

**NANOFIBRES IN CARTILAGE ENGINEERING**

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Tissue engineering is a modern approach in therapy. Production and development of cartilages belongs to the most advanced among preparations of artificial tissues. Chondrocytes embedded in biocompatible scaffolds are reported to have the capacity to repair osteochondral defects. Biodegradable polymers, such as collagen, fibrin, PGA, PLA, hyaluronic acid (HA) are broadly used. However, the artificially prepared cartilages must also show appropriate biomechanical properties. In principle, the biomechanical properties should closely approximate the native tissue properties. This task is not simple even from the point of accurate description of the tissue. We have developed a unique biophysical technique based on a sample exposure to ultrasonic waves. A novel laser system for sample response detection is constructed to describe in a very short time period complex biomechanical properties of very small samples like a tiny piece of native cartilage or artificial tissue. A novel detection system has revealed that tissues need special scaffolds to keep a proper biomechanical backing and to secure the constructed system with enough nutrition both in vitro and in vivo. Hence, we have developed a proper support based on different biodegradable materials with enough space to supply the whole artificial construct with nutrition. Combination of nanofibers with other structures has opened a novel approach in tissue engineering. The desired biomechanical properties of the artificial tissue can be modulated and modified as well as the nutrition regulation of new implanted tissue even in vivo. We have studied the biophysical properties of the newly prepared composite scaffold based on combination of nanofibers from biodegradable materials, which are of potential interest for tissue engineering. In addition, isolated chondrocytes were cultured in a three-dimensional system. Different growth factors, ascorbic acid and other supplements were used for cell proliferation and differentiation in vitro. We tested the development and properties of such developed artificial cartilages.

**THE EFFECT OF HYPERCHOLESTEROLEMIA AND SIMVASTATIN ON ISCHEMIC-REPERFUSION INJURY IN RAT HEARTS WITH EXPERIMENTAL DIABETES MELLITUS.**

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Hypercholesterolemia (HCH) and diabetes mellitus (DM) are important risk factors in pathogenesis of ischemic heart disease. Unlike in clinical trials, experimental studies report a reduction in sensitivity of the diabetic heart to ischemia/reperfusion (I/R) injury, however, ischemic tolerance has not been evaluated in a combined (DM and HCH) model. Since statins are known to protect rat hearts against I/R injury (1), our aims were: 1. to characterize the effect of combination of DM and HCH on I/R injury in the rat heart; 2. to investigate the effect of simvastatin on I/R injury in DM/HCH hearts. Control group contained male Wistar rats fed a standart diet. DM was induced by the application of streptozotocin (STZ, 80 mg/kg, i.p.). In the third (DM/HCH) group, diabetic rats were fed a fat-cholesterol diet (FCHD). The fourth group contained DM/HCH rats treated with simvastatin (S), which was applied (10 mg/kg daily) as a part of a FCHD. On the eighth day the hearts from all groups were isolated and perfused according to Langendorff. Test I/R was induced by 30 min occlusion of left anterior descending (LAD) coronary artery and 2 h reperfusion, and the size of myocardial

infarction served as the end-point of injury. The size of infarction was delineated by double staining with potassium permanganate and 2,3,5 triphenyltetrazolium (2) and evaluated on the thin slices by a computerized planimetric method as a ratio of infarct size (IS) to the area at risk (AR) size (IS/AR). Results: administration of STZ led to elevation of blood glucose, and a combination of DM and FCHD resulted in significantly increased cholesterol levels. In the diabetic group IS/AR was significantly lower than in the control group ( $15.1 \pm 3\%$  vs.  $37.6 \pm 2.8\%$ ;  $p < 0.05$ ). FCHD had a negative influence on the extent of I/R injury in the diabetic myocardium leading to an increase in IS/AR to  $37.3 \pm 3.1\%$  ( $p < 0.05$  vs. DM group). The size of infarction was significantly smaller in S-treated DM/HCH group (IS/AR  $14.2 \pm 1.3\%$  in comparison with the non-treated DM/HCH group;  $p < 0.05$ ). In conclusion: our results suggest that diabetic hearts are more resistant to I/R injury, however the combination of DM and FCHD causes the loss of this resistance. Thus, hypercholesterolemia increases the sensitivity of the diabetic heart to I/R injury. Simvastatin exerts cardioprotective effect in the model of DM/HCH suggesting that treatment with statins can decrease the risks of cardiovascular complications in the diabetic patients with disorders of lipid metabolism.

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**ADHESION AND GROWTH OF VASCULAR SMOOTH MUSCLE CELLS ON COLLAGEN I MODIFIED BY MAST CELLS**

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Mast cells play an important role in the remodeling of the pulmonary blood vessels during chronic hypoxic pulmonary hypertension, especially by their production of proteases which degrade the vascular extracellular matrix (1). In the first set of experiments, collagen I adsorbed on polystyrene dishes was pretreated for 24 hours with rat mastocytoma RBL-2H3 cells in a normoxic atmosphere (i.e., 95 % of air and 5 % of CO<sub>2</sub>; sample "N") or under hypoxia (i.e., 10 % of O<sub>2</sub> in the cultivation atmosphere; sample "H"). Dishes coated with unmodified collagen ("C") served as control samples. After removal of RBL-2H3 cells by EDTA, the samples were seeded with rat vascular smooth muscle cells (VSMC; passage 3 to 8; 2500 cells/cm<sup>2</sup>, medium DMEM with 10 % of fetal bovine serum). Growth curves revealed that from day 3 after seeding, the VSMC on "N" and "H" grew faster, reaching significantly higher population densities on day 7 (by  $58 \pm 2\%$  and  $88 \pm 8\%$ , respectively, compared to "C"). On the sample "H", the VSMC also contained a significantly lower concentration of vinculin, a focal adhesion protein (by  $29 \pm 6\%$  and  $33 \pm 5\%$  compared to the values on the samples "C" and "N", respectively), as well as alpha-actin, a contractile protein and important marker of VSMC differentiation (by  $48 \pm 8\%$  and  $48 \pm 12\%$  in comparison with both "C" and "N", respectively; measured by ELISA per mg of protein). In the second set of experiments, the collagen was exposed for 24 h to an extract of mast cells isolated from the rat lung and pre-incubated under normoxic ("N") or hypoxic (10 % O<sub>2</sub>; "H") conditions. In serum-free medium, the cell-material contact area on "H" and "N" was significantly smaller (by  $33 \pm 4\%$  and  $22 \pm 5\%$ ) than that on the unmodified collagen, respectively. Moreover, in the serum-free medium, the fastest VSMC

proliferation was found on the collagen "H". These results suggest that the mast cells decrease the strength of adhesion of VSMC to collagen, promote their transition from the contractile to synthetic phenotype and stimulate their proliferation. These effects are probably mediated by the production of proteolytic enzymes, as well as various cytokines, hormones and growth factors in the mast cells, especially those activated by hypoxic conditions.

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#### **EFFECT OF N-ACETYLCYSTEINE ON MYOCARDIAL INFARCT SIZE, PKC EXPRESSION AND PHOSPHOLIPID COMPOSITION IN RATS ADAPTED TO CHRONIC HYPOXIA**

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Adaptation to chronic hypoxia protects the heart against ischemia-reperfusion injury. We have shown previously that this process is associated with increased oxidative stress, remodelling of phospholipid (PL) fatty acid composition and upregulation of protein kinase C (PKC) isoform  $\delta$ . The aim of this study was to determine whether the inhibition of oxidative stress by antioxidant treatment (N-acetylcysteine, NAC) affects cardiac ischemic tolerance, PKC expression, antioxidative enzymes activities, the level of conjugated dienes and PL composition in left ventricular myocardium of rats adapted to chronic hypoxia. Our results demonstrate that chronic intermittent hypobaric hypoxia (7000 m, 8 hours/day, 25 exposures) reduced infarct size by 50 % as compared with normoxia. It increased the proportion of n-3 PUFA and decreased the proportion of n-6 PUFA in PL (by 23.1 % and 12.2 %, respectively). Chronic hypoxia enhanced the relative amount of PKC  $\delta$  in the particulate fraction and lowered the relative amount of PKC  $\epsilon$  as compared with normoxic controls. NAC treatment (100 mg/kg daily before each hypoxic exposure) decreased infarct size in the normoxic group by 25 % but it abolished the protection induced by chronic hypoxia. NAC increased the proportion of n-3 PUFA in the normoxic tissue and prevented the hypoxia-induced upregulation of PKC  $\delta$  in the hypoxic tissue. Activities of antioxidative enzymes (catalase, superoxide dismutase, glutathione peroxidase) and the level of conjugated dienes were affected by neither hypoxia nor NAC treatment. It is concluded that oxidative stress associated with chronic hypoxia plays an important role in the development of increased cardiac ischemic tolerance. Antioxidant treatment abolished both cardioprotective effect and PKC  $\delta$  upregulation induced by chronic hypoxia. The infarct size-limiting mechanism of chronic hypoxia seems to involve PKC  $\delta$ -dependent pathway.

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#### **INHIBITORS OF PROTEIN KINASE PATHWAYS AS REVERSAL AGENTS OF P-GLYCOPROTEIN-MEDIATED MULTIDRUG RESISTANCE IN L1210/VCR CELLS**

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The transmembrane transport pump P-glycoprotein (P-gp) causes the efflux of chemotherapeutic agents from cells and is an important system

that secures multidrug resistance (MDR) of neoplastic cells. In the present study drug sensitive L1210 and multidrug resistant L1210/VCR mouse leukemic cell lines were used as an experimental model. We found that LY294,002 (LY), a specific inhibitor of PI3K/Akt kinase pathway, significantly reduced the degree of vincristine (VCR) resistance in L1210/VCR cells in concentration dependent manner. This was accompanied by decrease in LC<sub>50</sub> value to VCR. For sensitive cells represented IC<sub>50</sub> to VCR 0.010  $\mu$ mol/l and the resistance index (ratio of IC<sub>50</sub> for resistant and sensitive cells) changed from 313 for L1210/VCR cells cultivated in the absence of LY to 48 for cells in the presence of 8  $\mu$ mol/l LY. For lower concentrations of LY were the changes in the degree of resistance not so high (for 4  $\mu$ mol/l LY was value of resistance index 95). Fluo-3/AM (Fluo) represents a good substrate for P-gp and this substance may be used for measurement of P-gp transport activity. We found that the presence of P-gp in resistant cells prevents the cells to be loaded by Fluo. In contrast, sensitive cells were extensively loaded by this fluorescent dye. This effect was not altered by presence of VCR and also LY did not significantly influence the loading with Fluo. Western blot analysis revealed that in sensitive cells is increased content of the cleaved caspase-3 and the presence of VCR induced further increase in levels of the 17/19 kDa fragments. In resistant cells induced the presence of LY increased cleavage of caspase-3 and increase in the content of 30 and 55 kDa proteins. The presence of LY reversed also the activation of Akt kinase in resistant cells. Our data show that LY294,002, an inhibitor of PI3K/Akt kinase pathway, reversed the MDR resistance of L1210/VCR cells and that the development of this resistance is connected with lower degree of caspase-3 activation in comparison to parental L1210 cells. These findings point to the possible involvement of PI3K/Akt kinase pathway in modulation of P-gp mediated multidrug resistance in L1210/VCR cell line.

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#### **THE EFFECT OF A JUVENILE HORMONE ANALOGUE ON ADIPOKINETIC HORMONE ACTIVITY IN BUGS (HETEROPTERA, INSECTA)**

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Insect energy metabolism is controlled by adipokinetic hormones (AKHs) that are synthesised, stored and released into the haemolymph by neurosecretory cells of the corpora cardiaca (CC), a neuroendocrine gland situated near the insect brain. The AKHs are octa-, nona- or decapeptides with both termini blocked. They control processes on subcellular, cellular, organ and organismic levels (mobilisation of energy stores, activity of enzymes, inhibition of synthetic reactions, stimulation of locomotion (1, 2), stimulation of heart beating etc.) that are involved in stress reactions. In bugs the AKHs stimulate above all mobilisation of lipid stores that serve as a main source of energy in this group of insects (2, 3). In this paper we have studied an effect of a juvenile hormone analogue - the juvenoid methoprene - on a role of *Pyrrhocoris apterus* (Heteroptera, Insecta) adipokinetic hormone (Pyrap-AKH) (2) in lipid metabolism. We investigated how methoprene modifies lipid mobilisation elicited by Pyrap-AKH and how affects level of AKHs in CNS and haemolymph of flightless bug *P. apterus* and flying cotton bug *Dysdercus cingulatus* (Heteroptera, Insecta). It seems that juvenile hormone, which has crucial role in insect development, metamorphosis and reproduction, affects adipokinetic characteristics by probable inhibiting of both AKH synthesis in CC and their release into the haemolymph, as well as by inhibiting of lipid mobilisation from reserves in fat body. Intensity of the suppressing effects depends on insect age and on other physiological parameters.

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#### COMPARISON BETWEEN CONCENTRATIONS OF FABPS AND SIGNIFICANT MARKERS OF METABOLIC SYNDROME IN SUBCUTANEOUS ADIPOSE TISSUE IN PATIENTS WITH DIABETES 2 TYPE AND HEALTHY PERSONS

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Fatty acid-binding proteins (FABPs) belong to the multigene family of the intracellular lipid-binding proteins. Various functions have been proposed for these proteins, including the promotion of cellular uptake and transport of fatty acids, the targeting of fatty acids to specific metabolic pathways, and the participation in the regulation of gene expression and cell growth. The aim of this study was: 1) to assess and compare the concentration of FABPs and other markers in serum and in fat of two investigated groups. 2) to determine correlations between FABPs and other markers in serum and in fat in each group. This study included two groups: 11 non-diabetic persons (NDP) and 22 patients with diabetes 2 type (PWD), diabetes duration from 1 to 28 years. PWD were treated mostly with insulin and antihypertensive drugs and/or diuretics etc. Markers in serum and in fat homogenates were measured using ELISA method. Fat was obtained by means of the Bard Magnum System and stored at -80 °C. In serum of NDP, significant correlations were found among FABPs, resistin and leptin. In serum of PWD, significant correlations were found only between FABPs and leptin. In fat of NDP significant correlation was found among FABPs and adiponectin. However in fat PWD significant correlations were found among FABPs, adiponectin and leptin.

#### CHANGES OF EXTRACELLULAR UNIT ACTIVITY DURING DEVELOPMENT OF PHOTOTHROMBOTIC LEASION IN RAT SENSORIMOTOR CORTEX

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The aim of this study was to continue examining acute changes during development of photothrombotic ischemic lesion. In our previous study we described acute changes in somatosensory evoked potentials (SEPs). Now we used this model for examining extracellular unit activity. Methods: The experiment was performed in 30 adult male albino rats (30 cells) of the Wistar strain, which were housed under standard conditions. The animals were divided into three groups: Control (C, n=8), ipsi-lateral (IL = unit activity recording and laser beam on the same side, n=11) and contra-lateral (CL, n=11). Surgical openings of the skull and placing surface ECoG electrode, stimulatory electrodes and glass microelectrode filled with NaCl (2 mol) for unit recording in the sensory-motor cortex was done under urethan anesthesia (20 % solution). Almost immediately after i.v. (tail vein) application of 20 % rose Bengal solution (1 ml/1 kg) (control group get saline) the brain was exposed to laser beam (532 nm, 50 mW/mm<sup>2</sup>, area <1 mm<sup>2</sup>) for 9 min. Unit activity (and ECoG) was recorded before and during rose Bengal application and during and after laser irradiation. 16 min after the end of irradiation the contralateral cortex was 3 times electrically stimulated (bursts of monopolar pulses 20 s, 8 Hz, 10 mA). Evaluation: Stability of the unit frequency, average frequency of the discharges and sequence Poincaré plot of the discharge intervals were evaluated. The response to stimulation and ECoG were also evaluated. Results: During the early development of ischemic lesion we observed substantial changes of the unit activity in cortex of both hemispheres.

Ipsi-laterally were the changes in the unit activity during laser beam exposure and after it much more marked than contra-laterally. The stimulation 16 min after laser exposure depressed unit activity on the contralateral side.

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#### EFFECT OF CHRONIC STRESS ON VASCULAR RESPONSES IN RATS WITH BORDERLINE AND SPONTANEOUS HYPERTENSION

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Chronic stress is a risk factor in the etiology of civilization diseases including hypertension. The aim of this study was to investigate the effect of chronic social stress produced by crowding on blood pressure and vascular function in normotensive Wistar, borderline hypertensive (BHR, offspring of SHR dams and Wistar sires) and spontaneously hypertensive (SHR) rats. Male rats (12 weeks old) of all phenotypes were exposed to crowding for eight weeks (200 cm<sup>2</sup> per rat, 5 rats per cage). Control rats were kept 4 rats per cage (480 cm<sup>2</sup> per rat). Blood pressure (BP) of Wistar, BHR and SHR before experiment (determined by tail-cuff method) was 111±3, 133±2 and 184±2 mm Hg, respectively (p<0.001 between phenotypes). After 8-week crowding, the elevation of BP was observed only in BHR and SHR (p<0.01 vs. control). Stress increased the left ventricle-to-body weight ratio and the adrenal gland-to-body weight ratio only in BHR (p<0.02 vs. control). Significantly reduced NO synthase activity was observed in the aorta and the left ventricle of crowded SHR but partial reduction of NO production was found also in BHR. Stress had no effect on NO synthase activity in the aorta and left ventricle in Wistar rats. Basal noradrenaline (NA)-induced constriction was lower in BHR vs. Wistar and SHR (p<0.02) and stress increased NA-induced constriction in all phenotypes (p<0.05). Acetylcholine (ACh)-induced relaxation of control Wistar was lower than that of BHR and SHR. Crowding partially improved acetylcholine (ACh)-induced relaxation in Wistar, had no effect in BHR, and reduced ACh-induced relaxation in SHR. Acute administration of the low dose of NO synthase inhibitor L-NAME (10<sup>-6</sup> mol/l) into incubation bath reduced ACh-induced relaxation in stress exposed W and both control and stressed SHR but had no effect in BHR. In conclusion, while the effect of crowding was associated with the elevation of vasoconstriction in all phenotypes investigated, its effect on endothelium-dependent relaxation was different in Wistar, BHR and SHR. Results suggest that endothelium-dependent relaxation of BHR rats was more resistant to acute NO deficiency than that of their Wistar and SHR progenitors.

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#### MONITORING OF THE PHYSIOLOGICAL STATUS VARIABILITY IN MEDICAL STUDENTS DURING STUDY

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Cardiovascular and tumorous diseases belong to the most frequent causes of death in developed countries of the world. It is alarming that these diseases move to the younger age groups. Some risk factors such as food composition, smoking, stress, insufficient movement, obesity, excessive alcohol consumption can be affectable and after long-term action they lead to serious health consequences. Nutrition plays an important role in the development of the human society and for

sustaining of good health status. At the judgement of nutrition we observe changes in the values of nutritional markers that reflect the status of cardiovascular and renal functions with application in the primary as well as secondary prevention. 33 men and 52 women, students of the medicine at the age of 18–23 years formed a set. They were examined in the 1st and 6th year of their study. We observed their anthropometric parameters, body weight and height from which the body mass index – BMI ( $\text{kg/m}^2$ ) was calculated. Of the biochemical and immunological parameters the serum concentration of transferrin (Trf), prealbumin (Prea), ceruloplasmin (Cpl) and orosomucoid (ORM) were determined by commercial sets of Fy Sevapharma, Czech republic. Concentrations of vitamin C were determined using the spectrophotometric method of Roe and Kuether. No statistically significant changes were recorded in the mean values of BMI in the 1st and 6th year of study in the group of women, but in the group of men in the 6th year of study the mean value of BMI significantly increased in comparison with the 1st year of study ( $p < 0.05$ ) as well as at comparison of men and women in the last year of study ( $p < 0.001$ ). The mean values of the immunological parameters observed are presented in the table. Pronounced difference of the mean values were recorded in vitamin C. A statistical increase in the values of vitamin C occurred in men as well as women in the last year of study and between groups compared with the 1st year of study ( $p < 0.01$ ).

Parameters	MALE		FEMALE	
	1st year of study	6th	1st	6th
Trf (g/L)	3.29±1.18	3.47±1.16	3.54±0.96	3.62±1.16
Prea (g/L)	0.34±0.09	0.37±0.05	0.32±0.08	0.33±0.05
Cpl (g/L)	0.33±0.08	0.39±0.09**	0.36±0.06	0.35±0.09
ORM (g/L)	0.87±0.20	0.77±0.33	0.84±0.22	0.68±0.23***

\*\* $p < 0.01$ , \*\*\* $p < 0.001$

At monitoring of the nutrition state it is important to observe not only the biochemical and immunological parameters, but also to correlate them with the anthropometric values. Regarding the quality of nutrition and eating habits BMI is of great importance (1). Results of our dynamic study aimed at the observation of the nutritional status of the student of medicine, whose observed parameter values ranged within the physiological limit, testify to their positive approach to their health.

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## USE OF CAPILLAROSCOPY TO STUDY OF HUMAN PERIPHERAL MICROCIRCULATION

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**Introduction:** Capillary microscopy (capillaroscopy) is a noninvasive method for investigation of the human microcirculation (1,2,3). Evaluation of skin capillaries is generally performed at the nailfold because that area is easily accessible for examination and here the major axis of the capillaries is parallel to the skin surface, while in other areas it appears to be perpendicular. **AIM:** To introduce the method in our laboratory and to measure red blood cell velocity (CBV) in capillary limbs and to obtain morphometric parameters of nailfold capillaries using capillaroscopy in healthy individuals. **Methods:** Our system consisted of an optical microscope with CCD camera connected to PC allowing online video recording and offline processing of the examined capillaries. In vivo nailfold capillary microscopy was performed on 15 apparently healthy subjects, 6 males and 9 females, age 23 to 32 years (mean 26). Subjects were examined in sitting posture at room temperature of 21–25 °C for 30 min. The fourth finger was fastened by

plastic holder with temperature 37 °C during the whole measurement. To increase transparency, a drop of immersion oil was placed on the nailfold before examining the capillaries in the nail bed. Then CBV in arterial, venous and transition limb and morphometric parameters such as length and diameter of capillary limbs were calculated using frame-to-frame analysis. Data concerning CBV of subjects are the mean of three measurements. Results: CBV in arterial limb (789  $\mu\text{m/s}$ , range 227–2096  $\mu\text{m/s}$ ) was nonsignificantly higher compared to CBV in venous limb (567  $\mu\text{m/s}$ , range 154–1907  $\mu\text{m/s}$ ). Significant difference was shown between CBV in arterial and transition limb (789/298  $\mu\text{m/s}$ ). Analysis of morphometric parameters such as length and diameter of capillary limbs showed similar values in both arterial and venous limb of the capillaries. **Conclusion:** We demonstrated the use of capillaroscopy for investigation of cutaneous microcirculation, and our data are comparable with those already published.

