50 ± 0.35 μmol/l, 3$^{rd}$ h, 1.35 ± 0.59 μmol/l, vs. control 1.66 ± 0.55 μmol/l, p = 0.03).
Conclusion: Reperfusion of occluded coronary artery by PCI in AMI leads to immediate decrease of TAC which gives the indirect evidence of reactive oxygen species formation. However, the dynamics from MDA level, instead of expected increase following reperfusion revealed significant decrease.

**THE ROLE OF MITOGEN-ACTIVATED PROTEIN KINASES IN DIAZOXID-INDUCED CARDIOPROTECTION IN RAT MYOCARDIUM**

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In this study, we aimed to clarify the role of mitogen-activated protein kinases and other protein systems in diazoxide (a selective opener of the mitochondrial ATP-sensitive potassium channel)-induced cardioprotection. Isolated Langendorff-perfused rat hearts were subjected to 25 min global ischemia followed by 35 min reperfusion (index ischemia-II). In diazoxid (D)-treated hearts, D (50μm) was applied 15 min before II. The levels and activation state of specific proteins were determined by Western blot assay with specific antibodies. The activities of matrix metalloproteinases (MMP) were determined by zymography using gelatine as a substrate. It was found that the hearts pretreated with D showed better recovery of contractile

function after II. II induced increased release of cytochrome c from mitochondria and activation of caspase-3 as well as decrease of Bcl-2 levels. D-treatment did not significantly influence these II-induced changes. However, D-pretreatment reduced the cytosolic levels of pro-apoptotic Bax protein. Application of D increased activation of extracellular-signal regulated protein kinases (ERK) and we also found moderate increase in Raf-1 activities in D-treated hearts after II. The effect of D on ERK pathway points to the involvement of this signaling cascade in D-mediated adaptive responses of rat myocardium to ischemia. The actions of D were also linked with inhibition of MMP-2. The results also suggest possible relationship between ERK pathway and modulation of MMP-2 activities.

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## GENETIC POLYMORPHISMS IN GLUTATHIONE-S-TRANSFERASE GENES AND PROSTATE CANCER

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Glutathione-S-transferases (GSTs) belong to a superfamily of detoxification enzymes that provide critical defences against a large variety of chemical carcinogens and environmental toxicants. Deletion polymorphisms in the genes *GSTM1* and *GSTT1* and a base transition polymorphism at codon 105 (Ile-->Val) in *GSTP1* were investigated in relation to prostate cancer risk. We have investigated the potential functional significance of these polymorphisms and their association with prostate cancer susceptibility in 145 men with histologically confirmed prostate cancer and 219 healthy men. The presence of the GSTM1 null and GSTT1 null polymorphisms were screened by using a multiplex PCR procedure. A PCR-RFLP method was used to detect polymorphism of the *GSTP1* gene. We observed non-significant association in null alleles of the *GSTM1* (OR = 0.92; 95% CI = 0.59-1.42) and *GSTT1* (OR = 0.62, 95% CI = 0.35-1.08) with risk of prostate cancer. For *GSTP1*, the data were suggestive of a trend of increasing risk with higher numbers of codon 105 valine alleles compared with isoleucine alleles, a 1.46-fold increased risk of prostate cancer (95% CI = 1.46-4.55) was associated with Val/Val homozygosity. There was no significant link between Ile/Val genotype and risk of prostate cancer (OR - 1.0; 95% CI = 0.64 – 1.56).
Our findings suggest that GSTP1 (Val/Val) genotype may affect the risk of prostate cancer and tumour aggressiveness. Further, GSTM1 null and GSTT1 null genotypes did not appear to influence the susceptibility to prostate cancer.
This work was supported by grants UK/264/2006, MVTS Bil/ČR/SR/UK/06 and AV 4/0013/05.

## EFFECTS OF FEEDING GRAINS CONTAMINATED WITH *Fusarium* MYCO-TOXINS ON BIOCHEMICAL PARAMETERS OF BROILER CHICKENS

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Contamination of feed grains with *Fusarium* mycotoxins has resulted in economic losses in the animal industries. It has been reported that chicks have a high tolerance to deoxynivalenol-contaminated grains (1). It has been observed, however, that broiler chicks fed diets containing 1 to 3 mg DON/kg had changes in haematology (2,3,4). Limited information, however, is available on biochemical alterations in chicks caused by chronic ingestion of mixture of *Fusarium* mycotoxins. The study was conducted, therefore, to investigate the effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on biochemical parameters of chicks. Sixty, 1-d-old broiler chicks were fed 1 of 3 diets containing grains contaminated with *Fusarium* mycotoxins for 28 days. The diets included (1) control (0.60 mg/kg deoxynivalenol (DON), 0.07 mg/kg zearalenone (ZEA); (2) low level of contaminated grains (3.48 mg/kg DON, 3.36 mg/kg ZEA) and (3) high level of contaminated grains (8.19 mg/kg DON, 8.28 mg/kg ZEA). Plasma potassium, magnesium, phosphorus, total proteins, albumin, triglycerides and free glycerol were decreased and calcium, ALP, ALT, AST and cholesterol were elevated in chicks fed low level of contaminated grains. Plasma potassium, magnesium, total proteins, albumin, triglycerides and free glycerol were decreased and ALT and AST activities were increased in chicks fed high level of contaminated grains. It was concluded that chicks are susceptible during extended feeding of grains contaminated with *Fusarium* mycotoxins.
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## ANALYSIS OF PRODUCTS OF LIPID DEGRADATION IN ADIPOSE TISSUES