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## ANOXIA IN SOME LOWER VERTEBRATES.

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Understanding of long time resistance of some lower vertebrates originated in Czech (Czechoslovak) environment, state of the art may be summarized:

I. Energetic metabolism. In two models (Europe – fish g. Carassius, US and Canada - turtles g. Chrysemys) it was confirmed, that they survive complete anoxia for several months below 5 °C. In both groups energetic metabolism (measured as production of carbon dioxide, rate of ATP synthesis) is drastically reduced and at the same time energy consumption required for maintenance of concentration gradients on biological membranes and for the synthesis of proteins drops down and concentration of ATP remains on the normoxic level 1). Fish and turtles differ in anaerobic end-products: in Crucian carp ethanol was found, its stoichiometry to  $\text{CO}_2$  excreted is however not clear, while turtles form lactate which is deposited in the carapax. Fish maintain decreased but continuing reactivity, while turtles become turbid in anoxia immediately 2). II. Functioning of brain and circulatory system is a second condition of survival. Their importance is stressed very enthusiastically, but the results are far from being complete. III. Recently we got relatively extensive data on excretion of ammonia by Crucian carp; in contrary to previously published data we have found a highly significant drop of the rate in anoxia or deep hypoxia but not a complete stop. Nitrogen excretion in deep hypoxia and anoxia corresponds to a very minor fraction of  $\text{CO}_2$  excretion. Fatty acids are from the very beginning considered as a plausible end-product of long term anaerobic metabolism. Incorporation of labelled acetyl-coenzyme A was found in eel anaerobic mitochondria, same for Crucian carp mitochondria in our lab; moreover the process shows a seasonal relationship. Survival of various species of the genus Carassius in anoxia was repeatedly confirmed, but there is no systematic study. The early observation was done in the stunted form of Crucian carp (C. carassius), much work was done also in gold-fish (C. auratus), which is readily available in aquarium pet shops, but its survival in anoxia is about 1/10 of the Crucian carp. Recently we got data which show survival of the triploid gynogenetic form of C. auratus (C.a. gibelio – the Prussian or German carp) comparable to Crucian carp and its seasonal relationship.

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## BENEFIT AND RISK OF GOECKERMAN'S THERAPY OF PSORIASIS.

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**Objective.** Goeckerman's therapy of psoriasis (GT) includes combine dermal exposure to coal tar and UV radiation (UV-A, UV-B). Coal tar and UV-B radiation represent mutagenic and carcinogenic agents. The study of GT was aimed at differences of benefit and risk between adults and children. **Methods.** Observed group consisted of 49 patients (23 adults and 26 children) with psoriasis, undergoing GT. The benefit of GT was evaluated by PASI score (Psoriasis Area and Severity Index). The risk was evaluated by chromosomal aberrations in peripheral lymphocytes. The aberrations were determined before GT and after last application. **Results.** GT significantly decreased the PASI score ( $p < 0.001$ ) in both, adults and children. Chromosomal aberrations in both groups were significantly elevated after the therapy ( $p < 0.001$ ). **Conclusions.** Decreased level of PASI score confirmed high efficiency of GR for adults and children. However, significantly elevated chromosomal aberrations indicated the presence of genotoxic risk (1, 2). We did not find any significant differences between adults and children.

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## MOTOR ABILITIES OF NEURODEFECTIVE LURCHER MUTANT MICE OF THE STRAINS C3H AND C57BL/7 AFTER THE CEREBELLAR TISSUE TRANSPLANTATION

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Lurcher mutant mice are a natural model of hereditary olivocerebellar degeneration (1). These mice lose completely Purkinje cells and most of the cerebellar granule cells and inferior olive neurons postnatally (2). They suffer from cerebellar ataxia. Lurcher mutant mice are used for investigation of functional and morphological consequences of the neurodegeneration and of its therapeutical influencing. The aim of the work was to assess the effect of embryonic cerebellar tissue transplantation on motor coordination in adult Lurcher mutant mice derived from two strains – C3H and C57BL/7. As donors 12-13 days old mice embryos producing green fluorescent protein (GFP) were used. Embryonic cerebellar tissue was applied as a solid graft with a microcapillary into the cerebellum of adult Lurcher mutant mice under general anaesthesia. Motor coordination was tested with a set of three methods (horizontal bar, ladder and rotarod). All tests were repeated four times in one session. The tests were performed before the surgery and then once a week in the 2-7th week after the surgery. Results of the experimental mice were compared with sham-operated control animals. Finally, cerebella of experimental mice were examined histologically. The graft was detected according to its GFP fluorescence. Graft survival was higher in C57BL/7 mice. In mice of the C3H strain slight positive effect of the transplantation on motor skills was observed. Mice of this

strain improved their results during the course of the experiment. In C57BL/7 mice no differences between animals after the transplantation and controls were found and these mice did not show significant amelioration of motor coordination ability during 2-7th week after the surgery. Transplantation of the cerebellar tissue had slight functional effect only in Lurcher mutant mice of the C3H strain. On the other hand, in mice of the C57BL/7 strain the graft survival was more frequent.

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## MAGNETIC FIELD OF POWER FREQUENCY – THE INFLUENCE OF LOW LEVEL INDUCTIONS ON CELL-MEDIATED IMMUNITY IN PATIENTS WITH HEAD AND NECK CARCINOMA

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We were comparing the adherence ability of T lymphocytes gained from blood of patients with head and neck carcinoma before launching the treatment before and after their exposure to magnetic field of power frequency of 50 Hz with induction of 10 mT, 1 mT, 0.5 mT and 0.1 mT. The gained results have proved that magnetic field has a statistically significantly increases the ability of adherence in unhealthy T lymphocytes in all applied inductions, including a very low-level induction of 0.1 mT. In case of 0.5 mT induction, namely statistical level of importance worth 0.001 is in question. There is no statistically significant difference among the average values measured in all groups without the influence of magnetic field. For values received by measuring in magnetic field a statistically significant difference has been proved among the NAI values in inductions of 0.1 mT and 10 mT (level of importance of 0.05), and inductions of 0.5 mT and 10 mT (level of importance of 0.001). If we consider the lymphocytes adherence ability a sign of cell-mediated immunity, it is clear that magnetic field of power frequency has an influence on cell-mediated immunity in patients with head and neck carcinoma, namely statistically significantly in all tested inductions (including a minimum induction of 0.1 mT).

## PHYSIOLOGICAL EFFECTS OF CONTINUAL POSITIVE AIRWAY PRESSURE THERAPY IN PATIENTS WITH SLEEP APNEA

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**Introduction:** Continuous positive airway pressure (CPAP) is the primary therapy administered for patients with obstructive sleep apnea/hypopnoea syndrome (OSAHS), which is frequently associated with cardiovascular, neuro-behavioral, endocrine, metabolic, and other disorders. **Methods:** 67 patients with severe OSAHS were recruited for CPAP treatment from more than 600 subjects investigated in our Sleep laboratory. Ambulatory blood pressure measurement (ABPM) was performed parallelly with whole-night polysomnographic recording

(PSG) in 7 severe OSAHS patients (average age  $48.1 \pm 4.8$  years, average BMI  $36.3 \pm 8.2$ , average RDI  $12.2 \pm 8.8$ ) treated by CPAP. In 2 patients parallel continuous glucose monitoring (CGMS) was performed. Clinical and physiological data were evaluated in all patients, compared to 7 healthy control subjects (average age  $56.9 \pm 20.4$  years, average BMI  $26.6 \pm 3.1$ , average RDI  $2.5 \pm 1.4$ ). Statistical analyses included paired Student's t-test. Results: Application of CPAP practically eliminated apnoeic episodes, normalized oxygen saturation, and decreased the average diastolic nocturnal blood pressure (BP) significantly ( $78.3 \pm 4$  mm Hg), not different from control values ( $p=0.13$ ;  $n=7$ ), but without changes in average systolic BP values. The positive effect on diastolic BP persisted only during CPAP use, which also eliminated nocturnal cardiac dysrhythmias in several patients (frequent bigemina, atrial fibrillation). Changes in glycemic excursions before and after CPAP treatment determined by CGMS will be demonstrated. The case study of a 38 year old patient will illustrate the contribution of CPAP therapy on body weight reduction, in addition to improvement of quality of life observed in all 67 CPAP treated patients by increasing the motivation to more compliant therapy (diet, physical activity, changes in life-style, etc.). Conclusions: CPAP therapy showed important positive physiological effects in cardiovascular, neuro-behavioral, endocrine, and metabolic areas in OSAHS patients.

#### EFFECT OF LIGHT-DARK CYCLE ON THE DEVELOPMENT OF CIRCADIAN RHYTHMICITY IN GENE EXPRESSION WITHIN THE RAT SUPRACHIASMATIC NUCLEUS.

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The mammalian circadian clock within the suprachiasmatic nucleus (SCN) develops before birth and is synchronized by maternal signals. The early postnatal entrainment has also been attributed mostly to maternal cues. However, the rat SCN is sensitive to light immediately after birth. The aim of the present study was to elucidate whether the presence of external light-dark cycle (LD) affects the development of the circadian rhythms in expression of clock genes, namely of *Per1*, *Per2*, *Cry1* and *Bmal1*, and of *c-fos*, a marker of neuronal activity, within the rat SCN during the fetal and early postnatal stages. Pregnant Wistar rats or mothers with their pups were maintained under LD conditions with 12h of light and 12h of darkness. On gestational day 20 and on day of birth, the morning light was not turned on and 20-day-old fetuses (E20) and 3-day-old pups (P3), respectively, were reared in complete darkness (DD) or left under LD conditions. Their brains were sampled in 2h intervals throughout the whole circadian cycle and processed for in situ hybridization to detect levels of *Per1*, *Per2*, *Cry1*, *Bmal1* and *c-fos* mRNAs within the SCN. On E20, no significant difference between the profiles of *Per1*, *Per2*, and *c-fos* mRNAs were detected between the LD and DD conditions. In pups at P3, a significant rhythm in *Per1*, *Per2* and *Cry1*, but not in *Bmal1*, expression was detected under DD. Time of the rise and the decline of *Per1* and *Per2* mRNA levels corresponded roughly to that in pups maintained in LD. The results demonstrate that during early postnatal period, the circadian rhythms in clock gene expression develop endogenously without contribution of external LD conditions and the phase of the SCN rhythmicity is set mostly by maternal cues.

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#### ROLE OF 11 $\beta$ -HYDROXYSTEROID DEHYDROGENASE DURING INFLAMMATION

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Glucocorticoids are known to modulate a number of immunological processes including inflammation. They influence development and effector functions of immune system, trafficking of immune cells, their maturation, differentiation and activation. Intracellularly, the glucocorticoids mediate their action by binding the glucocorticoid receptors. Traditionally glucocorticoids have been known to induce immunosuppression. Recently, however, it was shown that glucocorticoids influence also the shift from Th1 to Th2 pattern of immunity. The biological activity of glucocorticoids depends not only on their plasma concentration, the number of receptors, and the responsiveness of the target cells, but also on local metabolism of glucocorticoids that is predominated by 11 $\beta$ -hydroxysteroid dehydrogenase (11HSD). The isoform 11HSD1 operates in as a NADPH-dependent reductase that locally increases glucocorticoid concentration (cortisol, corticosterone) by reduction of their 11-oxo derivatives (cortisone, 11-dehydrocorticosterone). The isoform 11HSD2 is a sole NAD<sup>+</sup>-dependent dehydrogenase that inactivates biologically active glucocorticoids to their 11-oxo derivatives. As endogenous glucocorticoids might play an essential physiological role in regulation of the immune system, we have investigated the glucocorticoid metabolism in immune cells and in inflamed tissues of rats and mice with colitis and arthritis. To determine metabolism of corticosterone we studied mRNA levels of both isoforms of 11HSD in Wistar and Lewis rats using RT-real time PCR. These experiments indicated that T cells and macrophages express 11HSD1 but not 11HSD2, but B cells are without any 11HSD. Similarly, we have found 11HSD1 transcript in lymphatic nodes and inflammation up-regulated the level of transcript. Experimentally induced colitis stimulated the expression of 11HSD1 in mesenteric lymphatic nodes and experimental arthritis the expression in inguinal and caudal nodes and peritoneal macrophages. Administration of lipopolysaccharides (LPS) to animals increased 11HSD1 mRNA in peripheral leucocytes and the incubation of isolated lymphocytes in vitro with serum isolated from these animals had similar effect on 11HSD1. We conclude that the cells of lymphoid tissue express only 11HSD1 and that this expression is up-regulated during activation of immune system. These data suggest that inflammation increases local concentration of glucocorticoids that might counterbalance the inflammatory response via paracrine and/or intracrine effect.

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#### BRAIN REGION SPECIFIC CHANGES IN $\alpha_1$ -ADRENERGIC RECEPTOR LEVEL IN ACETYLCHOLINESTERASE KNOCKOUT MICE

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The acetylcholinesterase knockout (AChE <sup>-/-</sup>) mouse has no AChE activity and no AChE protein which leads to the increase in the acetylcholine level (1). Hyperstimulation of muscarinic receptors (MR) by this neurotransmitter results in their down-regulation and regulation of other G-protein coupled receptors (2). The aim of this work was to examine the  $\alpha_1$ -adrenoceptor ( $\alpha_1$ -AR) distribution within the central nervous system (CNS) of these animals. Adult (over 60 days old) wild-type and AChE <sup>-/-</sup> mice were used in the study (3). Animals were euthanized, brains removed and different functional regions were dissected (medulla oblongata, cerebellum, frontal, parietal and occipital cortex, striatum, thalamus and hypothalamus).  $\alpha_1$ -AR were quantified by radioligand binding study, using a specific ligand [<sup>3</sup>H] prazosin. There was a substantial decrease in  $\alpha_1$ -AR observed in AChE <sup>-/-</sup> occipital and parietal cortex comparing to wild-type animals. However

the level of  $\alpha_1$ -AR in AChE  $-/-$  thalamus was about fourfold higher than in wild-type counterparts. Other studied brain regions did not display any differences between two genotypes. It is supposed that AChE  $-/-$  mice are able to live due to multiple adaptation changes in their phenotype. Cholinergic adaptations were described (4, 5) in the past. Unfortunately only little attention has been paid to the non-cholinergic changes in AChE  $-/-$  CNS. We show in this work that  $\alpha$ -adrenoceptors are significantly changed in the CNS of AChE  $-/-$  mice while these changes are brain-region specific.

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#### RELATIONSHIP BETWEEN FLUIDITY TRANSMEMBRANE POTENTIAL AND FUNCTIONAL CHARACTERISTICS OF MITOCHONDRIA IN HEARTS OF ACUTE DIABETIC RATS

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**Introduction:** Remodeling of subcellular membrane systems also takes part in endogenous protective mechanisms (EPM) acting in the diabetic (DIA) myocardium. Present study is devoted to elucidation of the role of changes in mitochondrial (MIT) membrane fluidity (MF) and transmembrane potential (MP) in remodeling of the MIT, associated with EPM and leading to adaptation of the heart to DIA. **Experimental:** DIA was induced by a single dose streptozotocin (STZ, 55 mg/kg i.p.) to male wistar rats (220±20). Hearts were investigated on 8th day after STZ administration when the animals exhibited 300-330 % increase in blood glucose and triacylglycerole. MF of isolated MIT (with protease) was assessed by measuring fluorescence anisotropy of DPH (1,6-diphenyl-1,3,5-hexatriene). MP of the MIT was monitored by confocal microscopy using JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) as a fluorescent indicator. Mg-dependent and DNP (2,4-dinitrophenol)-stimulated ATPase (the total MIT ATPase) was assessed by estimation of P<sub>i</sub> liberated from ATP splitting. Content of conjugated dienes (CDI) in MIT membrane lipids was estimated spectrophotometrically at 230 nm. **Results and Discussion:** Increased formation of radicals in DIA heart MIT, manifested by a ~17 % (p<0.05) increase in oxidized Q<sub>10</sub> and was also coupled with lowered oxygen consumption, RCI and the rate of phosphorylation (all p<0.01) and MP, but an effective decrease in the ADP/O ratio and elevation in CDI content remained absent (p>0.05). Oppositely, MF, the total MIT ATPase and the formation of membrane transition pores increased (all p<0.05). The amount of created radicals was neither capable to induce a considerable oxidation, nor a decrease in MF. Original finding is, that in 46 % of variability of cases, with 5 % confidence interval, regression analysis revealed a significant association between the increase of MF and decrease in MP. **Conclusion:** Remodeling of functions and physical properties of the MIT membranes is involved in EPM that yield in adaptation of the myocardium to DIA.

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#### ARTIFICIAL SCAFFOLDS IN CARTILAGE REGENERATION

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Cartilage has a poor reparative capacity. It can be stimulated by autologous chondrocytes implantation, especially if the chondrocytes are embedded in a proper biodegradable scaffold. The proper scaffold promotes chondrocyte proliferation and extracellular matrix synthesis, enables their nutrition, and provides them with an appropriate mechanical stability. Polyglycolic acid (PGA) is a biodegradable polymer, which is intensively studied as a potential material for tissue engineering, especially as a co-polymer with other substances, such as polylactic acid or chitosan (1). We have prepared several types of artificial scaffolds seeded with chondrocytes. The isotropic scaffold from collagen/ hyaluronate/fibrin allowed homogenous cell distribution, promoted re-differentiation of chondrocytes and collagen type II synthesis, and was able to repair osteochondral defect in a six-week study in rabbits. Subsequently, the scaffolds with improved biomechanical properties were prepared. The woven scaffolds from polyglycolic acid (PGA) were made by knitting, and non/woven scaffolds from PGA and polyvinylalcohol (PGA/PVA) were prepared by a wet-laid method. Supplementation with nanofibres was also employed. Chondrocytes were seeded onto the scaffolds at density of 80 x 10<sup>3</sup> cells/cm<sup>2</sup>. Proliferation and viability of chondrocytes were testing using MTT test, fluorescence microscopy, and confocal microscopy. Immunohistochemical staining for collagen type II was used for evaluation of the chondrocyte differentiation. Both woven and non/woven scaffolds allowed good chondrocyte adhesion and proliferation, although only a two-dimensional net of chondrocytes was observed in the woven scaffolds. The three-dimensional cell distribution was observed in the non-woven scaffolds. According to the previous observation, the acidification of the medium of the PGA scaffolds was observed.

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#### GROWTH OF VASCULAR ENDOTHELIAL CELLS ON FIBRIN ASSEMBLIES

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Fibrin assemblies are used to seal woven vascular prostheses in order to prevent bleeding. Fibrin can also improve adhesion and growth of endothelial cells (EC) on vascular prostheses (1). In this study, we tested the cultivation of vascular endothelial cells on polystyrene surface coated with two-dimensional fibrin structures (2DFb) and three-dimensional fibrin networks (3DFb). The 2DFb were prepared by repeating three-times a procedure consisted in the successive adsorption of fibrinogen (Fbg), treatment with thrombin (T), and inhibition of thrombin with D-phenylalanyl-L-prolyl-L-arginyl chloromethyl ketone (PPACK) and hirudin. Different 2DFb were obtained using different concentrations of Fbg in solutions. The 3DFb of various thicknesses were prepared from fibrinogen and thrombin solution and from the solution inhibited with antithrombin III (AT) and heparin (H). The morphology of 2DFb and 3DFb was investigated using transmission

electron microscopy. The culture medium was supplemented with aprotinin, an inhibitor of fibrinolysis. Pure polystyrene without plasma treatment, i.e. the carrier of the assemblies, was used as a control sample. In comparison with this sample, the number of EC on day 1 after seeding was significantly higher only on 3DFb. However, the cells proliferated more quickly on 2DFb. Thus, their population densities were similar or even higher than on 3DFb assemblies on the day 7 after seeding. Immunocytochemical staining of  $\alpha$ -v integrin, which is known to mediate EC-fibrinogen interaction (2), and vinculin showed well developed focal adhesion plaques on EC growing on all fibrin assemblies. Most of EC were positively stained for von Willebrand factor, an important marker of EC differentiation. It can be concluded that the cell behaviour can be regulated by various configurations of fibrin structures attached to a solid surface. The modification of artificial vascular prostheses with these fibrin structures, itself or followed with pre-cultivation of endothelial cells on the surface, could be potentially applied for the treatment of vascular prostheses before their introduction into the patient's organism.

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#### LACIDIPINE INHIBITS THE ATHEROGENESIS IN APOE-DEFICIENT MICE.

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**Background:** Calcium channel blockers slow the progression of atherosclerosis. The purpose of present experiments was to examine the action of lacidipine in a condition that accelerates the development of atherosclerosis in order to test the hypothesis that the protective action of lacidipine in atherosclerosis is unrelated to the reduction of blood pressure. **Materials:** Male ApoE-deficient mice (6 week old) were exposed either to normal chow (ND) or to a Western-type diet (WD, adjusted calorie diet containing 42 % from fat) for 8 weeks, and they were treated with lacidipine 1 and 3 mg/kg/day. **Methods:** After exposure to treatment animals were anesthetized with thiopental and both kidneys were excised. Kidneys were examined to renal morphology, glomerular abnormalities, and interlobular arteries using standard histological methods. **Results:** In mice exposed to WD lacidipine reduced extension of atherosclerotic lesions, renal injury and increase in blood pressure. Kidneys histology of WD ApoE-deficient mice showed severe alterations characterized by accumulation of extracellular matrix proteins in glomeruli and structural changes in intrarenal arteries, mainly a reduction of the lumen of intrarenal vessels. **Conclusion:** Experimental data indicate that inhibition of Western-type diet evoked alterations is related to both antioxidant and vasoactive properties of lacidipine.

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#### HUMAN OSTEOBLAST-LIKE MG 63 CELLS ON CARBON - AND SILICON-CONTAINING MATERIALS FOR BONE TISSUE ENGINEERING

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Artificial materials are of growing importance in medicine and various biotechnologies. Carbon- and silicon-based materials are promising especially for the orthopaedic and dental surgery. Therefore, the adhesion and proliferation of human osteoblast-like MG 63 cells in cultures on carbon-polymer composites and bioglass fibres containing SiO<sub>2</sub> were studied. The polymeric part of the composites was represented by a terpolymer containing polypropylene, polytetrafluorethylene and polyvinylidene fluoride (CP0). By addition of carbon component, six groups of the following composites were constructed: terpolymer reinforced with carbon fibres (CP4) or carbon fabric (CP5), terpolymer reinforced with carbon fibres and with pores created by addition and following dissolution of alginate fibres (CP6) or powder (CP7), terpolymer reinforced with carbon fibres and containing the alginate powder (CP8) or fibres (CP9). Bioglass fibres were used in four types different in their thickness (diameter of 13 or 26  $\mu$ m) and amount of SiO<sub>2</sub> (20 or 30 %). On day 1, 3 and 7 after seeding, the cells were either fixed with ethanol and stained with propidium iodide or trypsinized and counted in the Bürker haemocytometer. On day 7 after seeding, the number of cells on carbon-polymer composites increased in the following order: CP0<PS<CP6<CP8<CP7<CP5<CP4<CP9 (17 766 cells/cm<sup>2</sup> on CP0 and 67 002 cells/cm<sup>2</sup> on CP9). On the same day, the density of MG 63 cells on the bioglass fibres ranged only from 20 150 to 23 970 cells/cm<sup>2</sup> (no significant differences among the four groups were found). However, the cells were viable and capable of proliferation. Their spindle shape with the long axis oriented in parallel with the fibres suggested that their lower number was due to the relatively small diameter and surface curvature of the fibres (i.e., a topography less appropriate for cell spreading) rather than to a cytotoxic action of the material. Thus, the carbon-polymer composites, including those enriched with alginate, could be used for bone implantation, and SiO<sub>2</sub>-containing bioglass fibres could serve for reinforcement of newly constructed materials for bone tissue engineering.

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#### AMBULATORY BLOOD PRESSURE MONITORING IN HYPERTENSIVE PATIENTS WITH SLEEP DISORDERED BREATHING

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**Introduction:** Obstructive sleep apnea/hypopnoea syndrome (OSAHS) is often associated with important changes in blood pressure (BP), ranging from acute increases in BP at the time of the apnoeic episodes to sustained nocturnal and/or daytime hypertension. A 24-h ambulatory blood pressure monitoring (ABPM) has made it easy to investigate the predictive role of multiple BP measurements outside of the clinical setting. **Methods:** Thirty adults were studied by overnight polysomnography using Alice 3 in sleep laboratory and with non-invasive parallel discontinuous measurement of BP by Cardiotens 1.34. The subjects were divided into 4 groups according to apnoea/hypopnoea index (AHI) and Min Sa O<sub>2</sub> % and nocturnal systolic and diastolic blood pressures (SBPn, DBPn). **Results:** 1) patients with hypertension (HT n=7), Min Sa O<sub>2</sub> 86 $\pm$ 4 %, SBPn 135 $\pm$ 9 mm Hg, DBPn 73 $\pm$ 6 mm Hg and without OSAHS, apnoea/hypopnoea index = AHI < 4/h, SBP was increased significantly during the night (P<0.01) compared to the controls, 2) In 9 patients (with HT, Min Sa O<sub>2</sub> 74 $\pm$ 14 %, SBPn 128 $\pm$ 15 mm Hg, DBPn 72 $\pm$ 9 mm Hg, and with moderate OSAHS AHI 5-40/h), SBP was increased significantly during the night (P<0.05) compared to



the controls, 3) In 7 patients (with HT, Min Sa O<sub>2</sub> 49±10 %, SBPn 134±13 mm Hg, DBPn 79±8 mm Hg, and severe OSAHS, AHI > 40), both systolic and diastolic BP were increased significantly during the night ( $P<0.01$ ) compared to the controls, 4) In control group = 7 subjects (without both HT and OSAHS, Min Sa O<sub>2</sub> 87±2 %, SBPn 113±8 mm Hg, DBPn 66±7 mm Hg with AHI < 4/h). Min SaO<sub>2</sub> negatively correlated were in with DBP during the night. Correlation results between saturation and blood pressure parameters suggest that the decrease of blood saturation with oxygen increases initially the diastolic blood pressure. Conclusions: The results indicate that 24 hour monitoring of BP has important implications mainly for screening of non-diagnosed HT, and for differential diagnostic and monitoring of therapy in patients with OSAHS and HT.

#### PPAR- $\alpha$ AND INSULIN SENSITIVITY: THE ROLE OF ENDOCRINE FUNCTION OF ADIPOSE TISSUE

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We studied the significance of adipose tissue-derived hormones resistin and adiponectin in the development of insulin resistance induced by lipogenic, simple carbohydrate diet and in the insulin-sensitizing effects of PPAR- $\alpha$  activation. Male C57BL/6J mice were fed normal chow or lipogenic, simple carbohydrate diet (AIN-93G, Dyets Inc., USA) for 12 weeks. Mice on both diets were then treated with PPAR- $\alpha$  agonist fenofibrate (100 mg/kg) administered in the food for 14 days. Untreated mice on both diets served as controls. Biochemical and hormonal parameters were measured using commercial RIA and ELISA kits and insulin sensitivity was assessed by euglycaemic-hyperinsulinemic clamp using [3- H]glucose and 2-deoxy-D-[1-<sup>14</sup>C] as tracers. Lipogenic diet (LD) significantly increased body weight, gonadal fat pad weight and insulin levels relative to chow-fed group. Fenofibrate treatment decreased body weight and fat pad weight in both chow and LD-fed mice with concomitant reduction in blood glucose, free fatty acid, triglyceride and serum insulin levels. Euglycaemic-hyperinsulinemic clamp demonstrated the development of whole body and liver insulin resistance in LD-fed mice which was both normalized by fenofibrate treatment. Resistin levels tended to be lower in LD-fed mice relative to chow-fed group (12.6±0.7 ng/ml vs. 20.3±2.9 ng/ml,  $p=0.07$ ) and were two-fold increased by fenofibrate treatment on both LD and chow-fed mice (24.9±4.2 ng/ml and 36.3±2.6 ng/ml, respectively). Adiponectin levels were not affected by LD feeding and increased after fenofibrate treatment in chow-fed but not LD-fed mice. In conclusion, changes in adiponectin and resistin levels were not involved in LD feeding-induced insulin resistance. Fenofibrate treatment prevented development of obesity and improved insulin sensitivity in LD-fed mice despite marked increase in serum resistin levels suggesting that resistin may not be the major factor in the development of liver insulin resistance in mice.

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#### CLOCK GENES EXPRESSION IN BRAIN STRUCTURES INVOLVED IN BLOOD PRESSURE REGULATION IN HYPERTENSIVE TGR[mREN27]27 RATS

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gene (mREN2) and represent a model of hypertension characterized by an inverted circadian pattern of blood pressure. As a result of changed function of the rennin-angiotensin system rhythmic 24hr blood pressure (BP) profile in TGR has an elevated mesor value, higher amplitude and opposite phase in comparison with Sprague-Dawley control rats. To study physiological bases of the changed circadian profile of BP we measured expression of clock gene *per2*, *clock*, *bmal1* and clock controlled gene *dbp* in the nucleus suprachiasmatic (SCN), nucleus tractus solitarius (NTS) and C1 neurons of rostral ventrolateral medulla (RVLM) in TGR and control rats. Rats were entrained to LD 12:12 and samples were taken at age 10 weeks in 4hr intervals over 24h period. Brain nuclei were dissected by punching technique and expression of clock genes was measured by real time PCR. In SCN we observed the typical pattern of *per2* and *dbp* expression in both groups and increased expression of *clock*. In contrast with SCN, expression of *per2* in NTS and RVLM was higher during the dark-time in both groups and the rhythm was delayed in NTS of TGR rats in comparison with control. Role of NTS, RVLM and possibly other brain structures is suggested in mediating or gating information about internal circadian time from SCN to periphery.

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#### OXIDATIVE STRESS AND HEAVY SMOKERS

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Cigarette smoking is one of the major public health problem of our times with a negative impact upon the progression of various diseases. Cigarette smoke has two phases- a gas phase and a solid phase (tar). The both phases contain and induce generation of the free radicals. The dysbalance between generation and removal of free radicals produces oxidative stress. The organism has its own defensive antioxidant mechanism in order to eliminate the production of free radicals or in case that radicals have already been produced, to decrease any consequences of their negative effects. Vitamin C (ascorbic acid) as one of the major extracellular antioxidant in the body, the levels of which were examined at the different risk levels of total cholesterol in 109 smokers and 103 nonsmokers. The average age of probands was 49.6 years. Probands smoked 15 cigarettes and more per day. Vitamin users were excluded from the study. The degree of the oxidative activity was evaluated from the detection of malondialdehyde formation (MDA-TBARS) assayed with thiobarbituric acid. Deficit of vitamin C in smokers vs. nonsmokers in the low risk group (control group with total cholesterol less than 4.69 mmol/L) was 13.5 % and had an increasing tendency in individual risk groups. In heavy smokers with a total cholesterol more than 6.7 mmol/L in comparison with the control group the deficit of ascorbic acid ranged from 35-55 %. Oxidative activity measured by MDA is significantly increased ( $p<0.01$ ) in smokers with total cholesterol more than 6.7 mmol/L. This results indicate that insufficient intake of vitamins with antioxidative effect have contribute to negative effect of smoking itself when smokers are unable to quit of smoking or to increase saturation of organism with vitamins. The prevention is more effective than cure of consequences of diseases caused by smoking.

Transgenic TGR[mRen27]27 rats harbor the mouse salivary gland renin

## COMPUTER VISION: TRACKING RATS IN THE MORRIS WATER MAZE

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We have designed and implemented a tracking system prototype capable of tracking rats in video sequences to automate the processing of behavioral animal studies. Computer vision technology using an overhead mounted digital video camera allows tracking of the animal, recording its trajectory and quantifying the rat's behavior in real time. Whilst available commercial solutions depend on segmentation of a swimming rat in individual frames, we also explore interframe relations. Three mechanisms of rat detection run in parallel. These are motion detection (and interframe motion prediction), robust salient region detection, and shape classification. Firstly, the rat's movement in relation to its background is detected by the running average motion detection algorithm. Secondly, the color of the rat is distinct from the background and is detected by the Maximally Stable Extremal Regions (MSERs) algorithm (1). Thirdly, rats have a certain shape which can be used as a feature with which to perform statistical classification. Training of the classifier has been performed off-line: the user does not need to specify the color or shape interactively. Finally, the tracking is performed by Beleznai's fast mean-shift algorithm (2). Tracking data can be exported to the Wintrack public domain analysis software produced by Wolfer (3) to calculate the total path length, the latency before the goal is reached, the initial direction taken by the subject, and to provide publication-quality printouts. Our computer vision public domain system is not affected by animal color and copes with imperfect lighting conditions. To the best of our knowledge, in its technical parameters our system outperforms the state-of-the-art solutions.

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## EFFECT OF CHRONIC HYPOXIA ON SUBCELLULAR LOCALISATION OF PKC IN RAT MYOCARDIUM

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Adaptation of rats to chronic hypoxia increases the expression of protein kinase C (PKC) isoforms  $\delta$  and  $\epsilon$  in the myocardium. It is known that the translocation of inactive PKC from the cytosol to the particulate fraction and its activation depend on fatty acid (FA) composition of membrane phospholipids. The aim of this study was to analyze effects of diets with different FA composition on the expression of PKC  $\delta$  and  $\epsilon$  in normoxic and chronically hypoxic rat hearts. Adult male Wistar rats were fed non-fat diet enriched by 10 % of lard (saturated FA, SFA), fish oil (n-3 PUFA, n-3) or corn oil (n-6 PUFA, n-6) for 10 weeks. After 4 weeks on diets, each group was divided into two subgroups that were either exposed for 6 weeks to intermittent high altitude hypoxia of 7000 m in a barochamber for 8 h/day, 5 days/week or kept under normoxic condition for the same period of time. The immunoanalysis of PKC isoforms was performed in particulate and cytosolic fractions

(differential centrifugation, 105x10<sup>3</sup> g) from the left ventricles, followed by Western blotting and chemiluminescent ECL technique with the aid of Image Quant software. In normoxic tissue, the diet composition had no effect on relative amounts of PKC  $\delta$  in any fraction. The proportion of PKC  $\epsilon$  was higher in the particulate fraction of n-6 group as compared with SFA and n-3 groups. Chronic hypoxia increased the relative amount of PKC  $\delta$  in the particulate fraction of SFA and n-3 groups (by 40.0±2.6 % and 82.7±11.27 %, respectively) but not in n-6 group. In contrast, chronic hypoxia did not influence PKC  $\epsilon$  of SFA and n-3 groups but it decreased the relative amount of this isoform in the particulate fraction of n-6 group (by 41.4±2.46 %). In conclusion, the diet lipid composition significantly modulates the effect of chronic hypoxia on cardiac PKC isoform expression and subcellular distribution.

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## TEMPOL REDUCES WARM ISCHEMIC DAMAGE OF LUNG FUNCTIONS IN NON-HEART-BEATING DONORS IF ADDED PRE-ARRESTLY BUT DOESN'T HAVE ANY PROTECTIVE EFFECT ON ISCHEMIA – REPERFUSION INJURY

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Lungs retrieved from non-heart-beating donors (NHBD) may alleviate the critical shortage of suitable organs for transplantation. We tested the role of cell permeable reactive oxygen species scavenger – Tempol – in protection of warm ischemic and reperfusion injury of lung functions in experimental model of NHBD. We measured perfusion pressure, weight gain and arterio - venous difference in O<sub>2</sub> partial pressure in Salt solution + Ficol perfused lungs isolated from Wistar rats in two experiments: A: Experimental rats underwent the protocol of NHBD lung harvesting: 1 hour of warm ischemia after pentobarbital euthanasia followed with 90 min of cold ischemia (12 °C). Under these conditions we compared room-air ventilated rats with non-ventilated during warm ischemia and we were interested in possible protective effect of Tempol (100 mg/kg) added pre-arrestly. In controls, lungs were harvested immediately after euthanasia. B: We used the same protocol as in A and observed the effect of Tempol added into perfusate at the beginning of reperfusion of NHBD lungs. Ventilation during warm ischemia deteriorates lung functions by the effect of reactive oxygen species. This mechanism was in part A inhibited by pre-arrest administration of Tempol, which prevented development of lung edema and improved oxygen transport ability. In part B Tempol added into the perfusate deteriorated lung functions by causing pulmonary edema. This could be caused by Tempol-mediated inhibition of hypoxic pulmonary vasoconstriction.

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## MULTIMEDIAL EDUCATION IN TRAINING COURSES OF PHYSIOLOGY

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The recent methodological approach enables broad exploitations of standard knowledge and information, however, high quality presentations are of a great importance to promote education at the university level. This creates a basis to tackle any biomedical topic and also to merge into a systemic interdisciplinary view. Rapid progress and research in preclinical disciplines and applied biomedicine call for a new concept and methodical approach in university education, using

available high-tech equipment. Physiology as the science about body functions deals with lot of experiments and broad practical demonstrations. Thus, we particularly focused on training courses to facilitate and improve practical education in physiology for veterinary and pharmaceutical students. The complex study material involves electronic version of text files dealing with theoretical introduction to each topic and methodical descriptions of experiments performed. Schematic displays, pictures, videos are followed by suggested practical applications of results obtained during the course. Enough space is given to self-work of students leading to final evaluation of results, correct conclusions, discussion and successful understanding of investigated physiological functions. The study electronic material was created as a hypertext file which enables to jump quickly among areas of interest, to google particular definitions and to jigsaw different areas of physiology. Moreover, the text is simply interconnected with corresponding pictures, schemes and movies. These materials are available on CD and also on the university website in frame of Intranet and will be exploited by 330 students each academic year, directly in the training room as well as for self-education.

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#### **GREATER INTIMA-MEDIA THICKNESS IS ASSOCIATED WITH HIGHER AGE, BODY MASS INDEX, BLOOD PRESSURE AND REDUCED BAROREFLEX SENSITIVITY.**

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Association of greater intima-media thickness (IMT) in the carotid bulb with higher age, body mass index (BMI), and blood pressure was investigated. We examined 27 treated hypertensives (47.2±8.7 years) and 23 normotensives (44.1±8.1 years), age difference was insignificant. Mean blood pressure (MBP) was determined from 24-hour recordings of blood pressure, IMT was measured ultrasonographically, and baroreflex sensitivity (BRS) was evaluated by spectral method (1) from 5-minute continuous recordings of finger blood pressure (Finapres). Significant differences (hypertensives versus normotensives) were observed in IMT (0.60±0.08 vs. 0.51±0.07 mm;  $p<0.001$ ) and BRS (3.5±1.8 vs. 5.6±2.1 ms/mmHg;  $p<0.001$ ). In the hierarchical multiple regression analysis, IMT in normotensives (multiple correlation coefficient  $R=0.7037$ ,  $p<0.01$ ) correlated positively with age ( $p<0.001$ ) and with BMI ( $p<0.05$ ), influence of MBP was insignificant. Increased IMT in hypertensives was additively influenced neither by age nor by BMI nor by MBP. IMT in all subjects together (multiple correlation coefficient  $R=0.4816$ ,  $p<0.01$ ) correlated positively with age ( $p<0.01$ ) and with MBP ( $p<0.05$ ). A dependence of a BRS-decrease on age and IMT was also evaluated: BRS decreased with age ( $p<0.01$ ) and with IMT ( $p<0.05$ ) in normotensives, but not in hypertensives. In all subjects, BRS decreased with age ( $p<0.01$ ) and with IMT ( $p<0.01$ ). The study brought evidence that age and BMI play an additive role in the growth of carotid intima-media in healthy subjects. On the other hand, in hypertensives, high blood pressure renders the effect of age and BMI on IMT negligible. The influence of blood pressure on IMT predominates in hypertensives, as documented by a comparison of IMT between hypertensives and controls. BRS is low in hypertensives and its age-related decrease is weakened in this group.

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#### **DIETARY INTAKE OF PROTEINS, LIPIDS AND CARBOHYDRATES IN COMPARISON WITH NUTRITIONAL, BIOCHEMICAL AND BIRTH PARAMETERS OF PREGNANT WOMEN: LONGITUDINAL STUDY.**

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Recent research suggests that alterations in fetal nutrition and endocrine status result in developmental adaptations that permanently change structure, physiology, and metabolism, thereby predisposing individuals to cardiovascular, metabolic, and endocrine disease in adult life (1). This longitudinal study evaluated dietary intake of proteins, lipids and carbohydrates in interrelationship between nutritional, biochemical and gynecological parameters. 694 pregnant women (in age of 26±4 years) in the second trimester were studied. For determination of nutritional parameters was used program Nutricom. Cholesterol, HDL, LDL lipoproteins and triglycerides were measured in serum. Weight gain in pregnancy, duration of pregnancy, duration of periods delivery, extent of blood loss during delivery, and weight of a newborn were observed. General evaluation demonstrates high dietary intake of lipids (79.9 g/day, 107 % RDA), low intake of animal proteins (44.85 g/day, 89.7 % RDA), low intake of vegetable proteins (32.00 g/day, 80.01 % RDA), low intake of carbohydrates (292.7 g/day, 73.5 % RDA) and high dietary intake of cholesterol (310.2 g/day, 103.39 % RDA). Plasma levels of cholesterol, LDL and HDL lipoproteins were within normal range. Plasma levels of triglycerides were increased (1.993 mmol/l). A significant positive correlations ( $p<0.05$ ) were observed between dietary intake of proteins and total plasma cholesterol, and plasma triglycerides; between dietary intake of lipids and plasma cholesterol; between dietary intake of carbohydrates and triglycerides and LDL lipoproteins; between dietary intake of lipids and preconceptive weight and weight before the delivery. Other observed negative correlations: between carbohydrates and preconceptive weight and weight before the delivery. (2) These results suggest high dietary intake of saturated fatty acid, low dietary intake of polyunsaturated fatty may cause to increase the risk in fetal development of central nervous system.

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#### **BEHAVIOR OF WISTAR RATS IN THE OPEN FIELD FOLLOWING REPEATED RESTRAINT STRESS**

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In the previous studies we have demonstrated that behavioral alterations are dependent on the stress type as well as rat strain. Some stresses or repeated stresses can induce long-lasting changes in neurobiological responses of the organism; and therefore some stress protocols can be used as animal models for posttraumatic stress disorder (PTSD). In this presentation we describe differential behavior of Wistar rats in the open field in dependence whether the three times repeated acute stress was applied with or without immediately followed test in the open field device. Male Wistar rats were exposed for three consecutive days to 60 min lasting immobilization (restraint) alone (IMO) or IMO combined with water immersion at 21 °C (IMO+C). In the experimental series 1 (Ex1) the animals were exposed to the open field test immediately after application of stressors, in the series 2 (Ex2) the open field test was performed for the first time after one week from the first stress exposure (day 8). The rats were tested in the open field device (Coulbourn Instruments Inc., PA, USA) and we measured the overall activity as

total movement distance (TMD), vertical exploratory activity (rearing) and time spent in the center of arena (CT) as possible indicator of anxiety. Surprising results we obtained in the Ex2 where three times repeated stresses without immediate open field tests induced persistent stress behavioral effects; on day 8 we observed a slight decrease of TMD which became more pronounced in the following testing on days 9-10, 15 and 22. Thus, the inhibition of movement activity continued for at least three weeks. The impairment of exploratory activity measured as rearing and CT were even more pronounced than the parameter TMD. On the contrary, in Ex1, 60 min after the end of each stress exposure we performed open field tests; both stressors induced highly significant behavioral deterioration, and the effect IMO+C was much stronger than the effect of IMO. On days 8-10, 15 and 22 we did not find any persisting effects of stress and the parameters were very similar to controls. Our data indicate that sequential application of restraint stressors produce differential response depending on whether the open field test was or was not applied immediately after the stressors. In Ex1, where experiments were performed without the open field tests after the exposure to stressors, we observed long-lasting changes of behavior that could possibly be used as an animal model of PTSD.