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The quality of fat obtained from slaughter animals is affected by both intravital factors and stress factors, acting immediately before slaughter, which increase consumption of energy and subsequently increase the number of free radicals in cells resulting in considerable post mortem acceleration of oxidative degradation of fat (1, 4). The final product of this process, which develops in substantial quantities, is malonaldehyde (3, 2). Changes in the basic lipid constants, the peroxide value (PV), acid value (AV) and thiobarbituric acid reactive substances (TBARs) in pork meat of normal quality and in that of altered quality (PSE: pale, soft, exudative), were observed in dependence on the length of freezing storage. We recorded a significantly higher level of acid value (AV) in pigs with PSE meat. However, we failed to observe significantly higher differences in levels of products of oxidative degradation of fat (PV) in fresh meat between the experimental and control group which is in agreement with the results published in (1). The differences in PV values between groups were insignificant, although they were generally higher (PV: PSE 2.35; control 2.31 $mmol.kg^{-1}$) and indicated that the damage is conditional on additional factors besides the stress load. Statistically significant changes in the basic lipid constants in frozen pork fat occurred in general after 6 months of freezing storage in both groups.
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## CARDIOPROTECTIVE EFFECT OF CHRONIC CONTINUOUS HYPOXIA IS MEDIATED BY $ß_1$-ADRENOCEPTORS

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Chronic hypoxia is associated with increased sympathetic activity and improved cardiac tolerance to acute ischemia/reperfusion injury. The aim was to determine whether the stimulation of the $ß_1$-adrenoceptor ($ß_1$-AR) pathway is involved in the cardioprotective mechanism of chronic hypoxia. Adult male Wistar rats were exposed to chronic continuous hypoxia (CCH, $FIO_2$ = 0.1, 24 h/day, 3 weeks). Half of the animals received a selective antagonist of $ß_1$-AR metoprolol (50 mg/kg/day). Control rats were kept under normoxia and treated in a

corresponding manner. 24 hours after withdrawal of metoprolol, anesthetized animals were subjected to 20-min coronary artery occlusion and 3-h reperfusion. Adaptation to CCH reduced infarct size from 67.6 ± 5.6 % of the area at risk in the normoxic controls to 36.6 ± 3.0 %. Metoprolol had no effect on infarct size in the normoxic animals (64.0 ± 3.9 %), but it completely prevented protection provided by CCH (to 68.6 ± 3.9 %). In addition, CCH reduced both the total number of ischemic premature ventricular complexes and duration of tachyarrhythmias; these antiarrhythmic effects were not affected by metoprolol. We conclude that catecholamines produced during the adaptation to CCH play an important role in the development of increased cardiac ischemic tolerance (infarct size reduction), which appears to be mediated by the $\beta_1$-AR pathway.

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## MORPHOLOGICAL CHANGES IN THE UTERUS OF SHEEP AFTER APPLICATION OF CLOPROSTENOL AND EQUIN CHORION GONADOTROPINE

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The investigations were carried out on 40 sheep in the period of physiological anoestrus. Oestrus induction in anoestrus sheep was achieved by intravaginal instillation of fluorogestonacetate. During inducted oestrous cycle in sheep on 7 day were administered of Cloprostenol ($PGF_2\alpha$). Next day after first application of Cloprostenol was administrated of the equine chorione gonadotropine (eCG) hormone in dose of 750, 1000 and 1500 IU.

Our investigations showed that the administration of $PGF_2\alpha$ resulted in a less significant decrease in the mass of uterus ($p<0.05$) than that observed after the application of eCG ($p<0.001$). An increase in the uterus cervix mass, observed following the administration of $PGF_2\alpha$ was of low significance ($p<0.1$) however that recorded after the administration of eCG (750 and 1500 IU) was more significant ($p<0.01$) and the most significant increase was elicited by administration of 1000 IU eCG ($p<0.001$). Cervical epithelial cilia of experimental sheep were many times elongated in comparison with those in the control animals. Secondary and tertiary projections were also observed. SEM examinations showed that the cervical epithelial surface was covered with dense microvillus and cilia and secretion blebs cud be observed in many places. The administration of oestrus inducing preparations elicits responses in the uterus of anoestrus sheep similar to those observed during the natural oestrus (1,2).