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#### INNOVATION OF CLINICALLY USED VASCULAR PROSTHESES BY PROTEIN ASSEMBLIES ON THEIR LUMINAL SURFACE

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Damaged blood vessels are frequently bypassed with synthetic vascular prostheses. Their thrombogenic surface may lead to a failure in low diameter grafts. Surface modifications including cell seeding are carried out for better haemocompatibility. Knitted tubular vascular prostheses of 6 mm inner diameter (VÚP® Joint-Stock Comp., Brno, Czech Republic) made of PET (polyethylene terephthalate) with type I collagen impregnation were modified on the luminal surface by the immobilization of an extracellular matrix protein laminin (LM) or coating with fibrin network (FB). Human saphenous vein endothelial cells obtained from coronary bypass surgery were harvested, cultured (M199 medium with 20 % foetal calf serum, Heparin, b-FGF, Penicillin, Streptomycin) and seeded in the density of  $1.5 \times 10^5$  cells/cm<sup>2</sup> on both unmodified (UM) and modified (LM, FB) grafts. After each step, the cell lining was visualized by Live/Dead Kit staining (Molecular Probes®) of a prosthesis fragment and quantified by trypsinization and cell counting. After 2 days of maturation, the cell retention was 21 %, 37 % and only 2 % of the seeding density on the UM, LM- and FB-coated grafts, respectively. These seeded prostheses were exposed to laminar shear stress (SS) of 15 dynes/cm<sup>2</sup> for 40 (UM, LM, FB) and 120 (UM, LM) minutes (') in a haemodynamic bench that simulates arterial blood circulation. Static control graft was submitted to the same conditions except for shear stress. After 40'SS the cell numbers were 78 %, 27 % and 72 % for the UM, LM and FB prosthesis compared to the static control. The cell densities were 61 % and 57 % on UM and LM after 120'SS. To conclude, laminin immobilized on the collagen impregnated prosthesis improved endothelial cells adhesion but not their flow resistance. Reverse effect was observed on fibrin coating. The cells seeded on the unmodified prosthesis appeared to be the most resistant to shear stress.

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#### ANTIOXIDANT STATUS AND LIPIDS PEROXIDATION IN ROMANY CHILDREN POPULATION

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Romanies are the second most numerous minority in the Slovak Republic. Among Romany children there is generally a higher prevalence of infectious disease, injuries, poisoning and burns caused by environmental hazards.(1) The aim of study was to investigate the activity of antioxidant enzymes and lipids peroxidation in Romany children population and compare with majority children population. We determined activity of red cell enzymes (superoxide dismutase - SOD, glutathione peroxidase - GPX, catalase - CAT) and plasma thiobarbituric acid reacting substances (TBARS) in 32 Romany and 32 majority children (age between 0-3 years). SOD and GPX were measured by spectrophotometric methods (RANSOD and RANSEL from Randox, UK) on Cobas Mira automatic analyzer, CAT by UV spectrophotometric method according to Luck and TBARS by spectrofluorometric methods according to Yagi. The results are summarised in Table as mean  $\pm$ SD

Group	Cat mkatal/gHb	GPX U/gHb	SOD U/gHb	TBARS μmol/l
Romany children	4.48 $\pm$ 1.18	42.8 $\pm$ 5.5	1057 $\pm$ 116	2.08 $\pm$ 0.45
Majority children	3.75 $\pm$ 0.74 <sup>a</sup>	41.7 $\pm$ 9.3	1041 $\pm$ 191	2.08 $\pm$ 0.66

<sup>a</sup>  $p < 0.01$

There was no difference in the activity of red cell SOD and GPX neither in the concentration of TBARS between the majority and Romany children. The activity of catalase was significantly elevated in Romany children. Probably the increase of activity CAT in Romany children is result of "non healthy" life style of Romany mothers. Further carefully designed studies are needed to clarify the causal relationships between increase activity catalase in Romany children and state of health in Romany pregnant women.

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#### EXPRESSION OF NPY MRNA IN THE HEART OF QUANETHIDIN AND STREPTOZOTOCIN TREATED RAT

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Neuropeptide Y (NPY), a 36 amino acid peptide produced by cleavage from a large precursor preproNPY, is co-stored and co-released with noradrenaline in sympathetic nerve fibers. In the rat heart, NPY mRNA is expressed in the cell bodies of intrinsic neurons, in endothelial cells, and in the sympathetic nerve fibers. The actions of NPY are extensive and include practically every cardiac cell type, e.g. NPY induces vasoconstriction and exerts hypertrophic effects on adult cardiomyocytes. Investigations to date have implicated the role of NPY in the pathology of a number of diseases including diabetes (1). Here,

we investigated the contribution of sympathetic nerve fibers to the total NPY production in the left atrium of rat heart by using chemical sympathectomy (20 days guanethidine application in dose 50 mg/kg/day). Furthermore, we measured level of mRNA for NPY in the left atrium to assess involvement of the neurotransmitter in the events underlying development of diabetic cardiomyopathy in the rat model of streptozotocin (STZ)-induced diabetes by means of real-time RT-PCR and immunohistochemistry. Wistar rats were sacrificed by decapitation one day after a series of guanethidine injections, and/or 6, 9 and 12 months after the application of STZ (65 mg/kg body weight). Relative gene expression was expressed as a ratio of target gene concentration to housekeeping gene. The results were considered significantly different when  $p < 0.05$ . Expression of preproNPY mRNA in sympathectomised animals was significantly lower ( $n=8$ ; mean=0.39) than in control animals. It was similar to STZ treated animals, where mRNA for preproNPY significantly decreased 6 months after the induction of diabetes ( $n=5$ ; mean=0.42); it returned to control levels after 9 months ( $n=5$ ; mean=1.39) and remained at the control levels after 12 months of the disease ( $n=6$ ; mean=1.15). Indirect immunofluorescence showed reduction of NPY-immunoreactive (IR) nerve fibers in the hearts of diabetic animals.

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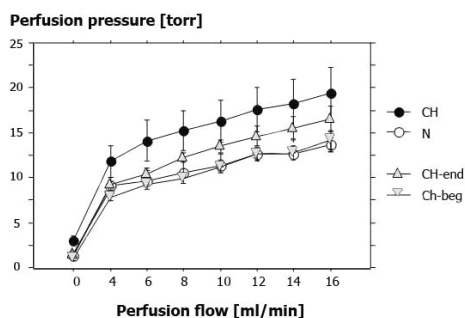
Supported by grants: GACR 305/03/D180, GACR 305/04/0144, and D-CZ 26/05-06

#### EFFECT OF ANTIOXIDANT TEMPOL ON DEVELOPMENT OF HYPOXIC PULMONARY HYPERTENSION (HPH)

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Exposure to chronic hypoxia (CH) results in hypoxic pulmonary hypertension. We hypothesize that oxidant injury to pulmonary vascular walls of prealveolar vessels participates in the pathogenesis. Two experiments with inhibition of tissue oxidant injury by superoxide dismutase mimetic Tempol were performed in male rats exposed to CH ( $F_{iO_2}=0.1$ ). The presence of CH – induced vascular changes was in isolated PSS perfused lungs determined by analysis of perfusion pressure increments induced by stepwise increase of perfusion flow (P/Q relationship). In the first experiment the treatment with Tempol (80 mg/kg b. w. in drinking water) during 5 days exposure to CH inhibited the development of HPH. In the second experiment rats exposed to CH for 3 weeks. Group CH-beg ( $N=8$ ) obtained Tempol during the first and group CH-end ( $N=7$ ) during the last 4 days of hypoxia. CH ( $N=8$ ) and N ( $N=8$ ) were hypoxic and normoxic controls. The P/Q relationship in CH-beg did not differ from normoxic controls and it was significantly shifted ( $P < 0.002$ ) to low perfusion pressures compared to CH. Treatment at the end of exposure (CH-end) was less effective. We conclude that oxidant tissue stress plays important role in the initiation of HPH development.



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#### EFFECTS OF MAGNETIC FIELD 0.05–10 mT ON LEUKOCYTE ADHERENCE INHIBITION

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Effects of exposure of T lymphocytes from healthy humans and cancer patients before and after medical treatment to magnetic field were studied. Adherence of T lymphocytes to solid substrates was investigated using Leukocyte Adherence Inhibition Assay which is understood to correlate with the cell mediated immunity (1-3). Specific (tumor) and non-specific (LDH virus) antigens were used. Adherence properties of T lymphocytes were evaluated as the number of non-adherent cell, as the non-adherence index, and as the index of positivity. Exposure to the magnetic field alters adherence properties. After exposure to the magnetic field T lymphocytes from healthy humans have greater adherence for induction 0.5 mT. Exposure to the magnetic field strongly increases adherence of T lymphocytes from cancer patients before medical treatment (in particular at 0.5 mT). T lymphocytes taken from cancer patient after medical treatment behave like those from healthy humans. Effects of AC and DC magnetic field 0.05 mT do not display large differences. The results are consistent with suggestion of magnetic field effects on immune function in humans. The effects are not adverse.

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#### INFLUENCE OF MAGNESIUM ON HYPOXIA INDUCED INCREASE OF THE NITRERGIC NEURONS DENSITY IN HIPPOCAMPUS

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Using histochemical analysis (NADPH-diaphorase staining) we studied the influence of magnesium pre-treatment during long-lasting hypoxia on the brain structure of rats. NADPH-diaphorase occurs concurrently with NO-synthase that is responsible for NO synthesis. NO participates in hypoxic-ischaemic injury of the brain. Magnesium was repeatedly administered in consecutive days from the 2nd till the 11th day of postnatal life in a hypobaric chamber (for 8 hours per day). At the age of 12 days, the animals were transcardially perfused with 4 % buffered neutral paraformaldehyde under the deep thiopental anaesthesia. Cryostat sections were stained to identify NADPH-diaphorase positive neurons that were then quantified in five hippocampal regions. In comparison with control animals, intermittent hypoxia brought about higher density of NADPH-diaphorase positive neurons in CA1 and CA3 areas of the hippocampus and in dorsal and ventral blades of the dentate gyrus. In the hilus of the dentate gyrus, on the contrary, the number of nitrergic neurons was lowered. Magnesium pre-treatment during hypoxia reduced number of NADPH-diaphorase positive neurons. These results indicate that magnesium can have a neuroprotective effect.

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# ASSOCIATION OF eNOS GENE POLYMORPHISMS T-786C AND G894T WITH BLOOD PRESSURE VARIABILITY

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**Introduction:** Increased blood pressure variability (BPV) is considered as an independent risk factor for end-organ damage and development of cardiovascular diseases such as atherosclerosis and hypertension. The aim of this study was to evaluate association of polymorphisms (SNPs) T-786C and G894T in gene encoding eNOS with blood pressure variability in men. **Methods:** Blood pressure was recorded beat-to-beat 3 times in periods of one week (5 min, Finapres, breathing at 0.33 Hz) in 129 subjects (19-24 years). Relative/absolute systolic ( $SBPV_{rel}/SBPV_{abs}$ ) and diastolic ( $DBPV_{rel}/DBPV_{abs}$ ) blood pressure variability was determined by cross-spectral method as spectral power in a frequency range of 0.067-0.133 Hz. Genotype was detected by means of polymerase chain reaction and restriction analysis using enzymes Msp I and Ban II. We compared BPV among genotypes of each SNP. Then, the difference in BPV among combined genotypes of both SNPs was tested (Kruskal-Wallis). **Results:** The frequency of genotypes for T-786C SNP was 41.2 % (TT, n=54), 44.4 % (CT, n=58), 13.0 % (CC, n=17) and for G894T SNP 57.3 % (GG, n=75), 36.6 % (GT, n=48), 4.9 % (TT, n=6). We found significant differences in BPV among genotypes of each SNP (T-786C,  $p<0.05$  and G894T,  $p<0.05$ ). Homozygotes in less frequent (mutant) alleles showed greater BPV comparing carriers of other genotypes (T-786C:  $DBPV_{rel}$  - TT:  $0.04\pm0.01$ , TC:  $0.043\pm0.013$ , CC:  $0.052\pm0.016$  r.u.; G894T:  $SBPV_{rel}$  - GG:  $0.022\pm0.01$ , GT:  $0.02\pm0.008$ , TT:  $0.036\pm0.02$  r.u.). The determined SNPs were in linkage disequilibrium ( $D'=0.37$ ;  $p<0.01$ ). We found significant differences in BPV among combined genotypes of both SNPs ( $p<0.05$ ). The greatest difference was found between genotypes CCTT and TTGT ( $DBPV_{rel}$ :  $0.059\pm0.01$  vs.  $0.029\pm0.006$  r.u.;  $SBPV_{rel}$ :  $0.05\pm0.01$  vs.  $0.015\pm0.004$  r.u.). No differences in  $SBPV_{abs}$  or  $DBPV_{abs}$  were observed. **Conclusion:** We found significant associations of T-786C and G894T polymorphisms in gene encoding eNOS with systolic and diastolic blood pressure variability. Heterozygosity or homozygosity in more frequent alleles of T-786C or G894T SNPs change BPV from highest to lowest values. Less frequent alleles are thought to cause decreased activity of eNOS. Thus genetically determined decreased activity of eNOS suppresses the NO related blood pressure buffering.

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# HUMAN OSTEOBLAST-LIKE CELLS MG 63 IN CULTURES ON METALLIC BONE IMPLANTS WITH DIFFERENT SURFACE MODIFICATIONS

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Metallic materials are widely used in the hard tissue surgery, e.g. for construction of joint prostheses. The integration of the bone-anchored end of the implant with the surrounding osseous tissue is strongly influenced by physical and chemical properties of the material, such as its surface roughness or chemical composition. Therefore, samples of pure Ti or Ti6Al4V alloy were treated by machining or subsequent polishing by diamond paste and seeded with human osteosarcoma-derived cells of the line MG 63 ( $13230$  cells/cm<sup>2</sup>, medium DMEM with 10 % of fetal bovine serum). In addition, a newly developed Ti5Al2.5Fe alloy, treated either with electro-erosion or plasma-spraying with Ti, was also used. On day 1 after seeding, the lowest numbers of initially adhering cells (about  $10000$  cells/cm<sup>2</sup>) were found on both polished Ti and Ti6Al4V, respectively, i.e. surfaces with the lowest roughness

( $R_a=0.17\mu\text{m}$ ,  $S=0.03\mu\text{m}$  for both materials). However, the cells on the smoothest surfaces had the shortest population doubling time (20.8 h and 18.6 h on the polished vs. 25.5 h and 21.9 h on the unpolished Ti and Ti6Al4V, respectively), so that on day 4, their population densities equaled the values on the corresponding non-polished samples of a higher surface roughness (Ti:  $R=0.63\mu\text{m}$ ,  $S=0.05\mu\text{m}$ ; Ti6Al4V:  $R=0.89\mu\text{m}$ ,  $S=0.07\mu\text{m}$ ). The cell number also depended on the chemical composition of the sample, being significantly higher on Ti6Al4V ( $157400\pm7200$  and  $150000\pm5800$  cells/cm<sup>2</sup>) than on Ti ( $105400\pm3300$  and  $112000\pm3600$  cells/cm<sup>2</sup> on unpolished and polished material, respectively; day 4). Both Ti5Al2.5Fe samples were relatively good for the initial cell adhesion on day 1 (i.e., all seeded cells adhered to these samples), but the cell population doubling times were the longest (28.5 and 29.2 h on electro-eroded and plasma-sprayed Ti5Al2.5Fe, respectively) and the final cell population densities reached on day 4 were the lowest ( $77401\pm7478$  and  $73806\pm2117$  cells/cm<sup>2</sup>). These samples displayed the highest surface roughness (electro-eroded:  $R_a=15.27\mu\text{m}$ ,  $S=332.85\mu\text{m}$ ; plasma-sprayed:  $R_a=39.72\mu\text{m}$ ,  $S=332.85\mu\text{m}$ ). These results suggest that both polished and unpolished machined Ti6Al4V samples are most appropriate for colonization with bone-derived cells.

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# ODOTOPY IN THE ANTENNAL LOBE OF THE SPHINX MOTH MANDUCA SEXTA

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The sphinx moth *Manduca sexta* is a well studied insect model to investigate central nervous processing of olfactory information. M. sexta primary olfactory center, antennal lobe (AL), displays a typical glomerular architecture where the olfactory information is processed in two parallel olfactory systems: 1) Macroglomerular complex (MGC) is present only in male moths and processes exclusively information about conspecific sex pheromone. 2) Ordinary glomeruli are present in both sexes and analyze all other non-pheromonal information. Using optical imaging to visualize calcium dynamics corresponding with neural activity, we studied pheromonal and host plant odor processing in AL of M. sexta. The individual odors elicited unique patterns of neuronal activity. Information processing was segregated based on chemical identity of analyzed compound in both - sex specific and "general" - olfactory systems. Non pheromonal information was processed equally in both sexes. Data support hypothesis about the odotopic organization of the moth primary olfactory center. However, the exact odotopic logic is not fully understood.

# HOW SMALL DOSES OF AMPHETAMINE INFLUENCE OPEN FIELD BEHAVIOR OF WISTAR RATS PREVIOUSLY EXPOSED TO REPEATED RESTRAINT STRESS

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In our previous study (1) we have described a persisting behavioral deterioration, tested in the open field device, of Wistar rats that have been exposed to restraint stress for three consecutive days and when the open field test was performed for the first time with one week delay from the first stressor application. We hypothesized that this experimental protocol could serve as an animal model of posttraumatic

stress disorder (PTSD). The individuals with this disorder are predisposed to use drugs of abuse and amphetamine (AMPH) especially. The aim of this study was to determine whether small doses of AMPH would be able to modify persisting behavioral changes following repeated stress application. Male Wistar rats were exposed for three consecutive days to 60 min lasting immobilization alone (IMO) or IMO combined with water immersion at 21 °C (IMO+C). In the open field device (Coulbourn Instruments Inc., PA, USA) we tested the overall exploratory activity (TMD), rearing as vertical exploratory activity and time spent in the center of arena as an indicator of anxiety (CT). Small i.p. doses of AMPH (0.3 and 1.0 mg/kg) were given 60 min before the open field test that was performed 2-3 weeks after the application of stresses. In experimental setting 1 where the effect of stressors survived (three stresses were given one week before the onset of tests), AMPH increased proportionally the behavioral parameters in controls as well as in stress treated groups with reduced behavioral parameters. The effect of AMPH did not persist to the next week testing and stress-induced effects were again evident. In experimental settings 2 where open field tests were performed immediately after stress applications we did not observe a persisting stress-effects and the effects of AMPH were equal in all groups of rats. In summary, in experimental setting 1, where three consecutive stresses induced the persisting effect in the open field behavior, AMPH enhanced behavioral parameters, however, its effect did not antagonize stress-induced changes permanently. In experimental settings 2, where stress did not produce long-lasting changes, the effect of AMPH was also transient and no significant differences in stress groups and controls were observed.

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#### POSITIVE RELATION BETWEEN QT LENGTH AND SYSTOLIC BLOOD PRESSURE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Background: Experimental studies reported prolongation of QT length in spontaneously hypertensive rats (SHR) and this has been associated with increased left ventricular mass (LVM). The aim of this study was to test the relationship of QT length with systolic blood pressure (sBP) and LVM, resp. in the early period of hypertension and left ventricular hypertrophy (LVH). Animals and methods: SHR were compared to Wistar-Kyoto rats (WKY). Tail-cuff sBP was measured in 12 and 20 weeks old conscious rats and Frank ECG was recorded under general anaesthesia. QT length was evaluated from the start of the QRS complex to the end of T wave in either of the simultaneously recorded leads. After recordings, rats were sacrificed and LVM was weighted. Results: In the age of 12 weeks SHR showed only tendency to QT prolongation in comparison to age matched WKY (WKY12: 82±9 ms and SHR12: 91±17; NS). Twenty weeks old SHR showed significantly higher values of QT length, compared to age matched WKY and to SHR12 (WKY20: 81±9 ms and SHR20: 98±10; p<0.05 vs. WKY20 and SHR12, resp.). There was a positive correlation between QT length and sBP in SHR both age, but we find positive relationship between QT and LVM only in 12 weeks old SHR, i.e. in the age, where the QT length only tended to prolong. There was no correlation between QT length and sBP or LVM in WKY. Conclusion: In our study, SHR showed progressive prolongation of QT length with the age. This prolongation was strongly related to arterial sBP, but not to LVM. This findings demonstrate, that QT prolongation is more associated with the increase of arterial sBP than with increase of LVM in the early stage of progression and stabilisation of hypertension and LVH in SHR.

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#### 11β-HYDROXYSTEROID DEHYDROGENASE IN THE LIVER OF HYPERTRIGLYCERIDEMIC RATS

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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 hypertension, glucose intolerance, hyperinsulinemia, hypertriglyceridemia, and obesity. Similar symptoms can be found in patients with Cushing's syndrome which is characterised by elevated plasma level of glucocorticoids and thus the disturbances of metabolic syndrome seem to be not only insulin- but also steroid-sensitive. There is no change in plasma levels of glucocorticoids in metabolic syndrome but the glucocorticoid action in target tissues might depend also on their local concentration. This concentration is determined by the activity of 11β-hydroxysteroid dehydrogenase type 1 (11HSD1) that is predominantly expressed in major insulin target tissues including liver. Its function is to regenerate corticosterone from its inactive 11-keto derivate. To determine whether metabolic disorders are associated with changes in liver metabolism of corticosterone we studied activity and mRNA levels of 11HSD1 in Prague hypertriglyceridemic rats (HTG), HTG on fructose diet (HTG+Fru) and normotriglyceridemic Wistar rats. In addition we measured mRNA levels of phosphoenolpyruvate carboxykinase (PEPCK), a key gluconeogenic enzyme, whose expression is stimulated by glucocorticoids. To demonstrate a metabolic dysfunctions of HTG rats we also measured body weight, serum triglyceride (TG) and insulin (IN) levels and arterial blood pressure. Oxidase activity of 11HSD1, which predominates in vitro in disrupted cells, was measured by radiometric assay. Transcript levels were determined by RT „real time“ PCR. Plasma levels of TG and IN were significantly increased in HTG rats than in control Wistar rats and dietary fructose further stimulated the levels of TG and IN; body weight remained unchanged in all groups. The activity and mRNA level of 11HSD1 was higher in HTG than in Wistar rats but stimulation of hypertriglyceridemia in HTG+Fru was not associated with further up-regulation of 11HSD1. In contrast to 11HSD1 mRNA the levels of PEPCK mRNA in HTG and HTG+Fru were down-regulated. The results indicate that the local concentration of glucocorticoids is increased in HTG rats but that the stimulation of gluconeogenic pathway is suppressed in these animals.

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#### STRUCTURE AND FUNCTION OF INSECT STRESS HORMONES FROM ADIPOKINETIC PEPTIDES FAMILY.

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Insect endocrine system is very well known, documented and investigated. Insects possess dozens of various hormones that control practically all aspects of their life. Based on their chemical structure and physiological activity the hormones can be divided into three groups: (a) ecdysteroids that control insect development, metamorphosis and reproduction; (b) juvenile hormones that control development of insect juvenile stages and in adults again some aspects of reproduction; and (c) neurohormones, a large group of peptide hormones that affect physiology of most processes in insect body. The last group includes also the family of adipokinetic hormones (AKHs) that control insect energy metabolism. AKHs are typical stress hormones: they stimulate catabolic reactions that make energy more available, while inhibiting synthetic reactions. They are synthesised and released by corpora cardiaca, an endocrine gland in CNS near the brain. We have studied these hormones in bugs *Pyrrhocoris apterus* (Heteroptera, Insecta),

where we isolated and characterised two octapeptide members: Pyrap-AKH (pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-NH<sub>2</sub>) (1) that can be found also in further species of Heteroptera (2), and Peram-CAH-II (pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-NH<sub>2</sub>) (3) that has been described also in cockroaches and beetles including *Leptinotarsa decemlineata* (Colorado potato beetle), an important pest of potatoes. Further experiments showed that adipokinetic characteristics are significantly enhanced when the experimental insects are exposed to various stressors (4).

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#### THE EFFECT OF CREATINE SUPPLEMENTATION ON THE METABOLIC OF CREATININE AND THIODIGLYCOLIC ACID IN URINE

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**Background:** Some people supplement their diet by high doses of creatine [CR] to increase their muscle performance. Thus intensify the biochemical processes on cell membranes (1). The organism must remove exogenously added CR probably in a similar way as in removing of xenobiotics via oxidation with cyt.P450 to 2C units in cooperation with GSH. The voltammetric method enables to estimate thiodiglycolic acid [TDGA] the natural product of oxidative catabolism of thiocompounds excreted to urine in regular catabolic processes. **Method And Results:** The present study was designed to assess the effect of creatine supplementation on the levels of TDGA and creatinine in urine. The study was completed by ten male volunteers, aged 22.6±3.5 yr, with obtained one dose of creatine monohydrate [CRM] per os [5 g]. TDGA and creatinine were determined in urine before treatment and 3rd, 4th and maximal 9th hour after application of CRM. The TDGA level in urine of healthy persons is below 20 mg.L<sup>-1</sup>. Creatine supplementation increased twice the values of TDGA concentration in urine, but the changes of creatinine were not significant (2, 3). The results suggest that: The values of TDGA concentration were plotted without correction for creatinine, which had been previously supposed to correct the varied dilution of urine samples. The correction for specific weight was based on a similar assumption. The position of the maxima and minima of the curves of creatinine and specific weight did not usually correspond to maxima and minima of TDGA (2, 3, 4, 5).

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#### SERUM CONCENTRATIONS OF APOLIPOPROTEIN B<sub>100</sub> AND SOME ANTIOXIDANTS IN ROMANY CHILDREN

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Apolipoprotein B<sub>100</sub> is an important and majority constituent of LDL-cholesterol. The elevated serum concentration of apo B<sub>100</sub> is a better discriminator and risk factor for premature atherosclerosis than the lipids (1). Depletion of the vitamin C is an important sign of the disproportion in the antioxidative activities (2). The aim of study was to find the serum concentration of apo B<sub>100</sub>, some lipids and antioxidants in children of ethnic Romanies, in addition to the risk of premature atherosclerosis. Besides apo B<sub>100</sub> were assayed the serum concentrations of total cholesterol (TC), triacylglycerols (TG) and vitamin C (vit C) in 40 1-3 years old Romany children (R). The concentrations of those determined parameters were compared with measured concentrations in 37 2-4 years old non-Romany children in the control group (C). Apo B<sub>100</sub> was determined by the electroimmunoassay with antibodies and standards of Behringwerke Germany. TC and TG were determined using the Czech biochemical sets of Pliva-Lachema company and vitamin C colorimetrically. In Romany children were found statistically increased concentrations of apo B<sub>100</sub> (p 0.001) TC and TG (p 0.001). The concentration of vitamin C was statistically decreased (p 0.01). The elevated serum concentration of apo B<sub>100</sub> with the high serum concentration of lipids is a very risky combination regarding the development of premature atherosclerosis. Significant depletion of vitamin C is unfavourable because it supports higher oxidative risk.

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#### DOES THE RETINAL DEGENERATION INFLUENCE TESTS OF MOTOR FUNCTIONS IN NORMAL AND NEURODEFFECTIVE LURCHER MUTANT MICE?

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Lurcher mutant mice are a natural model of olivocerebellar degeneration (1). They suffer from cerebellar ataxia and deterioration of cognitive functions. Their wild type littermates are healthy and serve as ideal controls. Lurcher mutant mice are used for investigation of functional and morphological consequences of the neurodegeneration and of its therapeutical influencing. In some mice of the C3H strain also a hereditary retinal degeneration develops and it changes results of some behavioural experiments, e.g. navigation in the Morris water maze (2). The aim of the work was to assess whether the retinal degeneration influences motor abilities and results of motor coordination tests and to compare motor abilities of C3H mice with both wild type and Lurcher mutant mice of the C57Bl/7 strain, in which the retinal degeneration does not occur. Motor coordination was tested with a set of three methods (horizontal bar, ladder and rotarod). All tests were repeated four times and mean latencies and criterion meeting to reach the latency at least 60 s were evaluated. Then retinas of the C3H mice were examined histologically (hematoxyline-eosine) to detect the retinal degeneration. Wild type mice reached significantly better results than Lurcher mutant mice of the same strain. Retinal degeneration did not affect significantly motor abilities neither in wild type nor in Lurcher mutants of the C3H strain. Strain differences were not found in wild type mice. C57Bl/7 Lurcher mutants showed better motor skills as compared with both with the retinal degeneration affected and unaffected Lurchers of the C3H strain. The experiment suggested that the retinal degeneration does not influence the performance in the motor coordination tests in mice of the C3H strain. Lurcher mutant mice are useful as a model of cerebellar ataxia regardless of the retinal degeneration.

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### INSULIN RESISTANCE IN CRITICALLY ILL: THE ROLE OF ENDOCRINE FUNCTION OF ADIPOSE TISSUE

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**Backgrounds:** Hyperglycemia and insulin resistance is frequently occur in critically ill patients (e.g. patients serious trauma, extensive operations etc.) and is connected with increased mortality and morbidity. Detailed molecular mechanism of insulin resistance induced by critical illness is not completely understood, but it is frequently associated with alterations in postreceptor insulin signalling cascade. Insulin sensitivity is also influenced by adipose tissue-derived hormones (leptin, adiponectin, resistin) and possibly proinflammatory cytokines produced by adipose tissue (TNF- $\alpha$ , IL-6). The aim of this study was to explore role of adipose tissue-derived hormones in the development of insulin resistance in critically ill. **Materials and methods:** Serum insulin, adiponectin, leptin, resistin, TNF- $\alpha$  and IL-6 concentrations were measured in 9 patients before and 6, 12, 18, 24, 48 and 120 hours after elective cardiosurgical operation. Leptin, adiponectin and resistin mRNA expression in subcutaneous and visceral adipose tissue sampled at the beginning and at the end of the operation were measured by real-time PCR method. **Results:** The operation markedly increased concentrations of proinflammatory cytokines TNF- $\alpha$  and IL-6 with peak 18 hours after operation. Leptin levels increased moderately, while marked 4-fold increase of resistin levels was detected. Serum adiponectin remained unchanged. The expression of leptin and adiponectin mRNA did not change during the operation. On the contrary, resistin mRNA expression markedly increased at the end of the operation both in the s.c. and visceral adipose tissue. **Conclusion:** Our data suggest that adipose tissue is the main source of resistin, which may participate in the development of insulin resistance in critically ill patients. The exact role of adipose tissue in the production of other proinflammatory cytokines is the scope of our current investigations.

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### INCREASED EXPRESSION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE IN ISOPROTERENOL-INDUCED MYOCARDIAL HYPERTROPHY IN THE RAT

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**Background.** Nitric oxide produced by the endothelial nitric oxide synthase (eNOS) may possess important cardioprotective properties. Expression of eNOS in the isoproterenol-induced cardiac hypertrophy has not been studied. **Aims:** To detect possible changes of eNOS expression in the heart and aorta of isoproterenol cardiac hypertrophy. **Methods:** Rats were treated with isoproterenol (ISO, 5mg/kg/d, i.p.) during 8 days (n=13). Control rats (n=14) received vehiculum. Systolic blood pressure (SBP) and heart rate (HR) were measured by tail-cuff method. eNOS protein levels were determined in the aorta, left and right ventricles using Western blot analysis. Relaxation of isolated aorta to acetylcholine and sodium nitroprusside were evaluated in norepinephrine-precontracted aortic rings. **Results:** After eight days, basal blood pressure and heart rate were decreased compared to control (SBP 110 $\pm$ 3 vs 126 $\pm$ 3 mmHg, P<0.05; heart rate 342 $\pm$ 8 vs 366 $\pm$ 6 beats per minute, P<0.05). ISO administration led to approximately 30 % increase in the mass of atria and ventricles (P<0.05). Western blot showed important increase of eNOS protein levels in the left ventricle (1.22 $\pm$ 0.07 vs 1.00 $\pm$ 0.05; P<0.05) and right ventricle (1.33 $\pm$ 0.10 vs 1.00 $\pm$ 0.09; P<0.05), as well as in the aorta (1.53 $\pm$ 0.12 vs 1.00 $\pm$ 0.03; P<0.001). However, studies on isolated aorta showed no evidence of

improved endothelial function or relaxations to NO donor sodium nitroprusside. **Conclusions:** Our results indicate a mild, but significant increase of eNOS expression in the ventricles and aorta after 8 days of isoproterenol administration in the rat. Increased eNOS in the aorta was not associated with an improvement of endothelial function *ex vivo*.

### DEVELOPMENT OF VISUAL MOTION PROCESSING IN CHILDREN

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In comparison with standard pattern-reversal visual evoked potentials (VEPs), the VEPs elicited by onset of a motion in the visual field (M-VEPs) exhibit distinct changes during the life. In childhood they display a marked shortening of latencies up to about 18 years of age ( $r = -0.85$ ;  $p < 0.001$ ) due to long maturation of the magnocellular system and/or dorsal stream of the visual pathway. Then they delay due to aging processes ( $r = 0.66$ ;  $p < 0.001$ ) (Langrová et al., Vision Res., 2005-in press). During childhood the M-VEPs also change their shape, especially those to more complex kind of motion (e.g. radial motion). In young children (up to the age of about 10 years), there is dominating positive peak that precedes the later negativity (dominant motion specific N2 peak in adult subjects with latency of ca 160 ms). This positive peak disappears completely in adults, providing that appropriate stimulus conditions (eliminating pattern-off effect at the beginning of motion) are used. The aim of this study was to elucidate the role of different parameters of motion stimuli that influence the shape, amplitudes and latencies of the M-VEPs in children. The motion-onset VEPs were tested with the use of two types of motion stimuli – linear (transitional) motion of vertical gratings or isolated checks and radial motion of concentric (circular) pattern. In both types of stimuli we manipulated with the pattern spatial frequency (0.2-1 c/deg), contrast level (0.1 and 0.95), contrast modulation (sinusoidal versus rectangular), velocity of motion (5-25 deg/s) and with stimulus extent/location (full field 28 $\times$ 37 $^\circ$ , central stimulus of 8 $^\circ$  and peripheral stimulus outside the central 20 $^\circ$ ). The VEPs to linear motion were more comparable to the “typical” adult response than VEPs to the radial motion. The deficit of the M-VEPs to radial motion was more distinct in peripheral stimuli. The N2 peak amplitude increased and its latency decreased when full contrast pattern with high spatial frequency and rectangular contrast modulation was used. This is in contradiction to the optimal stimulus conditions for the magnocellular pathway activation in adult subjects. The found critical stimulus parameters for functioning of visual motion processing in children must be respected in their electrophysiological examination, in particular in objective testing of dyslexia.

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### CHANGES OF FUNCTIONAL PROPERTIES OF CARDIO-MYOCYTES ISOLATED FROM SHR RATS

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**Background:** Relationship between hypertension and left ventricular hypertrophy is not fully elucidated. We investigated this relationship in the model of spontaneously hypertensive rats. **Aim:** To compare basic hemodynamic parameters *in vivo*, morphometric and functional parameters of isolated cardiomyocytes from Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats. **Material and methods:** We used 20 weeks old WKY and SHR rats. First, we studied biometrical and hemodynamical parameters of both animal groups. Second, we isolated

cardiomyocytes from left ventricle and determined relative cell shortening and frequency-shortening relationship after electric stimulation (0.5, 1, 2 and 3 Hz) [1,2] and cell volume using confocal microscopy. Results: We observed significant increase in systolic blood pressure (SHR:  $206 \pm 13$  mmHg, WKY:  $131 \pm 7$ ,  $P < 0.05$ ) as well as in heart rate (SHR:  $432 \pm 26$  bpm, WKY:  $335 \pm 34$ ,  $P < 0.05$ ) in SHR. Relative left ventricular mass was significant higher in SHR ( $2.84 \pm 0.22$  g/kg) compared to WKY ( $1.87 \pm 0.12$ ,  $P < 0.05$ ). Cell length of cardiomyocytes isolated from SHR rats was significantly higher (SHR:  $144.6 \pm 1.76$   $\mu$ m, WKY:  $134.4 \pm 1.8$   $\mu$ m;  $P < 0.05$ ) as well as cell volume (SHR:  $73.3 \pm 3.7$  pl, WKY:  $65.5 \pm 1.7$  pl;  $P < 0.05$ ). Relative cell shortening after electrical stimulation was lower in the SHR (SHR:  $5.53 \pm 0.47$ , WKY:  $8.9 \pm 0.55$  %;  $P < 0.05$ ). Conclusion: Significant increase in relative left ventricular mass suggests left ventricular hypertrophy in SHR, associated with an increase in left ventricular cardiomyocyte volume. Cardiomyocytes isolated from SHR show a significant decrease in relative cell shortening in compare with WKY rats.

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#### THE IMPACT OF NEONATAL SYMPATHECTOMY ON THE PEPTIDERGIC INNERVATION OF THE RAT HEART

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Intracerebral administration of 6-hydroxydopamine (6HD) induces catecholaminergic cell death by reactive oxygen species generation or direct inhibition of the mitochondrial respiratory chain (1). However, in the peripheral nervous system, 6HD effect is generally regarded to be different, leading to transient degeneration of the sympathetic nerve terminals only (2). The aims of our study were to reveal whether repeated administration of 6HD to newborn rats (100 mg/kg/day on postnatal days 1-7, 14, 21 and 28) causes long-term degeneration of the cardiac sympathetic innervation and to characterize the impact of neonatal 6HD treatment on the concentrations of calcitonin gene-related peptide (CGRP) and neuropeptide Y (NPY) in relation to norepinephrine (NE) levels in the heart compartments. Cardiac NE, NPY and CGRP concentrations were determined by radioimmunoassay in 6HD-treated rats and the age-matched controls (C) at the ages of 10, 20, 40, 60, and 90 days. In the atria of 6HD rats, NE levels reached ~50 % of C values at the age of 10 days, they did not exceed 8 % of C on postnatal days 20 and 40 and then they increased to ~30 % and ~60 % of C levels at the ages of 60 and 90 days, respectively. In contrast, ventricular NE concentrations of 6HD rats did not exceed 15 % of C values on postnatal day 10 and they were 1-5 % of C levels till the age of 90 days. NPY levels in the 6HD atria were ~40 % of C values on postnatal days 10 and 20 and then they increased, reaching 85 % of C concentrations at the age of 90 days. In the left ventricle, NPY levels did not exceed 30 % of the respective C values throughout the study. Despite marked atrio-ventricular differences in the impact of 6HD on NE and NPY concentrations, CGRP levels had similar developmental patterns in all heart compartments: they represented ~130 % of C values at the age of 10 days, ~400 % on days 20-60 and ~250 % of C on postnatal day 90. In conclusion, repeated administration of 6HD to newborn rats resulted in long-term sympathetic denervation of the ventricles and in the increased CGRP levels in all heart compartments. Different patterns of changes in NE and NPY levels in the atria suggest that the intrinsic ganglionic neurones containing both NE and NPY could compensate the loss of the extrinsic sympathetic nerve fibres.

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#### SERUM CYTOKINES AFTER INTERFERON ALPHA AND RIBAVIRINE TREATMENT IN CHRONIC VIRAL HEPATITIS

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Background and aims: Hepatitis viral infection represents an important public health problem. Several studies have suggested that a specific cytokine profile is related to chronic evolution of viral infection. The major biological effect of interleukin 10 (IL-10) is inhibition of proinflammatory cytokines synthesis in monocytes. Interferon alpha (IFN- $\alpha$ ) has been shown to induce IL-10 production in human peripheral blood mononuclear cells. The aim of the study was assessment of the effect of the IFN- $\alpha$  treatment on serum IL-10 levels in patients with Chronic hepatitis B (VHB) and C (VHC) and to investigate the effect of therapy with IFN- $\alpha$  on serum hyaluronic acid (HA) concentration as a potential biochemical marker of liver fibrosis. The relationship of the IL-10 pattern to short virological response to the treatment was also studied. Methods: Serum IL-10 was measured by a commercially available kit Quantikine® (R&D Systems). Blood samples for serum IL-10 analysis were obtained before the treatment, in weeks 4 to 8 of the treatment and in weeks 20 to 28 of the treatment. Hyaluronic acid (HA) concentrations (Hyaluronic acid „Chugaí“) before and after treatment with IFN-alpha (48 week) in patients were assayed. Results: Serum HA level before the treatment correlated with the extent of liver fibrosis ( $r=0.87$ ,  $p<0.001$ ). We observed statistically significant decrease in HA in all patients (good responders and non-responders as well), after the finishing of the treatment by IFN alpha. All patients with VHB and good responders in the VHC group had significantly higher pre-treatment IL-10 levels, when compared to controls. During the treatment, a constant decrease in IL-10 was observed in VHB good responders subgroup, reaching the significant difference only in month 6. In VHC patients in the good responders subgroup a significant decrease in IL-10 levels was observed in month 1, while an increase was observed in non-responders subgroup. Conclusions: Serum HA measurement is a good and clinically useful non-invasive marker of liver fibrosis. It could be therefore used for monitoring of the stage of fibrosis as a measurement of response to antifibrotic therapy. Serum IL-10 assessment might be used as a response-predicting marker in management of patients with chronic viral hepatitis treated with IFN- $\alpha$

#### THE EFFECT OF VOLTAGE DEPENDENT CURRENTS ON THE TIME PRECISION OF CA3 PYRAMIDAL NEURONS

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Different concepts about neuronal coding have been proposed until now. Some of them suppose information is encoded by precise occurrences of APs (temporal codes; (1)), the others state that only average frequency, measured during relevant time window, represents information (rate codes; (2)). For temporal codes to be plausible, neurons should be endowed with mechanisms enabling them to generate APs with sufficient reliability and time precision. To what extent are neurons able to employ temporal coding depends partly on the temporal richness of their inputs, on the arrangement of neuronal “hardware” and also on the neuronal noise interfering with the signal. The last feature causes the process of spike encoding is noisy, which results in variable timing of individual APs in response to identical inputs (3). In many sensory systems it is relatively easy to assess this time precision experimentally, however in other systems the neuronal activity do not correlate with available stimuli so apparently. In the software environment GENESIS 2.2, we constructed a multicompartmental model of rat CA3 hippocampal pyramidal neuron, consisting of soma, dendritic tree and axonal initial segment (IS). We implemented voltage dependent conductances (Na fast current, high-voltage activated-slowly

inactivating Ca current, delayed rectifier K current, A-type of transient K current, long-duration Ca-dependent AHP K current, short-duration voltage and Ca-dependent K current) that included the mechanisms of channel noise generation. The noise corrupted the propagation of postsynaptic signal at the soma-dendritic (SD) membrane and the AP initiation at the IS. Random spatio-temporal patterns of synaptic activity were presented to the neuron repeatedly and elicited sequences of APs were analyzed by peri-stimulus time histograms (PSTHs) and statistically processed. The channel noise affected the time precision (measured by standard deviation of AP time jitter) of neuronal responses that varied from milliseconds (due to the channel noise of voltage-dependent channels with slow dynamic) to submilliseconds or even few microseconds for the Na channel noise only. The variability of the spatio-temporal pattern of synaptic activity, causing different voltage slopes at the IS, influenced the precision of APs maximally by an factor of 10.

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#### CONTRIBUTION OF PUTATIVE VOLTAGE SENSORS IN DOMAINS I – IV OF THE CA<sub>v</sub>3.1 CALCIUM CHANNEL TO THE CHANNEL GATING

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Voltage activated calcium channels consist of four homological domains, each containing permanently charged S4 segment. These S4 segments form putative voltage sensor of the channel. According to current hypothesis opening and closing of voltage-dependent channels is accompanied by movement of voltage sensor. We have investigated the contribution of S4 segments in individual domains of the murine T-type Ca<sub>v</sub>3.1 channel to the channel gating by replacement of charged arginine residues by neutral cysteines. Four point mutants were constructed: R180C, R834C, R1379C and R1717C and four double mutants combining mutations in two neighboring domains. Whole cell current was measured from HEK 293 cells transiently expressing individual channels with 2 mM Ca<sup>2+</sup> as a charge carrier. Voltage dependence of steady-state inactivation was fitted by a single Boltzmann function. Voltage dependence of current inactivation was significantly shifted in all four mutants and the corresponding slope factor was significantly increased in all cases. Voltages for half-maximal inactivation ( $V_{0.5}$ ) were as follow: -67.0±1.3 mV (wild type), -74.0±1.7 mV (R180C), -76.7±1.5 mV (R834C), -84.2±0.9 mV (R1379C) and -75.0±1.5 mV (R1717C). Corresponding slope factors (dV) were: 4.6±0.3 mV (wild type), 7.6±0.4 mV (R180C), 6.6±0.2 mV (R834C), 8.3±0.2 mV (R1379C) and 6.4±0.3 mV (R1717C). Time constants of current activation determined from Hodgkin-Huxley fit of individual current traces were moderately accelerated at depolarizations just above the threshold for current activation. Time constants of current inactivation were not affected by above-mentioned mutations. All observed effects were approximately additive in double mutants.