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## FACTORS AFFECTING SEIZURE SUSCEPTIBILITY OF ADULT RATS EXPOSED TO METHAMPHETAMINE DURING DEVELOPMENT

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Stimulant drugs are often associated with increase seizure susceptibility. Inhibitory γ-aminobutyric acid (GABA) and excitatory N-methyl-D-aspartate (NMDA) systems may play a role in the effect of stimulants on epileptic seizures. Our previous studies demonstrated that prenatal methamphetamine (MA) exposure affects seizure susceptibility of adult rats. In addition, we demonstrated that MA administered during gestation and lactation periods impairs maternal behavior as well as the postnatal development of rat pups that may induce long-lasting consequences. The aim of the present study was to distinguish the extent of the drug-induced effect and the extent of the effect induced by impaired maternal care. Offspring of three groups of females: MA-(5mg/kg) exposed, saline-exposed and absolute controls were used in the present study. Cross-fostering was conducted on postnatal day 1, so that each mother received some of her own and some of the pups of mother with other two treatments. Three seizure models were examined: seizures induced by bicuculline (as GABAA receptor antagonist), NMDA (as NMDA receptor agonist) and flurothyl (as convulsion inducing drug, which mechanism of action is not fully explained yet). No differences between groups were found in bicuculline seizures. In NMDA-induced seizures, the latency to onset of stereotypy and clonic-tonic seizures was affected only by saline injection (prenatal or postnatal); the latency was increased in prenatally saline-exposed rats raised by control mothers and in prenatally MA-exposed rats raised by saline-treated mothers. In flurothyl seizures, the threshold of first focal clonus, of clonic seizures as well as of tonic-clonic seizures varied in animals of different prenatal exposure raised by control mothers. The threshold was increased in saline-exposed rats and decreased in MA-exposed rats raised by control mothers. Thus, it seems that perinatal exposure to stress induced by saline injections plays a role in seizure susceptibility. Moreover, NMDA seems to be more affected by prenatal or postnatal drug (or injection) exposure than GABA system.

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## FUNCTIONAL AND BIOCHEMICAL CHANGES INDUCED BY CHRONIC L-NAME TREATMENT IN RAT HEARTS

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Nitric oxide (NO) plays important and multiple roles in the cells mediating a number of physiological and pathological processes. Recently was observed that the chronic inhibition of NO synthases by L-NAME resulted also in reduction of extent of myocardial injury after acute ischemia/reperfusion. The aim of the study was to characterize the effects of chronic NO synthase (NOS) inhibition on the alterations of regulatory proteins of intracellular signaling pathways (mitogen-activated protein kinase (MAPK) and Akt kinase cascades and matrix metalloproteinases (MMP)). NO deficiency (NOD) in rats was induced by L-NAME treatment (40/mg/rat daily, 4 weeks). Levels and specific phosphorylation (activation) of proteins were determined by Western blot analysis and activities of MMP were analyzed by zymography in polyacrylamide gels. The development of NOD was connected with decreased activation of both Akt kinase and extracellular signaling regulated protein kinases (ERKs). The L-NAME treatment induced also down-regulation of aFGF and H-Ras (possible activators of ERKs). Study of MMPs showed that in L-NAME treated rat hearts activities of both tissue and serum MMP-2 were decreased. On the other hand, in serum of L-NAME treated rats significantly increased gelatinolytic activity of approximately 20 kDa proteinase was observed. The results point to the possible relationship between ERK and Akt kinase pathways and activation of MMP-2. It seems that the changes in activities of ERK and Akt are also connected with modulation of activation of anti- and pro-apoptic proteins. The observed changes point to the involvement of ERK and Akt kinase cascades in responses of rat myocardium to NOD.

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## CHRONOPHYSIOLOGICAL VIEW ON THE PH AND BLOOD GASES CHANGES AT THE CONTROLED VENTILATION IN WISTAR RATS