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#### REGULATION OF STRESS-INDUCED INCREASE IN TYROSINE HYDROXYLASE GENE EXPRESSION AND PROTEIN IN THE DORSOMEDIAL HYPOTHALAMIC NUCLEUS OF RATS

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The dorsomedial nucleus (DMN) has been suggested as an important coordinate center in the regulation of autonomic and neuroendocrine system activities, especially in elaborating of response to stress. Previous studies have shown an existence of tyrosine hydroxylase (TH) immunopositive cells in DMN. We studied gene expression of catecholamine biosynthetic enzymes and *in vivo* norepinephrine release in the DMN in rats under control and stressful conditions. Moreover, we investigated effect of transection of brainstem pathways, or posterolateral surgical deafferentation of the dorsomedial or paraventricular nucleus on tyrosine hydroxylase gene expression in DMN. Both, single and repeated exposure to immobilization stress (IMO) for 2 hrs/day elicited about 6-fold increase in TH mRNA. Moreover, a huge increase in extracellular norepinephrine levels in DMN was observed in acutely stressed rats. TH protein levels were only slightly elevated after repeated IMO. Dopamine-β-hydroxylase mRNA has also been detected in DMN but only in a very low level estimated by seminested PCR. In contrast, PNMT mRNA level was detectable, especially after stress. Surgical transection of ascending neuronal pathways at the lower brainstem, as well as knife cuts immediately caudal and lateral to the DMN abolished IMO-induced increase in TH mRNA level in the DMN. However, knife cuts rostral and lateral to the DMN (transection between the PVN and the DMN) did not influence the stress-induced increase TH mRNA level in the DMN. Our data indicate that TH gene expression is induced by a single and repeated IMO stress in dorsomedial hypothalamic neurons. The expression of TH in the DMN seems to be controlled by ascending inputs from lower brainstem or spinal cord neurons but not by hypothalamic paraventricular neurons.

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#### INFLUENCE OF SCAVENGERS OF REACTIVE OXYGEN SPECIES ON LEARNING IMPAIRMENT ELICITED BY FLUROTHYL EPILEPTIC SEIZURE.

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Wistar albino rats exhibited impairment of learning in water maze after one epileptic seizure elicited by Flurothyl. In our previous experiments we demonstrated that the learning could be preserved by hypoxic preconditioning (3 days prior the seizure). The same effect was observed after the application of melatonin 1 h prior the seizure. We suppose that this effect is probably related to the scavenger activity of melatonin. Therefore we tested time relation of its application and action of another scavenger – reduced glutathione. The experiments were performed on young male Wistar rats. Application of melatonin (100 mg/kg i.p.) 2.5 min after the seizure improved the last phase of learning since the 4th day. We observed similar effect after application of glutathione (50 mg/kg i.p.) 10 min before the seizure. The effect of melatonin application 6 h after the seizure was not apparent. Pretreatment with melatonin 1h prior the hypobaric hypoxia decreased preconditioning effect. Our results support the possibility of

involvement of free radicals in some functional changes caused by epileptic seizure.

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#### A BIOCHEMICAL AND HISTOCHEMICAL STUDY OF GAMMA-GLUTAMYLTRANSPEPTIDASE (GGT) ACTIVITY IN HUMAN BRAIN TUMORS

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Growth progression of tumors is controlled by complex interactions between the parenchymal transformed cells and the blood vessels. In brain gliomas, these interactions involve up-regulation of several receptor-ligand molecular systems, including cell adhesions, growth factor receptors, activity of some enzymes and other molecular species. In this study we examined catalytic activity of GGT, the specific molecular constituents of the normal brain vascular system and its astroglial envelopes, in biopsies of several types of human brain tumors of different WHO grade of malignancy (G- I to IV). In gliomas, activity of GGT was found to vary both within the individual biopsy samples and the malignancy grade scale. Within the tumor, GGT was localized primarily in the blood vessels. In G-IV glioblastoma multiforme, high enzyme activity was observed also in the in between tumor parenchyma, rich in the transformed astrocytes. The latter displayed often a hypertrophic morphology and GFAP immune-positive fibers. As a consequence, the average values of GGT activity in higher grade tumors, i.e. anaplastic astrocytoma (G-III) and glioblastoma multiforme (G-IV) samples, was higher then in those of lower WHO grades. Activity of GGT was relatively high also in the ventricle-appendages-derived tumors i.e. from the cells which are GGT strongly positive already under normal conditions as well as the samples with post-irradiation reactive gliosis. The tumor grade dependence of GGT activity was also evident, although at the much lower base line, in G-I and G-II meningiomas. We assume that up-regulation of GGT is primarily related to maintenance of redox potential homeostasis (GGT) of tumors and higher level of oxidative stress in their higher grade groups.

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#### CHANGES OF THE ACTIVITY OF CORTICAL NERONES IN OFFSPRING OF MOTHERS EXPOSED TO ETHANOL ABUSUS

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Prenatal exposure to ethanol can cause many disorders characterized by central nervous system dysfunction with anatomical abnormalities in several brain regions. It is probably an outcome of numerous mechanisms (ethanol-induced process of apoptosis, interaction with neurotransmitter systems, etc.). In the present study functional changes of cortical neurones in offspring of mothers exposed to chronic ethanol intake were tested. Mothers obtained ethanol during all pregnancy and lactation in the drinking water as a 10 or 20 % solution. Mothers of controls offspring did not received alcohol. Excitability of cortical

neurones was tested in young rats (18, 25 and 35-day-old) by repeated stimulation of the sensorimotor cortex (frequency 8 Hz, pulse duration 1 ms, intensity 3-5 mA and 15 s stimulation was repeated 5times always 1 min after the end of previous evoked cortical afterdischarge - AD). The duration of evoked cortical afterdischarges was measured and results were statistically evaluated. In offspring of mothers drinking 10 % solution of ethanol, the evoked cortical activity was effected only in minimal extend. In 18-day-old rats (mothers drinking 20 % solution of ethanol), the shortening of duration of cortical afterdischarges was statistically significant ( $p \leq 0.001$ ). In older animals (25 and 35-day-old) the duration of ADs did not differ from that in control animals. Alcohol drinking during pregnancy can affect the brain development of their offspring – from mild impairment of brain function to full-blown fetal alcohol syndrome (FAS).

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#### EFFECT OF TRANSGENIC RESISTIN EXPRESSION ON PROTEIN KINASE C ACTIVATION AND SKELETAL MUSCLE INSULIN SENSITIVITY

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Resistin has been proposed as adipokine involved in the etiology of insulin resistance. However, the mechanism(s) responsible for impaired glucose metabolism in skeletal muscle is still unclear. We have previously shown that transgenic expression of resistin in the adipose tissue of spontaneously hypertensive rats (SHR) was associated with increased serum free fatty acids, skeletal muscle triglyceride accumulation and resistance to insulin action. In this study we investigated if increased expression of resistin in adipose tissue is related to protein kinase C (PKC) activation and cellular localization in skeletal muscle. One year old male SHR expressing the mouse resistin gene under control of fat-specific aP2 promoter were used. Control group comprised age-matched genetically identical rats with absence of the transgene. All animals were fed a diet with 60 % fructose for 2 weeks before the end of the study. Tissue sensitivity to insulin action was measured in vitro without or with insulin (250  $\mu$ U/ml) according to basal and insulin-stimulated <sup>14</sup>C-U-glucose incorporation into muscle glycogen and oxidation to CO<sub>2</sub>. One year old transgenic rats displayed higher body weight (389 $\pm$ 6 vs 370 $\pm$ 5 g,  $p < 0.05$ ) and elevated epididymal fat pad weight (0.922 $\pm$ 0.035 vs 0.709 $\pm$ 0.043 g /100g BW,  $p < 0.02$ ) compared to the control group. Serum triglyceride concentrations were increased (1.99 $\pm$ 0.15 vs 1.34 $\pm$ 0.11 mmol/l,  $p < 0.01$ ). The transgenic expression of resistin substantially impaired the tolerance to the oral glucose load (AUC<sub>0-120</sub>: 1026 $\pm$ 131 vs. 725 $\pm$ 14 mmol/l/120 min.,  $p < 0.02$ ) and increased hyperinsulinemia. Expression of transgenic resistin was associated with almost total adipose tissue resistance to insulin action. Skeletal muscles isolated from transgenic rats exhibited a significant decrease in glucose oxidation, measured in vitro according to <sup>14</sup>C-U-glucose incorporation into CO<sub>2</sub>. Study of insulin signalling indicated that there was a significantly higher protein content of PKC  $\theta$  isoform by 39 % ( $p < 0.05$ ) and 318 % ( $p < 0.01$ ) in membrane and cytosolic fraction, respectively. The results indicate that chronic transgenic expression of resistin gene was associated in one-year old animals with increased serum triglycerides, hyperinsulinemia, markedly impaired glucose tolerance and suggest possible involvement of PKC  $\theta$  activation in development of insulin resistance.

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# CORTICAL PHOTOTHROMBOTIC ISCHEMIC LESION: INFLUENCE OF HYPOXIC PRECONDITIONING AND MELATONIN APPLICATION

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Rose Bengal (RB) technique of photothrombotic occlusion of blood vessels is a commonly used model of focal ischemic lesions. It is based on the sensitization of endothelial cells and the subsequent activation of blood coagulation, which are both closely related to the formation of reactive oxygen species (ROS). ROS are also involved in the general mechanisms of neural injury and hypoxic/ischemic preconditioning. We tested the sensitivity of this model of focal ischemia to preconditioning with hypobaric hypoxia and pre-treatment with the ROS scavenger melatonin. Experiments were performed on adult male Wistar rats. Controls were not exposed to any other experimental manipulation. One group of experimental animals was exposed to hypobaric hypoxia (1 hour, 9 000 m) 72 hours before irradiation. Other group was pre-treated by i.p. melatonin 1 hour before photothrombosis. Under ketamine/xylazine anesthesia the RB solution was applied via the tail vein. Immediately after application the skull over the left sensorimotor cortex was exposed to a laser beam (532 nm) for 9 min. 72 hours after the irradiation, the brains were perfused, 1 mm coronary slices were obtained and stained with TTC (2,3,5-triphenyltetrazolium chloride). In the control group, surface lesion was observed only in 1 animal, while 15 animals developed deep damage involving the striatum. In the group pre-exposed to hypoxia, 1 animal displayed a surface defect and 10 animals showed severe lesions. In the group of animals pre-treated with melatonin, surface lesion was observed in 10 animals, whereas only 4 animals developed deep lesion. Chi square test has shown highly significant differences among all three groups. Conclusions: Pre-exposure to hypobaric hypoxia did not induce significant protection of ischemic damage in our experiment. However, pre-treatment with melatonin significantly reduced the size of brain tissue damage in this model. We suppose that the scavenger effect of melatonin influenced primarily the pathological changes in the vascular endothelium and the subsequent blood coagulation during and after irradiation.

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# DISODIUM CROMOGLYCATE REDUCES HYPOXIA-INDUCED PULMONARY HYPERTENSION IN RATS

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Exposure to chronic hypoxia results in hypoxic pulmonary hypertension. We hypothesize that collagenolytic enzymes produced among others by mast cells can play an important role in remodeling of the peripheral pulmonary vessels. Present study was designed to determine whether disodium cromoglycate (DSCG) an inhibitor of the mast cell secretion prevents development of hypoxic pulmonary hypertension in rats exposed to chronic hypoxia. Four groups of adult male Wistar rats were studied. Experimental groups were exposed for 3 weeks to isobaric hypoxia (F<sub>1</sub>O<sub>2</sub> 0.1) and treated with DSCG (40 mg/kg, i.p.). DSCG was given first 4 days of hypoxic exposure (group DSCG + H, n=8) and last 4 days of exposure (H + DSCG, n=8). These groups were compared with untreated rats exposed to hypoxia (H, n=8) and with normoxic controls (N, n=8). Cardiac output (CO), pulmonary arterial blood pressure (PAP), systemic arterial blood pressure (SAP), right and left heart ventricle weight were measured. The results evaluated by ANOVA with Fischer's post-hoc test are summarized in Table.

Group	CO (ml)	PAP (mmHg)	SAP (mmHg)	RV/BW (mg/100 g)
N	61±3	15±1	114±4	46±1
H	37±3*	30±2*	112±4	110±7*4
DSCG + H	38±2*	25±2*#	111±5	100±7*
H + DSCG	35±2*	33±2*	118±4	89±2*#

*Data are means ± SEM, \*p<0.0001 groups differ from group N, #p 0.05 groups with DSCG differ from group H.*

We conclude that DSCG applied in the early phase of exposure to hypoxia significantly reduces the development of hypoxic pulmonary hypertension.

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# NEIGHBORHOOD OF T-TUBULES IN DYADS OF OXIDATIVE MUSCLES IN MICE

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Oxidative muscles are characterized by high content of mitochondria that are localized mostly near I-bands in skeletal muscle cells but along sarcomeres in cardiac muscle cells. In slow muscles, direct communication of mitochondria with membranes of T-tubules was described at sites of dyadic connections (1). Based on short distance between mitochondria and the dyadic complex in cardiac myocytes, possible role of mitochondria in sequestration of Ca<sup>2+</sup> ions was considered (2). The aim of this study was to analyze and to compare environment of the T-tubules in dyads of two distinct types of oxidative muscles – soleus and ventricular muscles. A stereological method of vertical sections was used for analysis of electron microscopic images (3). It was found that the volume and surface densities of membranes of T-tubules and of cisterns of the sarcoplasmic reticulum (SR) were similar in both types of oxidative muscles and the difference was not statistically significant. Analysis of the neighbourhood revealed similar occurrence of various organelles in the vicinity of T-tubules in both muscle types. Prevalently, membranes of SR cisterns (54 % soleus and 64 % ventricles), cytoplasm (20 % and 24 %), actin filaments of I-bands (12 % and 4 %), mitochondria (8 % and 4 %), and myosin filaments of A-bands (4 % a 3 %) were found in the nearest neighbourhood of T-tubules. Less frequently were found membranes of SR (1 % a 2 %), lipid droplets (0.4 % a 0.0 %), and membranes of the T-system (0.2 % a 0.0 %). Our stereological analysis provided the first quantitative data on the neighbourhood of T-tubules in dyads of muscle cells. Results are in agreement with understanding of the dyadic function in excitation-contraction coupling. However, they also point to substantial extent of interaction between membranes of T-tubules and of mitochondria that can be of significance for regulation of energetics in skeletal and cardiac types of oxidative muscles.

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# ALCOHOL ABUSE IN MOTHERS DURING GRAVIDITY AND CHANGES OF HIPPOCAMPAL NEURONS IN THEIR OFFSPRING

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A neurotoxic effect of alcohol on the CNS of laboratory rats in the prenatal and postnatal period was studied. Another aim of the experiment was to analyse structure of the hippocampus after the prenatal and postnatal exposure to alcohol and to identify the most vulnerable hippocampal regions. Pregnant Wistar rats of our own breed received 20 % alcohol, p.o. at libitum, every day since the conception to weaning of their offspring (the 28th day of postnatal life). Since the birth (the day 1) till the age of 28 days offspring were kept together with their mother and were exposed to postnatal alcohol effect (alcohol in breast milk). At the age of 18, 35 or 90 days animals were perfused under deep thiopental anaesthesia with buffered solution of paraformaldehyde. Serial sections were stained with Fluoro-Jade B and DNA specific dye bis-benzimide (Hoechst). The brain of young rats aged 18, 35 and 90 days was analysed under the light microscope. In CA1 and CA3 areas and in Gyrus dentatus of the hippocampus, groups of degenerating cells were observed. In 90-day-old offspring no degenerating cells were observed in the above mentioned areas of the hippocampus. In all offspring (in the age of 18, 35 and 90 days) some cells with fine granulated karyons were identified, which were accompanied with high numbers of glial cells. Our results demonstrate the neurotoxic effects of alcohol and the high vulnerability of the developing CNS. Remarkable is the observation of high numbers of dying cells one week after the last exposition to alcohol in 35-day-old offspring. It suggests that the process of neuronal circuit remodelling in the juvenile tissue is long-term and is probably triggered by apoptosis. The identification of cells with fine granulated karyons indicates the role of apoptotic mechanism in the cell death.

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# POSSIBILITIES OF IMPROVEMENT VIDEOCAPILAROSCOPY FOR ANALYSIS OF CHANGES OF THE VASCULATURES UNDER THE EXPERIMENTAL CONDITIONS

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**Background And Purpose:** This study was designed to investigate the changes of the diameter of vasculatures under local pharmacological intervention. **Methods:** The microcirculation in the rat mesentery of the 12 spontaneous hypertensive rats was visualized in real time with the used intravital videomicroscopy. Under total anesthesia the mesentery was carefully placed into a glass chamber with 0.9 % NaCl solution (with temperature 37 °C). The chamber was placed on the stage of the reflex microscope for *in vivo* microscopy recording. Norepinephrine NE (five different concentrations from 10<sup>-8</sup> to 10<sup>-4</sup> in 0.9 % NaCl solution) was locally applied as describes before (1). We started recording every 30 s for 6 minutes, when zero time was 1 minutes before application NE. Hearts were removed directly after observation and processed for histology. **Results:** In control conditions (before measuring and during application of NaCl solution without NE), the velocity of erythrocytes through capillaries was 298.68±48.92 microm/s (mean ± SD), and the diameter of the vessels studied was 45.15±29.9 microm. We observed the changes in diameter and speed of circulation after local application. It was different in time of observe for different concentrations of NE. Maximum percentual decrease of diameter was in the case of c = 10<sup>-5</sup> NE (as it was described before at arterioles (2), but not at venules) and one minute after application (decrease was 23.07±3.19 %). 5 minutes

after using NE the diameter of vessels got back to the value before application (difference was 1.14±17.32 %). **Conclusion:** We showed that the capillaroscopy could be used after adaptation for *in vivo* real-time measurement of red blood cell velocity and selected morphometric parameters in rats. The frame-to-frame method allowed quantifying morphometric parameters of selected microvessels. We found that for invocation of contraction of micro vessels is most effective concentration of 10<sup>-5</sup> NE and it expires after 5 minutes.

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# THE COMPARISON OF IMMUNOLOGICAL REACTION AGAINST HUMAN CA MAMMAE AND MICE LDH VIRUS ANTIGENS

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We observed the immunological answer on antigens obtained by means of high-pressure gel chromatography (HPGC) from the human malignant breast tumor and from the blood of inbred C3H/H2K strain mice infected by a mice LDH virus (LDV) (1,2). We used the ELISA method modified by us (3) for testing giving antigens. We selected 181 women patients with a various degree of a non-malignant diseases of the breast and 415 women patients with malignant breast tumor from total 631 women examined. We determined a titer of common specific antibodies and titer of IgG and IgM class antibodies in all blood samples tested of woman patients. The titer of antibodies was determined against the specific antigen prepared from the malignant breast tumor and against the non-specific antigen prepared from the mice LDV. Based on the knowledge regarding a protective influence of sexual hormones on the immunological state of the organism, which decreases with an increasing age, the set of women with a non-malignant breast disease was divided in two groups, with the criterion being the dividing value of 35 years of age. The modified ELISA method is suitable for early diagnosing and monitoring antibodies in the cases of malignant breast tumors simultaneously with senological examinations which include mammography and clinical examinations (4).

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# BRAIN REGION SPECIFIC CHANGES IN $\alpha$ -ADRENERGIC RECEPTOR LEVEL IN ACETYLCHOLINESTERASE KNOCKOUT MICE

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The acetylcholinesterase knockout (AChE -/-) mouse has no AChE activity and no AChE protein which leads to the increase in the acetylcholine level (1). Hyperstimulation of muscarinic receptors (MR) by this neurotransmitter results in their down-regulation and regulation of other G-protein coupled receptors (2). The aim of this work was to

examine the  $\alpha_1$ -adrenoceptor ( $\alpha_1$ -AR) distribution within the central nervous system (CNS) of these animals. Adult (over 60 days old) wild-type and AChE  $-/-$  mice were used in the study (3). Animals were euthanized, brains removed and different functional regions were dissected (medulla oblongata, cerebellum, frontal, parietal and occipital cortex, striatum, thalamus and hypothalamus).  $\alpha_1$ -AR were quantified by radioligand binding study, using a specific ligand [ $^3$ H] prazosin. There was a substantial decrease in  $\alpha_1$ -AR observed in AChE  $-/-$  occipital and parietal cortex comparing to wild-type animals. However the level of  $\alpha_1$ -AR in AChE  $-/-$  thalamus was about fourfold higher than in wild-type counterparts. Other studied brain regions did not display any differences between two genotypes. It is supposed that AChE  $-/-$  mice are able to live due to multiple adaptation changes in their phenotype. Cholinergic adaptations were described (4, 5) in the past. Unfortunately only little attention has been paid to the non-cholinergic changes in AChE  $-/-$  CNS. We show in this work that  $\alpha$ -adrenoceptors are significantly changed in the CNS of AChE  $-/-$  mice while these changes are brain-region specific.

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#### LIMB EXPLANT CULTURES IN APOPTOSIS RESEARCH

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Limb development provides an interesting model to study apoptosis during mammalian embryogenesis. Interdigital web regression is a prominent morphogenetic event resulting in digit separation. Morphogens engaged in this process involve e. g. members of BMP and Wnt superfamilies. Cysteine proteases called caspases mediate intracellular signal transduction and apoptosis execution. To investigate molecular machinery engaged in apoptosis signalling, *ex vivo* systems are of a great importance. Limb explant cultures are thus an useful tool to investigate particular steps of apoptotic cell death machinery and to modulate signalling pathways which can not be silenced by knock-out due to early embryonic lethality. Mouse front limbs, embryonic day 12.5 and 13.5, respectively, were mounted on filters supported by metal grids and cultured up to 5 days *ex vivo*. Inspection of general morphology of explanted limbs indicated normal digit separation and interdigital tissue regression as *in vivo*, just slightly delayed. Having established the limb explant culture system, pharmaceutical inhibition of caspases was performed and impact on digit separation evaluated. Two methods of inhibitor delivery were tested, implanted beads soaked in the inhibitor and inhibitor diluted in the culture medium. Inhibitor penetration throughout the tissue was confirmed using a biotinylated pan caspase inhibitor followed by tracing of biotin in the histological sections by alkaline phosphatase color reaction. The best results were achieved with inhibitor provided in the medium. In these samples, inhibition of interdigital web regression was achieved and the digits did not separate. Explant cultures represent an unique opportunity to perform "animal-friendly" functional studies. Thus, individual caspases engaged in interdigital apoptosis can be further followed and also cross-inhibitions revealing molecular signalling networks engaged in embryonic apoptosis can be easily investigated.

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#### ELECTROPHYSIOLOGICAL EFFECTS OF SIGMA LIGAND HALOPERIDOL

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Sigma receptor ligand haloperidol is a psychotropic drug with severe cardiovascular side effects, particularly ventricular arrhythmias. We investigated the effects of haloperidol on the time course of action potential and on the basic ionic currents, namely on the sodium current  $I_{Na}$ , calcium current  $I_{Ca}$ , and potassium currents  $I_{to}$ ,  $I_{K1}$  and  $I_{K,end}$  (potassium current at the end of 250 ms long pulse). The experiments were performed on enzymatically isolated rat ventricular cardiomyocytes using the whole cell patch clamp technique at room temperature. Haloperidol induced reversible and concentration-dependent inhibition of all investigated membrane currents. 1  $\mu$ mol/l inhibited  $I_{Na}$  by 39 %,  $I_{Ca}$  by 19.5 %,  $I_{to}$  by 23 % and  $I_{K,end}$  by 14 %. 10  $\mu$ mol/l haloperidol inhibited the same currents by 95 %, 22 %, 80 % 37 %, respectively, and  $I_{K1}$  by 29 %. We observed also reversible loss of action potential in presence of 10  $\mu$ mol/l haloperidol. Inhibition of  $I_{to}$ -amplitude by haloperidol was accompanied by acceleration of apparent inactivation. Both were voltage-independent. The time course of recovery of  $I_{to}$  from inactivation was decelerated in presence of drug and 9 % of  $I_{to}$ -channels recovered with slow time constant about 1.4 s. It corresponded with a cumulation of inhibition at higher stimulation frequencies (3.3 Hz). We conclude that haloperidol inhibited all the main ionic current in rat ventricular myocytes with profound inhibition of  $I_{Na}$  that corresponded with loss of action potential in presence of 10  $\mu$ mol/l haloperidol. Inhibition of  $I_{to}$  is very likely caused by interaction of haloperidol with  $I_{to}$ -channels in open and in inactivated states as supported by the results of mathematical simulations.

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#### RELATIONSHIP BETWEEN EJECTION FRACTION AND BLOOD PRESSURE AND BAROREFLEX SENSITIVITY IN CHILDREN AND ADOLESCENT AFTER ANTHRACYCLINE THERAPY

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Introduction: Anthracyclines are frequently used in a treatment of hematological malignities and some solid tumours in children and adolescents. Cardiotoxic side effect of anthracyclines was described. The decrease of systolic and diastolic blood pressure (SBP, DBP) after treatment, which appears some years after termination of antitumour therapy, was also observed. The aim of this study was evaluation of relationships between left ventricle (LV) function expressed as indices ejection fraction (EF) and fractionally shortening (FS), SBP and DBP and baroreflex sensitivity are present in this study. Method: Forty patients (mean age $\pm$ SD: 14.3 $\pm$ 3.7 years) after anthracycline treatment were examined. Mean period between termination of therapy and examination was 1.5 years. Ejection fraction and fractionally shortening of LV were measured by echocardiography. Baroreflex sensitivity in ms/mmHg (BRS) and in mHz/mmHg (BRSf) was determined with spectral method (5 minute non-invasive continuous blood pressure

recording by Finapres at metronome controlled breathing). The values of EF, FS, SBP, DBP, BRS and BRSf were normalized according to age by linear regression (k). Spearman's correlation coefficient (r) was calculated. Results: Following values of linear regression coefficients for standardisation to age 15 years were estimated: EF:k=-0.1859, FS:k=-0.1272, SBP:k=1.0184, DBP:k=0.5572, BRS:k=-0.2324, BRSf:k=-0.617. We found positive correlations between EF and SBP ( $r=0.359$ ,  $p<0.05$ ); FS and SBP ( $r=0.386$ ,  $p<0.05$ ); EF and DBP ( $r=0.296$ ,  $p=0.064$ , n.s.); FS and DBP ( $r=0.289$ ,  $p=0.071$ , n.s.). Relationship between EF and BRS was not found. Conclusion: The positive correlation between EF and blood pressure may be an indicator a link between the changes of blood pressure and anthracycline's cardiotoxicity. Baroreflex sensitivity is not influenced.

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#### AFFINITY OF FLUORESCENT INDICATORS Rhod-5N, MagRhod-2 AND MagFluo-4 TO CALCIUM AND MAGNESIUM

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The low-affinity fluorescent indicators Rhod-5N, MagRhod-2 and MagFluo-4 are used in confocal microscopy for measuring intracellular concentrations of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions. However, the association constants of their complexes with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  are not known with sufficient precision and often vary from batch to batch (1, 2). We have measured the calcium dependence of fluorescence of Rhod-5N, as well as the magnesium dependence of fluorescence of MagRhod-2 and MagFluo-4, using high temporal resolution confocal spot microfluorimetry. The fluorescence of Rhod- and Fluo-based indicators was excited by the 543nm HeNe and 488 nm Ar lines, respectively, and the fluorescent emission was measured in the range of 550-600 nm and above 505 nm, respectively. Because of the low affinity of the indicators and unavailability of low-affinity  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  buffers, the measurements were done in the absence of buffers, and the theoretical concentration dependence accounted for the material balance of all components. The properties of the indicators are summarized in Table 1. The affinity of all indicators to the studied cations was very low. Interestingly, the maximum fluorescence increase upon complexation  $(\Delta F/F)_{\max}$  was much smaller than that advertised by the supplier (3).

Table 1. The properties of fluorescent indicators

Indicator	Ion	$K_{\text{Me}}$ (mM)	$(\Delta F/F)_{\max}$	n
Rhod-5N	$\text{Ca}^{2+}$	$0.745 \pm 0.058$	$7.90 \pm 0.37$	7
MagRhod-2	$\text{Mg}^{2+}$	$1.72 \pm 0.35$	$1.98 \pm 0.04$	4
MagFluo-4	$\text{Mg}^{2+}$	$12.1 \pm 1.0$	$1.53 \pm 0.06$	4

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#### OPTICAL COMPUTER MOUSE AS A SENSOR OF SMALL MOVEMENTS FOR ANIMAL PHYSIOLOGY LABS

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Modern optical mice are equipped with very precise sensors of movement. Their common resolution is 800 dpi and the best available ones have resolution up to 2000 dpi. That means that even with older

models we can track motions with precision of 30  $\mu\text{m}$ . This is sufficient to isotonic muscle physiology exercises. The sensor monitors movements of the surface which is less than 1 mm distant, so it is necessary to transfer the muscle contraction to movement of some object in focus of the mouse sensor. Practical design is described on a prepared gastrocnemius muscle with an attached nervus ischiadicus of young *Xenopus laevis* adults ( $>3\text{cm}$ ). The muscle is held through remnants of femur. To the tendon of Achilles a thread is bound on which a strip of shaded transparent plastic film is tied, which moves in front of the sensor and which position in focus of the sensor is maintained by a smooth metal plate. Nervus ischiadicus is fixed on a pair of AgCl electrodes, which are stimulated by a simple digital to analog converter (DAC) attached to computer printer parallel port. Computer program can be programmed to stimulate the muscle by DAC at rate of 1 kHz, which is sufficient to achieve tetanic contraction. Mouse movements and potentials on electrodes are graphically displayed and logged to a file, which can be later processed by any suitable computer program. Software simulation proved insufficient in the past, so we decided to enrich the labs by a voluntary work with real muscles. This method is both instructive and cheap. The hardware cost for one work group is less than 2000 CZK (80 USD) and the size of experimental animals is limited by manual skill of each student.

#### CLINICAL UTILITY OF THE CONTINUOUS GLUCOSE MONITORING IN INTENSIFIED INSULIN TREATED PATIENTS

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Introduction: Clinical use of the continuous glucose monitoring (CGM) system provides significantly more information on glucose patterns than self-monitoring of blood glucose (SMBG). Our aim was to analyze CGM profiles in intensified insulin treated patients in order to uncover correctable factors hidden from detection with conventional SMBG. Methods: 47 type 1 poorly-controlled diabetics ( $\bar{X}$  age $\pm$ SD =  $23.47\pm 11.33$  years,  $\bar{X}$  duration of diabetes =  $7.83\pm 7.34$  years,  $\bar{X}$  HbA1c =  $9.56\pm 1.48$  %,  $\bar{X}$  BMI =  $21.98\pm 3.67$ ) were monitored using CGM system (Medtronic MiniMed) for several days during normal activity, in conjunction with SMBG tests conducted at least 4x per day. Duration, frequency and cause of hyper- and hypoglycemic excursions were analyzed. Results were presented as means $\pm$  SD and percentage of time period spent hypo- or hyperglycemic. We evaluated HbA1c at baseline and 3 months after CGMS-based therapy changes. Statistical analyses included correlation, mean absolute difference (MAD) and paired Student's t-test. Results: 51390 CGM values ( $\bar{X}$  CGM duration =  $97.67\pm 28.44$  h) compared with 949 capillary SMBG values ( $\bar{X}$   $r=0.86\pm 0.09$ ;  $\bar{X}$  MAD =  $16.37\pm 5.31$  %) showed 645 hypoglycemic episodes ( $<3.5$  mmol/l at least 10min). 63 % of nocturnal hypoglycemia was during 1am-4am period, 35 % of daytime hypoglycemia during 10am-1pm period. From 166 nights only 2,2 % were hypoglycemic, but 5 asymptomatic episodes were prolonged ( $>280\text{min}$ ). 92 % of nocturnal and 65 % of daytime hypoglycemia was undetected by SMBG. CGM uncovered lowered BG post-exercise, continued fall for 1,5h post-exercise and lower BG following day, in 3 cases causing nocturnal hypoglycemia. Subjects showed 38.8 % prevalence of hyperglycemia ( $\bar{X}$  duration 9.3 h/patient/day), mostly attributed to diet fault or insufficient lag time. 75 % of post-hypoglycemic hyperglycemia was caused by overeating, 20 % was attributed to rebound hyperglycemia. Dawn Phenomenon was detected in 4 subjects. Stress hyperglycemia tended to come down without additional insulin. HbA1c decreased significantly from  $9.56\pm 1.48$  % at baseline to  $8.86\pm 1.38$  % at 3 months follow-up ( $p=0.021$ ). Conclusions: The CGM completed picture of patients' glycemic responses to sleep, work, exercise, food intake and insulin dose, leading to improved glycemic control.



*The study provided additional support for the clinical usefulness of the CGM system in the intensified insulin management of the diabetic patients*

#### VASCULAR SMOOTH MUSCLE CELLS IN CULTURES ON SYNTHETIC POLYMERS PATTERNED WITH ADHESIVE MICRODOMAINS

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Clinically used blood vessel prostheses are often made of polyethyleneterephthalate (PET). However, a relatively high hydrophobicity of this material does not allow for introduction of the physiological cellular component into the prostheses. Thus, PET foils were irradiated by ultraviolet (UV) light generated by Xe<sup>2+</sup>-excimer lamp (Heraeus-Noblelight, Germany) for 10, 20 or 30 min in an acetylene atmosphere through a nickel contact mask (500 µm wide holes with distances of 2 mm) in order to achieve a regionally-selective cell adhesion. The material was seeded with vascular smooth muscle cells (VSMC) derived from the rat aorta (passage 3, 17 000 cells/cm<sup>2</sup>). On day 1 after seeding, the total cell numbers found on the modified material surfaces were similar or even significantly lower in comparison to the values obtained on the unmodified PET. On day 3 after seeding, the values on all irradiated samples became several times higher than those on the unmodified PET, but on day 7, these differences disappeared. In addition, the cells were distributed homogeneously without selective adhesion to a certain domains on the polymer surface. However, in our earlier experiments, when polytetrafluoroethylene foils were irradiated by UV light in an NH<sub>3</sub> atmosphere, the cells adhered preferentially to the irradiated domains, especially on the samples modified for 20 and 30 min. On day 1 and 3 after seeding, these domains contained from 70 to 90 % of adhering VSMC. The differences in formation of cell-adhesive domains in the acetylene or NH<sub>3</sub> atmosphere could be explained by different changes in the physicochemical properties of the material surface. The UV light-irradiation in acetylene atmosphere led to the formation of hydrogenated amorphous carbon (a-C:H). On the contrary, the use of the NH<sub>3</sub> atmosphere led to the formation of C=O and C-NH<sub>2</sub> groups, which are well known to promote cell adhesion. These results demonstrate a possibility to achieve highly selective cell adhesion on polymeric surfaces patterned with adhesive domains created by UV light-irradiation in an appropriate atmosphere. These surfaces could be used for guided cell colonization of biomaterials designed for tissue engineering, as well as for creation of cell microarrays for advanced research in genomics and proteomics.

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#### METALLOPROTEASES AND PROTEINS WITH DOMINANT IMMUNOMODULATORY ACTIVITY IN CHILDREN OF ROMANY ETHNIC

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Metalloproteases are structurally and functionally variable groups of proteins protecting tissue against oxidative stress, and acting as scavengers of oxygen radicals. Protections against destructive action of free radicals is a dominant role of ceruloplasmin (Cpl) as well as other proteins with a dominant immunomodulating function – orosomucoid (ORM) and alfa2-macroglobulin (α<sub>2</sub>M). 217 Romany children at the age

of 0-5 years were involved into the set of which 62 % were not breastfed and 38 % were breastfed. Of the immunological parameters the serum concentrations of ORM, Cpl and α<sub>2</sub>M were assayed by commercial sets of fy Sevapharma, CR. A set of children was divided into two age groups (0-1 year and 1.1-5 years) and according to sex. The mean values of the parameters observed did not show pronounced changes, only in case of boys to the age of 1 year in the values of ORM and in boys (1.1-5 years) in the values of α<sub>2</sub>M. At the comparison of both sexes at the age to 1 year there was no significant increase in the values of ORM, Cpl and α<sub>2</sub>M. Comparison of the mean values of selected parameters between boys and girls at the age of 1.1-5 years is presented in the Table.

Parameters	Romany boys		Romany girls	
	A (n=38)	B (n=23)	A (n=31)	B (n=24)
ORM (g/L)	1.15±0.39	1.30±0.30	1.20±0.41**	1.10±0.36*
Cpl (g/L)	0.31±0.09	0.34±0.05	0.33±0.10***	0.33±0.11
α <sub>2</sub> M (g/L)	3.61±0.91	3.23±0.67	3.29±1.04***	3.43±0.68

*A – non-breastfed, B – breastfed, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001*

The key role in the beneficial development of an individual has breastfeeding. In the study it has been found that 41.86 % of children were breastfed for 0-3 months, 40.8 % more than 9 months. Pronounced differences were also in the length of breastfeeding between boys and girls. Of the children breastfed for more than 9 months 45.2 % were Romany girls, it is by 8.7 % more than Romany boys. Determination of the given parameters could be helpful at revealing of inflammatory processes, immune deficiencies and judgement of homeostasis at the pathological processes. They have a wide spectrum of activities which contribute to the protective reaction of the organism and to reparation of impairment arisen during inflammation.

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#### EXPRESSION OF INTEGRIN BETA 1 TWO RAT MODELS OF CARDIAC HYPERTROPHY

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Background: Integrins are a large family of transmembrane heterodimeric receptors that are widely expressed on the cell surface and provide a physical and biochemical bridge between extracellular matrix and the intracellular physiological environment. Beta 1 integrins may play an important coordinating role in extracellular matrix synthesis and remodeling. Aims: To examine whether expression of integrin beta 1 is changed in the heart and aorta in two models of cardiac hypertrophy, isoproterenol-induced hypertrophy and the spontaneously hypertensive rats (SHR). Methods: Wistar rats were treated with isoproterenol (ISO, 5 mg/kg/day) for 8 days (n=11), control rats (n=12) received vehicle. SHR (n=5) were untreated and Wistar rats were used as control. We measured systolic blood pressure (sBP) and heart rate (HR) by tail-cuff method (1). Expression of integrin beta1 in left, right ventricles and in the aorta was examined by Western blot. Results: Cardiac hypertrophy was observed in both ISO and SHR groups, while ISO rats also had marked cardiac fibrosis. Blood pressure and heart rate in ISO vs control were decreased (sBP 110±3 vs. 126±3 mmHg; HR 342±8 vs 366±6 beats/min) in SHR group were (sBP 206±13; HR 432±26 beats/min). In isoproterenol-induced hypertrophy, expression of integrin beta1 in the aorta and left, right ventricles was unaltered. Interestingly, in the SHR we observed almost threefold increase of expression integrin beta 1 in right ventricle (SHR: 2.83±0.44, Wistar 1.0±0.38; P<0.05), but no change in the left ventricle. Conclusion: Our results showed that cardiac hypertrophy was present

both in the SHR and ISO groups, but beta1 integrin expression was markedly higher only in the right ventricle of the SHR.

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#### POTENTIATION OF CALCIUM RELEASE IN CARDIAC MYOCYTES BY PREVIOUS CALCIUM INFLUX

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Efficiency of calcium current ( $I_{Ca}$ ) to activate  $Ca^{2+}$  release is important for reliable function of cardiac muscle cells. We have studied the relationship between  $I_{Ca}$  activation and activation of  $Ca^{2+}$  release by voltage stimuli in rat ventricular myocytes using whole-cell patch clamp and confocal microscopy. Depolarization of cells to the  $I_{Ca}$  reversal potential was used to eliminate  $Ca^{2+}$  influx during specific time periods, so that calcium current flowed either only during the depolarization (5 or 70 ms), or only during the subsequent repolarization, or during both events. Under control conditions, the process of calcium release activation was very sensitive to the mode of calcium influx. Latencies of calcium release evoked by repolarization after a short prepulse were much shorter than those evoked during a 70-ms depolarization. If  $Ca^{2+}$  influx that flowed during the depolarizing pulse did not evoke substantial  $Ca^{2+}$  release, it shortened the latency of the subsequent tail  $I_{Ca}$ -induced  $Ca^{2+}$  release events. At less negative repolarization potentials, at which the tail Ca current deactivated more slowly, the tail  $I_{Ca}$ -induced  $Ca^{2+}$  release had elevated probability and slightly increased latency. When the open time of calcium channels was prolonged by means of the calcium channel activator S(-) BayK 8644, the latencies of tail  $I_{Ca}$ -induced calcium release were not affected; however, the latencies evoked during a 70-ms depolarization were strongly decreased. In the presence of the drug, the latency of tail  $I_{Ca}$ -induced  $Ca^{2+}$  release events did not depend on the tail potential, i.e., on the rate of deactivation of calcium influx. We conclude that under control conditions, single short calcium channel openings activate  $Ca^{2+}$  release with low fidelity. Therefore, subthreshold local  $Ca^{2+}$  influx has a potentiating effect on the  $Ca^{2+}$  release machinery. In the presence of the activator BayK 8644, the single-channel openings of the calcium channel are long enough to activate release with high fidelity.

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#### PRENATAL AND EARLY POSTNATAL METHAMPHETAMINE ADMINISTRATION TO RAT DAMS IMPAIRS DEVELOPMENT OF SENSORY-MOTOR FUNCTIONS OF THEIR OFFSPRINGS

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Our previous studies demonstrated that methamphetamine (MA) administration during prenatal and preweaning periods affects birth weight and sensory-motor coordination of rat pups. The present study investigated the hypothesis that the effect of MA induces long-term changes affecting even second generation of rats that were not exposed to the drug. There were tested three groups: pups of mothers with MA exposition during prenatal and preweaning period (MA), pups of mothers with saline exposition in the same time (Sa) and pups of mothers without exposition (Co). Pups were tested throughout the lactation period to examine their morphological and neuromotor development and their acute learning. Our data demonstrated no differences in litter characteristics, birth weight and weight gain of pups between groups. Interestingly, pups from mothers exposed to MA

during prenatal and preweaning period had altered sensory-motor coordination. They were able to accomplish righting reflex in mid-air later than both control groups. Additionally, they exhibited more falls in rotarod and bar-holding test when compared to pups from both control and saline-exposed mothers. In homing performance, pups from MA- and saline-exposed dams did worst in the learning to return to the home box than pups of controls. Thus, the present study demonstrated that MA abusing mothers may affect even second generation that was not exposed to the drug at all.

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#### THE ROLE OF TRPV1 RECEPTORS IN CUTANEOUS HYPERSENSITIVITY FOLLOWING SURGICAL INCISION AND THE EFFECT OF LOCAL CAPSAICIN TREATMENT ON POSTOPERATIVE PAIN.

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Tissue injury often leads to increased sensitivity to innocuous and noxious stimuli – allodynia and hyperalgesia. Peripheral nerve endings containing capsaicin receptors play an important role in this process. Local, high concentration capsaicin treatment can induce regional destruction of these endings. The aim of this study was to examine the role of capsaicin – sensitive nociceptive fibers and TRPV1 receptors in the development and maintenance of cutaneous hypersensitivity to mechanical and thermal stimuli following surgical tissue injury. A rat plantar incision model of surgical pain was used in this study. Paw withdrawal responses to mechanical stimuli of plantar skin with von Frey filaments and thermal stimuli with radiant heat were tested. In different groups of animals, plantar skin was treated with capsaicin 24 hrs before or 2 hrs after the incision was made, or with TRPV1 antagonist SB 366791 30 min before and immediately after the surgery. Responses to mechanical and thermal stimuli were tested before and at several time points after the surgery. Magnitude of hyperalgesia was also judged by the number of spinothalamic (STT) and postsynaptic dorsal column (PSDC) neurons in the dorsal horn of the lumbar spinal cord expressing c-Fos 2 hrs after the surgery using immunohistochemical staining. Vehicle injected rats treated identically to capsaicin injected animals were used as controls. In the control group of animals mechanical (allodynia, hyperalgesia) and thermal sensitivity increased significantly following the incision, when compared to the responses evoked on the intact paw. Capsaicin applied 24 hrs or 2 hrs after the surgery significantly attenuated the development of postincisional mechanical allodynia and reduced the presence of hyperalgesia. The capsaicin treatment done 2 hours after the incision reduced thermal hyperalgesia following the incision. Treatment 24 hrs before the incision induced hypoalgesia present also after the paw incision. SB 366791 attenuated significantly postincisional thermal hyperalgesia and mechanical allodynia, without affecting mechanical hyperalgesia. Capsaicin treatment reduced the number of STT and PSDC neurons expressing c-Fos in the dorsal horn ipsilateral to the incised paw and also in the contralateral dorsal horn when compared to control. Our results show that local application of capsaicin in high concentration can significantly reduce postoperative mechanical and thermal hypersensitivity and that TRPV1 receptors play an important role in this pain state.