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Maintenance of the internal environment stability is primary effort in *in vivo* studies especially in the experiments with controled arteficial ventilation. Problem is that the results are only comparised with the averaged reference values of the blood gases and pH often irrespective of the circadian dependence and in not least cause also time of the goining experiments. The aim of our study was refered to the blood gases and pH changes in the anaesthetized rats (ketamine/xylazine anaesthesia, 100 mg/kg /15 mg/kg, i.m.) at the manipulations with the pulmonary ventilation in the light-dark dependence. In the first phase of the experiments, the animals were adapted to the lighted regime 12:12 hours, with the dark part from 18.00 to 06.00 hour. In the second phase of experiments, blood gases and pH were analyzed after the inverse setting, with the dark part from 06.00 to 18.00 hour. The animals were ventilated arteficially using respiratory pump with the parameters of normal ventilation and reoxygenation $V_T$ = 1ml/100g, respiratory rate 40–50 breaths/min. The apnoic episode was simulated by the swiching off respirator for 2 minutes. Arterial blood gases and pH were analyzed after the surgical interventions (tracheotomy and thoracotomy) and 5 minutes of the artificial normal ventilation (period of stabilization), after 2 minutes of the apnoic episode and after 5. and 20. minute of reoxygenation. Significant light-dark differences were found in all followed parameters after the period of stabilization (pH light 7,37 ± 0,08 vs. pH dark 7,68 ± 0,04, $p < 0.001$; $pCO_2$ light 6.03 ± 1.03 kPa vs. $pCO_2$ dark 2.11 ± 0.14 kPa, $p < 0.001$; $pO_2$ light 8.25 ± 2.29 kPa vs. $pO_2$ dark 10.08 ± 1.46 kPa, $p < 0.04$). Apnoic episode minimized light-dark differences for pH and $pO_2$ with the preservation in $pCO_2$ (light 10,11± 1,82 kPa vs. dark 6,89 ± 2,52 kPa, $p < 0.006$). It is concluded that different reactions of animals for the interventions to the pulmonary ventilation, from the point of view of the internal stability, would be considered also in the light-dark dependence in *in vivo* rat experiments with the arteficial ventilation.
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## ECG CHANGES DURING ASPHYXIA AND REOXYGENATION IN THE LD DEPENDENCE IN WISTAR RAT.

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The aim of study was to refer to ECG changes during the apnoic episode and reoxygenation in the dependence on the light-dark cycle (LD cycle). The experiments were performed in the ketamine/xylazine anaesthetized female Wistar rats (ketamine 100mg/kg + xylazine 15mg/kg of b.w., i.m.) after the adaptation on the lightin regimen 12:12 h of the light and dark. The animals were arteficial ventilated by the respiratory pump with parameters of ventilation: tidal volume 1 ml/100 g of b.w., respiratory rate 40–50 breaths/min. Ventilatory apnoe was simulated by the swiching off respirator for 120 seconds. RR, PQ and QT intervals as well as R, P and T wave amplitudes were evaluated in the single experimental steps (intact animal, after tracheotomy, preparation of artery, thoracotomy, after 5 minutes of stabilization, after 30, 60, 90 and 120 seconds of apnoe and after 5, 10, 15 and 20 minutes of reoxygenation). Before ventilatory manipulations, the statistical significant LD differences were found in the RR and PQ interval duration with longer intervals in the light part of the day. Although QT interval duration was longer in the light part of the day, any significant difference was not record. R wave amplitude do not show any LD dependence a comparised to P and T wave amplitudes. Apnoe minimized LD dependence in RR interval duration at once the start of apnoic episode and 20 minutes of reoxygenation did not recover LD differences, which were found in the preapnoic period. LD differences in PQ interval duration were preserved only to the 60. second of apnoe in contrast of QT interval, where they were found only after 90 and 120 seconds of the apnoic episode with the longer duration in the dark part of the day. During asphyxia, R, P and T wave amplitudes showed dependence on the lighting regimen. Reoxygenation recoveried LD differences in all parameters, which were found before apnoe. It is concluded that 1. LD cycle influences significantly electrophysiological parameters and 2. apnoic episode disturbs and in some cases enlarges LD differences producing so predispositions for the onset and development of the ventricular arrhythmias which depend on the lighting regimen.
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## PROCESS OF AGING AND OXIDATIVE DAMAGE OF BRAIN MITOCHONDRIA

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Oxidative damage has been identified as major causative factor in the decline in physiological functions that occur during the aging process. The brain is especially vulnerable to oxidative damage as a result of its high oxygen consumption rate, its abundant lipid content, and relative paucity of antioxidant enzymes compared to other tissues. It has been suggested that mitochondria are strong producers of reactive species, and at the same time, particularly susceptible to the oxidative damage produced by their action on lipids, proteins, DNA. In the present investigation, the activity of mitochondrial enzymes Mn-superoxide dismutase, complex I, complex IV, and oxidative modifications of lipids and proteins were measured in brains from aging male Wistar rats (6-, 15- and 27-month old). The activities of these critical enzymes for mitochondrial function, decreased progressively with activity losses of 20.5%, 8.6% and 13.2% ($p<0.01$), respectively, in the brains of senescent rats compared with adults. The whole aging process was associated with an increased content of the oxidation products, malondialdehyde and 4-hydroxynonenal, by 15.3% ($p<0.01$) and 37.2% ($p<0.001$), and significant decrease in protein sulfhydryl group content 23.8% ($p<0.001$), in 27-month old rats. Binding of 4-hydroxynonenal to brain proteins was increased with age. The study suggests that mitochondrial dysfunction increases in age-dependent manner and is mediated, in part, by modification of specific mitochondrial proteins and/or lipids.
This work was supported by grant MVTS 39, COST B30/06 and VEGA 3380/06.

## GENE EXPRESSION OF ADRENOCEPTORS IN THE HEART OF RATS EXPOSED TO A NOVEL STRESSOR

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Changes in the heart rate and force of contraction are regulated by catecholamines via adrenoceptors (AR). In this work we measured gene expression of AR in the heart of rats exposed to long-term cold stress and in cold acclimated rats exposed to a novel stressor (immobilization - IMO). We found a significant increase in mRNA levels of $\beta_3$-AR in left ventricle of rats exposed to acute (1 day) and long-term (28 days) cold, but no changes in $\beta_1$- and $\beta_2$-AR mRNA levels. Application of novel stressor (IMO) to previously cold acclimated animals did not show additional changes in β-AR mRNA levels. The most prominent changes in the heart AR were detected in $\alpha_{1B}$-AR gene expression. Decreased levels of $\alpha_{1B}$-AR mRNA in the heart of rats exposed to cold and IMO were found. Exposure of cold acclimated rats to IMO was responsible

for additional decrease of $\alpha_{1B}$-AR mRNA levels in the heart. It seems that while β-AR undergo an adaptation, $\alpha_{1B}$-AR are probably prepared to modulate heart functions. Thus, we have shown that gene expression of different AR subtypes in the heart is regulated differently by various stressors. A role of $\beta_2$- , $\beta_3$-AR and $\alpha_{1B}$-AR in the process of heart adaptation to chronic stress exposure is proposed. *This work was supported by APVV grant No.0148-06 and VEGA 2/5125.*

## CARDIOVASCULAR REGULATION IN CHILDREN AND ADOLESCENTS WITH OVERWEIGHT AND OBESITY

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Aim: The childhood overweight and obesity is a very serious problem. The purpose was to study the changes in cardiovascular regulation using heart rate and blood pressure variabilities analyses in children and adolescents with overweight and obesity.
Method: Fifty subjects (12-18 years) were examined – 20 obese, 20 nonobese (control) subjects and 10 children and adolescents with overweight. Continual recording of electrocardiogram (CHIRASTAR 60, Slovakia) and peripheral blood pressure using FINAPRES (Ohmeda 2300, USA) was performed at rest in lying position during 50 min. Evaluated parameters: *Heart rate variability (HRV)*: the mean R-R interval, rMSSD, pNN50, spectral powers in high (logHF), low (logLF) frequency bands and total power (logTP). *Blood pressure variability (BPV):* the mean systolic and diastolic blood pressure, spectral powers in low (LF) and high frequency (HF) bands.
Results: The group with overweight has significantly shortened mean R-R interval compared to controls and significantly prolonged mean R-R interval compared to obese group. Obese subjects have significantly higher heart rate and mean blood pressure. *HRV parameters* – pNN50, rMSSD, logHF, logTP - were significantly lower in obese patients compared to control group. *BPV parameters:* No significant differences were found in these parameters.
Conclusion: Higher mean values of heart rate and blood pressure, lower parasympathetic heart control without alteration in sympathetic vasomotor control were found in obese children and adolescents. Higher heart rate was found already in the subjects with overweight. We suppose altered cardiovascular regulation in the children and adolescents with overweight and obesity.

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## EFFECT OF CHRONIC SOCIAL STRESS ON NEUROGENIC CONTRACTIONS OF MESENTERIC ARTERY IN RATS WITH FAMILY HISTORY OF HYPERTENSION

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Hypertensive rats are very susceptible to various forms of stress but not much is known about the stimuli from social environment, which may contribute to deterioration and stabilization of hypertension. The aim of the study was to investigate the effect of eight-week-lasting crowding stress on blood pressure and contractile responses of isolated rat superior mesenteric artery to adrenergic stimuli in Wistar rats, bordeline hypertensive (BHR) and spontaneously hypertensive rats (SHR) treated and not treated with Provinols™. Systolic blood pressure of unstressed rats in Wistar, BHR and SHR rats at the end of experiment was 111±2, 132±2 and 183±4 mmHg, respectively. Eight weeks of crowding stress exposure resulted in increase of blood pressure in BHR (145±3 mmHg, $P< 0.05$) and SHR (205±5 mmHg, $P< 0.001$). In the stressed hypertensive rats treated with Provinols™, blood pressure in BHR and SHR was lower (l35±2 and 194±2 mmHg, respectively, $P< 0.05$). Chronic crowding stress did not influence the contractions of mesenteric artery to exogenous noradrenaline but it reduced neurogenic contractions induced by endogenous noradrenaline released by electrical stimulation of perivascular nerves. Provinols™ alone reduced neurogenic contractions in Wistar rats, but it had no significant effect on frequency-response curve in hypertensive rats. In stressed BHR and SHR simultaneously treated with Provinols™ the neurogenic responses were significantly reduced. These results are consistent with the idea that the increased release of nitric oxide during chronic crowding stress in SHR attenuates the contractile response to perivascular nerve stimulation. Thus, the decreased activity of sympathetic nerve system in large arteries may represent the protective mechanisms in hypertensive animals exposed to stress.
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## ADAPTATION OF MYOFIBRILLAR ENVIRONMENT OF SLOW SKELETAL MUSCLE FIBRES IN MICE LACKINKG CREATINE KINASE

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Morphometric analysis of sarcomere lengths of slow skeletal muscle from mice lacking creatine kinase ($CK^{-/-}$) revealed adaptational changes at the level of sarcomeres (1). Invalidation of creatine kinase led to elongation of sarcomeres and A-bands in comparison to control mice. The aim of this study was to analyse whether changes in the length of actin and myosin filaments are accompanied by changes in myo-fibrillar environment in slow skeletal muscle of $CK^{-/-}$ mice. We have used a stereological method of vertical sections applied to electron microscopic images to analyse environ-ment of myofibrils. The results revealed, that in $CK^{-/-}$ slow skeletal muscle fibres significantly increased contact area of myosin filaments with mitochondria (1, 7×). The partici-pation of cytosol near A-bands significantly decreased (0,7×). The surface of A-bands surrounded with sarcoplasmic reticulum was significantly smaller (0,7×) than in control mice. Contribution of sarcomere subunits to the environment of sarcomeres neighbour myofibrils was generaly higher in $CK^{-/-}$ mice due to increased relative shift of myofibrils. Quantitative analysis of myofibrillar environment in $CK^{-/-}$ slow skeletal muscles revealed cytoarchitectural adaptation of myofibrils and organels in close vicinity. The substantial changes in myofibrillar environment of $CK^{-/-}$ mice are due to the shift and splitting of myofibrils.
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## LIVER FAT EVALUATION OF PATIENTS WITH LIVER RESECTION USING MR SPECTROSCOPY

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Liver steatosis increases surgical risks during resection, especially in patients with chemotherapy-associated steatohepatitis (CASH). Our main goal was to evaluate liver steatosis using a simple and precise routine protocol in proton magnetic resonance spectroscopy ($^1$H MRS) on a clinical scanner and correlate its results to biochemical analysis of resected liver tissue and semiquantitative histopathology. $^1$H MRS determined hepatic triglyceride and water contents in selected volume (VOI) by acquiring six spectra during one breathold (PRESS sequence, $VOI=(3cm)^3$), which allowed to correct for different relaxation times of fat and water. The fat mass percentage was calculated. Results for water

and fat signals obtained using MRS in ten patients well correlated with biochemical analysis. MRS result never differed by more than 2%. Histopathological assessment stated the liver fat contents being same for all the patients, less than 5% To conclude, MRS evaluation costs five extra minutes if added to a standard pre-operative MRI examination. It is performed very easily on a routine scanner, and brings valuable information about the fat contents of the liver tissue.
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## TERTIARY FOLLICLE ATRESIA OF EWES IN POSTPARTUM PERIOD

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The changes in reproductive apparatus of our sheep breeds are described in the literature (1, 2). Laparotomy with following ovariectomy was realized after lamb weaning (spring) on day 17, 24 and 32 *post partum* (pp). The ovaries were fixed in 10 % formalin. The sections of the ovary tissue were stained with H-E. Ovarian slides were then analyzed by LUCIA-G 4.71. On days 17 and 24 pp 86 – 87 % follicles underwent atresia in recruitment stage (< 3 mm), most that of early and contractive. On day 32 pp we found out 93 % late atretic follicles ($P<0.05$). In the selection (> 3 mm) there were 22 % healthy follicles on day 17 pp with maximal diameter 3.1 mm. On days 24 and 32 pp 85 – 86 % atretic follicles were found out but differences were not significant. Most early and contractive changes of atresia were found out. The cystic atresia appeared on day 24 pp (5 %) in the regression stage. On day 32 pp healthy follicles reached the preovulatory sizes (> 4 mm). This fact refers to renewal of the reproductive cycle after ewe parturition which is connected with the finishing of the involution processes in the uterus.

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## MOTOR MANIFESTATION OF OLIVOCEREBELLAR AND RETINAL DEGENERATION IN LURCHER MUTANT MICE OF THE C3H STRAIN

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Lurcher mutant mice of the C3H strain represent a natural model of the olivocerebellar degeneration. Postnatally, complete loss of Purkinje cells and secondary decreased number of granule cells and inferior olive neurons occurs. The degeneration is manifested by cerebellar ataxia and impairment of cognitive functions. Wild type littermates of these mice are completely healthy and serve as ideal controls. Moreover, some of the C3H strain mice are afflicted with retinal degeneration which leads to a massive extinction of retinal photoreceptors. In this work, we investigated the influence of the olivocerebellar and retinal degeneration on gait control and spontaneous motor activity.
Gait control was investigated using the CatWalk system. In this experiment the CatWalk system was used for evaluation of ataxia and visual handicap for the first time ever. Spontaneous motor activity was tested in the open field using the EthoVision system. Trajectory length and time spent in the central zone of the open field were measured. Presence of the retinal degeneration was identified post mortem by histological examination.
Gait control investigation revealed significant differences between Lurcher mutant and wild type mice in many parameters, especially in an angle of forepaws position, footfall pattern regularity, base of support and the relative distribution of paw combinations that were at the same time in contact with the walkway during walking. Retinal degeneration does not have any influence on the observed parameters. In the open field, no difference between blind and seeing wild type mice was found. In the Lurcher mutant mice, the blind animals spent almost two-fold more time in the central area. Compared with the wild type mice, the Lurcher mutant mice spent markedly more time in the central area regardless to the presence of the retinal degeneration.
It is possible to quantify the cerebellar ataxia in the Lurcher mutant mice. The ataxia manifests itself with significantly altered gait pattern and with parameters of single steps as well. Retinal degeneration does not have any influence on the walking manner. Neither olivocerebellar nor retinal degeneration does not have any marked influence on the spontaneous motor activity, but affects the preference of the central area of the open field, what is an indicator of the anxiety level.

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## CHANGES OF THE EXPRESSION OF ENDOTHELIAL NO SYNTHASE, HEAT SHOCK PROTEIN 90 AND CAVEOLIN-1 AS ONE OF THE POSSIBLE CAUSES OF ENDOTHELIAL DYSFUNCTION IN DIABETES

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We determined whether mild form of diabetes and the treatment with pycnogenol influences the expression of eNOS, Hsp90 and caveolin-1 in aorta and liver. Diabetes was induced by streptozotocine administered for 3 days at a dose of 25 mg/kg/day i.p. After 14 days diabetic groups were treated with pycnogenol in doses 10, 20 and 50 mg/kg/day p.o. for 8 weeks. The expression of eNOS, Hsp90 and caveolin-1 was determined by SDS-PAGE and immunodetection. The expression of eNOS in aorta of diabetic rats was significantly elevated compared to control rats. The treatment with pycnogenol (10 and 20 mg/kg/day) significantly decreased the observed elevated expression of eNOS. The expression of caveolin-1 wasn´t changed in any organ and any group. The expression of Hsp90 was decreased in aortas of diabetic rats. Pycnogenol in dose of 20 mg/kg had avoided this decrease. There were no changes in expression of eNOS and Hsp90 in the liver in any group.

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## SEXUALLY DIMORPHIC INDEX 2D:4D AND PAIN PERCEPTION IN MEN AND WOMEN

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Men and women differ in their perception of pain. Females are more sensitive to experimentally induced pain relative to males and their responses to noxious stimuli vary across the menstrual cycle. In adulthood, circulating sex hormones have an activational effect whereas during prenatal period they have an organizational effect and can also influence development of nociceptive system. Sexually dimorphic index 2D:4D (digit length ratio between the second and fourth finger) is believed to reflect prenatal effect of testosterone and estrogens. Higher index in women (= or > 1) results from prenatal exposure to lower level of testosterone whereas lower index in men (< 1) results from higher testosterone level. We decided to compare nociceptive sensitivity in adult men and women in dependency on digit ratio. We hypothesized that more masculine index will be associated with lower sensitivity and more feminine index with higher sensitivity to pain. 18 women (23.1 years) and 16 men (23.4 years) underwent two thermal nociciptive tests: modified tail-flick (fingers were stimulated with the beam of radiant heat until appearance of withdrawal reaction) and the cold pressor test

(CPT - immersion of non-dominant hand into water 2°C for 2 min). Intensity of perceived cold pain was assessed in 15 s intervals on visual analog scale. Digit ratios were calculated from the photocopies of both hands. Digit ratio, as expected, was higher in women than in men. We found no significant sex differences in the thermal pain and no correlation between withdrawal latency from the heat stimulus and index 2D:4D. Index 2D:4D correlated positively with pain intensity ($r = 0.38$, $p < 0.05$) of CPT. During CPT women experienced more intensive pain than men. In women, this index also correlated with the skin temperature ($r = 0.61$, $p < 0.01$). From these results it can be concluded that sexually dimorphic index 2D:4D is probably more associated with affective than sensory dimension of pain.

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**CARDIAC INTERVAL AND BLOOD PRESSURE VARIABILITY IN CHILDREN AND ADOLESCENTS**

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Introduction: The aim of the study was to show the influence of age and mean cardiac interval on blood pressure and cardiac interval variability.
Methods: Blood pressure was continuously noninvasively measured in 415 healthy subjects (11-20 years) for 5 minutes by Finapres. Mean values of cardiac intervals (CI) and systolic blood pressure (SBP) and their standard deviations (CISD, SBPSD) were calculated. Also, parameters of short-term cardiac intervals and systolic blood pressure variability in relative (CIrel, SBPrel) and absolute units (CI0.1Hz, SBP0.1Hz) in the range of 0.1 Hz of the spectra were estimated by means of spectral analysis.
Results: We found a significant increase of CI and SBP and a significant decrease of $SBP_{SD}$ with age; however, $CI_{SD}$, $CI_{0.1Hz}$, $SBP_{0.1Hz}$, $CI_{rel}$, and $SBP_{rel}$ did not correlate with age. We also found increase of cardiac interval variability ($CI_{SD}$ and $CI_{0.1Hz}$), decrease of $CI_{rel}$ and systolic blood pressure variability ($SBP_{SD}$, $SBP_{0.1Hz}$, $SBP_{rel}$) with CI prolongation. After standardization on CI, multiple regression analysis showed a significant decrease of $CI_{SD}$, $CI_{0.1Hz}$ and a significant increase of $CI_{rel}$, $SBP_{rel}$ with age, but $SBP_{SD}$ and $SBP_{0.1Hz}$ were age-independent.
Conclusion: Parasympathetic and sympathetic activity during adolescence increased, which is evident from cardiac interval and systolic blood pressure increase. Increased parasympathetic activity is also usually documented by higher cardiac interval variability; however, we found that this parameter decreased with age, which could be explained by the effect of other mechanisms (mechanical, humoral or neural) dependent on age. The influence of cardiac intervals on systolic blood pressure variability could explain the weak significant decrease of $SBP_{SD}$ with age, although standardized systolic blood pressure variability did not change with age. The results indicate a partially independent development of the reflex and tonic control of the cardiovascular system.
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**LONG-TERM TREATMENT OF YOUNG SPONTANEOUSLY HYPERTENSIVE RATS WITH MELATONIN PARTIALLY PREVENTED DEVELOPMENT OF HYPERTENSION**

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Melatonin, a primary hormone of the pineal gland, has been reported to decrease arterial pressure in hypertensive adult rats as well as in adult humans. The aim of the present study was to investigate the preventive effect of melatonin on the development of spontaneous hypertension in young spontaneous hypertensive rats (SHR) in which hypertension was just developing. Eight-week-old SHR and Wistar rats were divided randomly into two groups: 1/ a control group drinking just tap water, and 2/ a group receiving melatonin (12 mg/kg/day of body weight) in tap water for 4 weeks. Blood pressure was measured weekly by the indirect tail-cuff technique. Rings of thoracic aorta and mesenteric artery isolated from 12-week-old rats were suspended in organ baths containing modified Krebs solution and connected to a force-displacement transducer for the recording of isometric tension. Blood pressure of 8-week-old melatonin treated SHR and their age-matched SHR controls were 149±1 mmHg and 143±3 mmHg, respectively. The treatment of rats prevented hypertension development in SHR: increment of blood pressure at the end of 12th week was decreased by 11% as compared with untreated control SHR. Melatonin treatment had no significant effect on the heart weight/body weight ratio. Acetylcholine-induced relaxation in the thoracic aorta from both untreated and melatonin-treated SHR was fully preserved, and contrasted with the maintained high blood pressure. Vascular contractions induced by periarterial nerve stimulation and exogenous noradrenaline were increased in SHR. Melatonin significantly reduced neurogenic contractions in mesenteric artery induced by high frequency of periarterial electrical stimulation. In conclusion, the results showed that chronic administration of melatonin to SHR with developing spontaneous hypertension decreased pathological elevation of blood pressure, and slightly reduced neurogenic contractions.
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**SIGNALIZATION BY HEART MITOCHONDRIA IN HYPOXIA, ISCHEMIA AND DIABETES: ROLE OF SUCCINATE AND FREE RADICALS**

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Background: Streptozotocin (STZ)-diabetes (DIA), hypoxia (HY) and ischemic preconditioning (IP) induce both pathological and protective changes in rat heart (H) mitochondria (MIT). Aims: i) Elucidation of character and consequences of functional changes in H MIT induced by acute DIA, HY and IP; ii) Investigation of signaling implemented by MIT in crosstalk with subcellular particles in HY, IP and DIA. Materials & methods: Male Wistar rats, 220±20g. Models: DIA- (7 days, STZ single dose, 65 mg/kg i.p.); HY- isolated cardiomyocytes (ICM), incubator ($pO_2 \approx 10$ mmHg); IP- imitated in by diazoxide (DZO) in ICM; MIT isolated with proteases. Estimations: Metabolism; In MIT: Functional parameters (FP), conjugated dienes (CD) and membrane fluidity (MF). MIT signaling to HY-genes (HG) was indicated by carbonyl anhydrase IX (Ca IX) expression. Modulators: tempol (TL), DZO and N-acetylcysteine (NAC). Results: Presence of DIA, HY and IP were confirmed by metabolic and FP. DZO and TL stimulated, NAC inhibited Ca IX expression. Conclusions: MIT from DIA, HY and IP H are signaling to HG by release of succinate and radicals. OH-radicals inhibit, superoxide radicals aggravate Ca IX expression. Grants: VEGA 2/0173/08, 2/7126/27, APVV 51-027404.