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# METHOD FOR ESTIMATION OF COOPERATIVE INTERACTION OF UNLABELLED ANTAGONISTS WITH UNLABELLED ALLOSTERIC MODULATORS AT MUSCARINIC ACETYLCHOLINE RECEPTORS

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Allosteric sites on muscarinic acetylcholine receptors represent novel drug targets. Search for suitable allosteric modulators is highly demanded to gain insight into the mechanism of ligand - receptor interaction, to determine the location of binding sites for different types of ligands, and to correlate between ligand structure and their affinity for particular receptor subtypes. Currently, a radioligand is not freely available to label an allosteric recognition site, and thus, direct competition experiments with unlabelled allosteric modulators are not possible. Similarly, an availability of the labelled orthosteric ligand is strongly restricted. We developed a method permitting to quantify allosteric interactions between the unlabelled allosteric ligand A, and the unlabelled classical ligand X. Receptors R are incubated in the presence of fixed concentrations of the radiolabelled orthosteric antagonist L and of the investigated unlabelled antagonist X, and of various concentrations of the allosteric modulator A. The estimation of the other binding parameters (apparent dissociation constants for the complexes RL, XR and AR, together with the cooperativity factor  $\alpha$ ), may be carried out separately in standard experiments. Finally, the cooperativity factor  $\beta$ , which describes fold change of the equilibrium dissociation constant for complex RX induced by simultaneous binding of A, is calculated. The possible effects of impurities in the radioligand solution, and some examples of misappropriation of the method which give rise to a controversy, will be discussed.

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# EFFECT OF CHRONIC LOW DOSE L-NAME TREATMENT AND STRESS ON CARDIOVASCULAR SYSTEM OF BORDERLINE HYPERTENSIVE RATS

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The aim of this study was to investigate the effect of long-term low dose L-NAME treatment, crowding stress and their interaction in Wistar and borderline hypertensive rats (BHR). Male, 12 weeks old, BHR (offspring of spontaneously hypertensive dams and Wistar sires) and Wistar rats were exposed to crowding (200 cm<sup>2</sup> per rat, 5 rats per cage), L-NAME treatment (1.5 mg/kg/day in drinking water) or their combination for 8 weeks. Control rats were kept 4 rats per cage (480 cm<sup>2</sup> per rat). Blood pressure (BP)x of Wistar and BHR before experiment (determined by tail-cuff method) was 111±3, 136±2 mm Hg, respectively (p<0.001). Stress alone gradually elevated BP only in BHR. L-NAME elevated BP on weeks 3 and 6 vs. control in both W and BHR. After this period, BP decreased in both groups investigated. At the end of the experiment no differences in BP were observed in W (vs. control) while BP of BHR rats was still significantly elevated vs. control. Combined L-NAME+stress treatment resulted in significant elevation of BP in both W and BHR at the end of experiment. The left ventricle-to-body weight ratio was elevated in W exposed to L-NAME+stress as well as in all BHR groups vs. control BHR. Acetylcholine (ACh)-induced relaxation of control Wistar rats was lower than that of BHR (57±3 % vs. 68±4 %, p<0.001). Both crowding and L-NAME improved ACh-induced relaxation in Wistar rats but had no effect in BHR. Interestingly, the combination of chronic L-NAME+stress resulted in reduction of ACh-induced vasorelaxation in

both phenotypes. Noradrenaline (NA)-induced vasoconstriction was lower in BHR vs. W by about 60 % (p<0.003) and stress increased NA-induced constriction in both phenotypes (p<0.001). L-NAME alone as well as in combination with stress reduced vasoconstriction in W (p<0.01 vs. control) but no significant effects were observed in BHR. In conclusion, chronic low dose of L-NAME and stress led to development of hypertension only in BHR, however their combination led to hypertension in both BHR and W. Vascular function of BHR was more resistant to chronic L-NAME and stress exposure than that of Wistar but concurrent L-NAME+stress treatment was associated with the dramatic decrease of vasorelaxation in both phenotypes.

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# NON-WOVEN PGA/PVA SCAFFOLDS IN TISSUE ENGINEERING OF CARTILAGE

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Biodegradable polymers polyglycolic acid (PGA) (1) and polyvinylalcohol (PVA) (2) were investigated as artificial scaffolds in tissue engineering. In this study, new composite three-dimensional biodegradable scaffolds from PGA and PVA (PGA/PVA scaffolds), and PGA were developed. The scaffolds were prepared by a wet-laid method, the PGA/PVA scaffolds were subsequently treated with a PVA solution (PVA/PGA/PVA scaffolds) and PGA scaffolds with hyaluronic sodium solution (PGA/HA scaffolds) and/or subsequently processed by needle punching (PGA/PVA and PGA/HA scaffolds). Supplementation with nanofibres was also employed. Chondrocytes were isolated from rabbit, cultured for 28 days and seeded onto the scaffolds at density of 80 x 10<sup>3</sup> cells/cm<sup>2</sup>. Proliferation and viability of chondrocytes were testing using MTT test, fluorescence microscopy, and confocal microscopy. Immunohistochemical staining for collagen type II was used for evaluation of the differentiation of chondrocytes. The absorbance of PVA/PGA/PVA, PGA and polystyrene (PS) groups were significantly higher compared to the other scaffolds at 24 hours after seeding. After 7, 14, and 21 days, scaffolds containing PVA (PVA/PGA, PVA/PGA/PVA) showed the highest proliferation rate, comparable with polystyrene. A good pH stability of culture medium was observed. On the other hand, scaffolds prepared with HA showed the lowest proliferation of chondrocytes, accompanied with the acidification of the culture medium. This study showed the best proliferation of chondrocytes on three-dimensional non-woven PVA/PGA, PVA/PGA/PVA scaffolds. This proved their potential for cartilage repair.

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# ACTIVATION OF PI3-KINASE/AKT PATHWAY PROTECTS RAT HEART AGAINST INFARCTION, BUT IS NOT INVOLVED IN THE ANTIARRHYTHMIC EFFECT OF ISCHEMIC PRECONDITIONING

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Endogenous protection against prolonged ischemic insult can be achieved in the myocardium by preceding brief episodes of intermittent ischemia (hypoxia) or by long-term adaptation to chronic hypoxia, however, the role of pro-survival cascades underlying protective mechanisms of the adaptive phenomena is not completely elucidated. We explored the role of phosphatidylinositol 3-kinase (PI3K)/Akt activation in cardioprotection conferred by ischemic preconditioning (IP) and by adaptation to chronic hypoxia. Enhanced phosphorylation of Akt was detected before the onset of 20-min occlusion/3-h reperfusion (test ischemia, TI) in the in vivo rats adapted to chronic intermittent hypobaric hypoxia (IHH) simulating high altitude in a hypobaric chamber (7000 m, 8 h/day, 25 exposure) and in the Langendorff-perfused hearts acutely preconditioned by 2 cycles of 5 min ischemia/reperfusion, prior to similar protocol of TI. In the open-chest hypoxic and normoxic rats, PI3K/Akt inhibitor LY294002 (LY) given 5 min before TI (0.3 mg/kg, i.v.) partially attenuated infarct size (IS)-limiting effect of IHH (59.7±4.1 % of the area at risk (AR) vs. IS/AR 51.8±4.4 % in the hypoxic rats and 64.9±5.1 % in the normoxic controls). In Langendorff-perfused hearts, LY (5 µM) applied 15 min before TI completely abolished anti-infarct protection by IP (IS/AR 55.0±4.9 % vs. 15.2±1.2 % in the preconditioned hearts and 42.0±5.5 % in the non-preconditioned controls;  $P<0.05$ ), as well as inhibited Akt phosphorylation. Administration of LY did not modify the size of infarction in the normal hearts, however, it markedly suppressed arrhythmias. Application of LY in the Langendorff-perfused hearts significantly decreased the number of ventricular premature beats (VPB) and the incidence of ventricular tachycardia (VT) from 518±71 and 100 % in the controls to 155±15 and 12.5 %, respectively ( $P<0.05$ ). Moreover, bracketing of IP with LY did not reverse antiarrhythmic effect of preconditioning: (VPB 77±20, incidence of VT 14.3 % vs. VPB 195±40 and VT 22 %, respectively, in the non-treated preconditioned hearts). The results suggest that activation of PI3K/Akt cascade plays a role in the infarct size-limiting mechanism in the rat heart, however, it is not involved in the mechanisms of antiarrhythmic protection.

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# RELATIONSHIP BETWEEN ACIDOBASE BALANCE AND VENTRICULAR ARRHYTHMIA THRESHOLD AT THE DISORDERS OF VENTILATION IN WISTAR RATS

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The arise and development of the ventricular arrhythmias are closely connected also with changes in the arterial acidobase balance resulting of pulmonary ventilation decrease (1,2). The aim of this methodological study was to verify the relationship between changes of acidobase parameters and ventricular arrhythmia threshold (VAT) in anesthetized Wistar rats at the changes of pulmonary ventilation. The experiments were performed in ketamine/xylazine anaesthesia in female Wistar rats (100 mg/kg + 15 mg/kg, i.m., open chest experiments). The changes in acidobase balance were followed after adaptation to light-dark cycle

(LD cycle) of 12:12 hour, with the dark part from 06.00 to 18.00 h. The experiments were performed only in the dark period. The rats were artificial ventilated by respirator at ventilatory parameters: 1 ml/100 g of body weight and respiratory rate 40-50 breaths/min. We compared mainly changes in  $pO_2$ ,  $pCO_2$ , pH and  $O_2$  saturation in animals after surgical interventions (tracheotomy, artery preparation and thoracotomy) and 5 min stabilization, after 2 min. of apnoic episode and after 5., 10. 15. and 20 min. of reoxygenation. At the end of 2 min apnoic episode, the arterial pH and  $pO_2$  were decreased significantly ( $p<0.01$ ),  $pCO_2$  were increased ( $p<0.001$ ) compared with the values after surgical intervention and 5 min stabilization with parameters of normal ventilation.  $O_2$  saturation on the end of apnoic episode was increased significantly ( $p<0.001$ ), too. After 20 min of reoxygenation the acidobasic parameters were in the range of arterial alkalosis and normoxic hypocapnia. After 2 min apnoe, the VATs were decreased significantly, the negative correlation between pH and VAT ( $r = -0.52$ ), and positive correlation between  $pCO_2$  ( $r=0.3$ ) and VAT were obtained. There was not any significant dependence between  $pO_2$ ,  $O_2$  saturation and VAT ( $r = -0.09$ ,  $r = -0.24$ ). During reoxygenation was found significant relation between  $pO_2$  ( $r = -0.4$ ) and  $O_2$  saturation and VAT ( $r = -0.3$ ). It is concluded that probably in conditions of the acute respiratory systemic hypoxia, hypercapnia and acidosis, the heart responds positively to acidosis and hypercapnia by the activation of the cardioprotective mechanism(s). These mechanisms are probably active also during reoxygenation and protect ventricles also against the reoxygenation injury.

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# NICOTINE AND KAINIC ACID ADMINISTRATION - CHANGES IN THE DENSITY OF NITRERGIC NEURONS AND IDENTIFICATION OF NEURONS EXTINCTION

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Using histochemical analysis (NADPH-diaphorase) we have investigated the influence of intraperitoneal administration of kainic acid (10 mg/kg), or nicotine and combination of both these two factors on neurons of the hippocampus in male rats of the Wistar strain. Kainic acid was administered to 25- and 35-day-old animals which were or weren't pre-treated with nicotine (1 mg/kg) 30 minutes before the kainic acid administration. Two days after the application, animals were transcardially perfused with 4 % paraformaldehyde under deep thiopental anaesthesia. Cryostat sections were stained to identify NADPH-diaphorase positive neurons that were then quantified in CA1 and CA3 areas of the hippocampus, in the hilus, dorsal and ventral blades of the dentate gyrus. To identify the most vulnerable hippocampal regions staining Fluoro Jade B and Hoechst (bisbenzimidazole) were used. The findings were as follows: Nicotine brings about higher numbers of NADPH-diaphorase positive neurons in CA3 area of the hippocampus and hilus of the dentate gyrus in the comparison with either group of control animals. Fluoro-Jade staining did not reveal any degenerating neurons in the hippocampus after the nicotine administration. We can speculate that nicotine can be a neuroprotective agent, but next studies are necessary.

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# INTRACEREBRAL EVENT-RELATED DESYNCHRONIZATION AND SYNCHRONIZATION DURING MOTOR RESPONSE IN VISUAL ODDBALL PARADIGM

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**Objectives:** Intracerebrally recorded event-related desynchronization (ERD) and synchronization (ERS) in frequency range of 5.5-15 Hz were studied during motor response to target stimulus in a visual oddball paradigm. **Methods:** In nine right-handed patients with medically intractable epilepsies depth electrodes were implanted to localize the seizure origin prior to surgical treatment. A total of 298 contacts were investigated. Visual oddball paradigm was performed. Each subject was instructed to respond to the target stimulus by pressing a microswitch button in the dominant hand and to count the target stimuli mentally. ERD and ERS expressed as a decrease and increase of power in a frequency range of 5.5-15 Hz was studied in a 3-s period starting 1 s before pressing the button. **Results:** ERD/ERS were identified in all patients in 62 sites of both hemispheres – amygdala, gyrus cinguli, gyrus fusiformis, hippocampus, putamen, gyrus parahippocampalis, gyrus temporalis superior, medius and inferior and frontoorbital cortex. ERD was observed in 32 sites and ERS in 23 sites and both were present before as well as after the motor response. In 7 sites both ERD and ERS were observed at the same time. Simultaneous scalp recoding revealed ERD/ERS in 6 out of 9 cases. **Conclusion:** Localization of ERD/ERS in various brain structures during the stimulus-dependent motor response extends recent knowledge about intracerebral distribution of this phenomenon. The existence of ERD/ERS before the motor response could represent not only the movement preparation and execution described in the literature but also memory mechanisms or sensory processing.

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## NHE3 ANTIPORT IN THE RAT INTESTINE

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Na/H exchangers are transporters that play a significant role in sodium absorption, and regulation of intracellular pH and cell volume. There are several isoforms of these transporters that differ in their physiological role. Three of them, NHE1, NHE2, and NHE3, were identified in the intestinal epithelium. The isoform NHE1 was found in the basolateral membrane of enterocytes and was shown that it participates in extrusion of hydrogen ions from cytosol during intracellular acidification. In contrast, the isoform NHE3 is localized in the apical membrane and is involved in transcellular transport of sodium ions. Previous investigations showed differences in regulation of NHE3 expression during ontogenetic development and during diurnal cycle in kidney. However, little is known about this regulation in colon. The present study investigated, therefore, (1) the profile of colonic NHE3 mRNA transcript in sucklings, weanlings, and adult rats and (2) the diurnal variations of this transcript in colon of adult animals. To determine the level of transcripts, quantitative RT-PCR of NHE3 was measured using SYBR Green method in Wistar rats kept on 12-hour light/dark cycle and fed ad libitum. The animals were killed 4 hours after the beginning of the light cycle in ontogenetic study, whereas in case of diurnal variation of NHE3 expression, the expression profile was examined every 4 hours starting at the beginning of the light cycle. To analyze the effect of reverse feeding on diurnal cycle of NHE3, some rats were fed only during the light but not dark cycle. We found that the level of NHE3 mRNA is very high in suckling animals, decreases dramatically during weaning period and then partially increases again. Nevertheless the

level of transcript in adult animals is significantly lower than in immature colon of suckling rats. By analyzing the level of NHE3 transcript during the dark/ light cycle we found rhythmic expression of NHE3 that was shifted by reverse feeding. In control animals the peak of NHE3 mRNA was found at the beginning of dark cycle whereas during reverse feeding the peak was found 4 hours earlier. We conclude that NHE3 is temporally regulated during ontogenetic and diurnal cycle.

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## APPLICATION OF ELISA FOR MYOSIN HEAVY CHAIN ISOFORMS QUANTIFICATION

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Quantitative information is essential for analyzing myosin heavy chain (MyHC) content changes. We have developed a 2-D stereological method using the principles of unbiased counting frame and point counting (C.A.S.T. Grid System, Olympus, Albertslund, Denmark) and analyzing fiber type composition in serial cross sections (1). Furthermore, we have used the SDS-PAGE technique to reveal proportions of MyHC isoforms in muscle homogenates; the individual MyHC isoforms have been densitometrically evaluated (AIDA 3.28 computer program, Germany). Unfortunately, the existence of hybrid fibers with mixed reactions and incomplete separation of 2a and 2x MyHC bands often hampered the exactness of evaluation. We have therefore adopted an immunocytochemical approach similar to the ELISA method to quantify the MyHCs isoform contribution. Polystyrene microplates were used for adsorption of partially purified myosin extracts from soleus and extensor digitorum longus (EDL) muscles of inbred Lewis strain rats in control euthyroid rats in comparison with the MyHC isoform content of hypothyroid and hyperthyroid rats. The total amount of adsorbed myosin was quantified with a "general" antimyosin antibody (aS MyHC-slow and aF MyHC-fast, provided by Biotrend or Medac/Novocastra, Germany) and the content of specific isoforms with isoform-specific antibodies (mAbs BA-D5 (MyHC-1), SC-71 (MyHC-2a), BF-35 (all isoforms except MyHC-2x/d) and BF-F3 (MyHC-2b), (2). Primary antibodies were gauged with peroxidase-labeled secondary antibodies. Using our "MyHC ELISA" method, we have confirmed that: (1) the MyHC-2a content in the soleus muscle was increased in hyperthyroid and decreased in hypothyroid status; the relative changes were smaller than those determined by SDS-PAGE or by stereological determination of muscle fiber types, (2) in the EDL muscle, corresponding changes were found by all 3 methods: i) MyHC-1 was mildly decreased in hyperthyroid and increased in hypothyroid status, ii) MyHC-2a was increased in both experimental conditions, iii) MyHC-2b was enhanced in the hyperthyroid status and decreased in the hypothyroid status, iv) unfortunately, 2x/d MyHC isoform cannot be determined at present due to the lack of a specific positive marker. Our results suggest that our method can provide additional "quantitative" information besides already existing methods.

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## MELATONIN AND MT1 MEMBRANE RECEPTOR IN RAT INTESTINE

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Melatonin (N-acetyl-5-methoxytryptamine) is localized both in central nervous system and in many peripheral tissues. High amounts of melatonin as well as binding sites for 2-I-melatonin were found in various parts of the gastrointestinal tract. Two main subtypes of melatonin membrane receptors were identified: the high-affinity receptors (MT1, MT2) belonging to the rhodopsin family and the low-affinity receptor (MT3) of the quinone-reductase family. The role of these receptors and their ligand has been shown in various functions of the gastrointestinal tract including ion-transport processes, motility, immunomodulation and blood-flow. Aim: (1) To characterize the expression of MT1 mRNA along the horizontal (duodenum, jejunum, ileum, colon) and vertical axis (epithelium layer vs. subepithelium layer after stripping colonic mucosa). (2) To examine the effect of the short-term (2 days) and long-term (7 days) fasting on the expression of MT1 mRNA. (3) To screen for the circadian profile of MT1 mRNA expression level. Methods: Adult male Wistar rats (LD 12:12) were sacrificed at 3 to 6 hours after the light onset or every 4 hours among the 24 hour cycle and RNA was examined using quantitative real-time RT-PCR and expressed as the ratio of MT1 to the level of  $\alpha$ -actin. Results: In all segments the expression of MT1 mRNA was higher in the subepithelial layer than in the epithelium. The highest expression was found in duodenum. Short-term fasting increased MT1 mRNA expression in all segments whereas MT1 mRNA after long-term fasting reached nearly control levels in proximal segments and maintained up-regulated only in the distal colon. No changes were observed in pituitary gland MT1 mRNA after fasting and expression in thymus was increased only after long-term fasting. In addition, no significant circadian rhythm of MT1 mRNA was observed in the subepithelium layer of the distal colon. Conclusions: Melatonin receptor MT1 is localized in all segments of intestine, predominantly in the subepithelial layer. Their expression is affected by changing physiological conditions. There are no rhythmic changes in MT1 mRNA expression during 24 hours.

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## EFFECT OF MESENTERIC ISCHAEMIA/REPERFUSION ON ENDOTHELIAL FUNCTION OF STRESSED RATS WITH DIFFERENT PREDISPOSITION TO HYPERTENSION

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The aim of this work was to investigate the effect of mesenteric ischaemia/ reperfusion on the crowding stress-induced changes in endothelium-dependent relaxation of the rat superior mesenteric artery (SMA) of normotensive and hypertensive rats. Experiments were performed on normotensive rats (Wistar) and spontaneously hypertensive rats (SHR). The animals were exposed to 8-week crowding stress (5 rats/cage 25/40/15 cm, cca 200 cm<sup>2</sup>/rat). Control rats were 4 per cage (35/55/20 cm, cca 480 cm<sup>2</sup>/rat). In anaesthetised rats, ischaemia of the mesentery was induced by occluding SMA for 60 minutes. After removal of the clamp, reperfusion lasted 30 minutes. Sham-operated animals served as controls. Blood pressure was measured using tail-cuff plethysmography. Endothelium-dependent relaxation of SMA rings was studied in vitro under isometric conditions. We evaluated the responses of phenylephrine-precontracted preparations (1  $\mu$ mol/l) to acetylcholine (0.01-10  $\mu$ mol/l) before and after inhibition of NO synthase (100  $\mu$ mol/l N $\omega$ -nitro-L-arginine methyl ester - L-NAME) and prostaglandin synthesis with indomethacin (10

$\mu$ mol/l). In control conditions, SHR rats had significantly higher blood pressure compared to Wistar rats (185 $\pm$ 2 and 111 $\pm$ 1 mm Hg, respectively). Moreover, SMA taken from SHR rats responded to acetylcholine with smaller relaxation than that from Wistar rats. Crowding stress induced the increase of blood pressure of SHR (193 $\pm$ 2 mm Hg), but not of Wistar rats (112 $\pm$ 2 mm Hg). In vessel functional studies responses of SMA to acetylcholine (the total response and NO-mediated portion of endothelium-dependent relaxation) were depressed in the SHR animals. Wistar rats responded to stress with depression only in L-NAME-resistant vasodilatation. Mesenteric ischaemia/reperfusion did not induce any further impairment of the response of SMA to acetylcholine either in Wistar or SHR group. The results showed damaging effect of crowding stress on endothelium-dependent relaxation of superior mesenteric artery of SHR rats. Ischemia/ reperfusion did not influence the changes in relaxation evoked by stress.

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## THE EFFECT OF STRESS ON THE HEART FUNCTION OF THE RATS WITH VARIOUS FAMILY HISTORY OF HYPERTENSION

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In this study the spontaneously hypertensive (SHR) and borderline hypertensive rats (BHR, offspring of SHR dams and Wistar sires) were compared to normotensive Wistar (Wis) rats. Male rats (12 weeks old) of all phenotypes were exposed to crowding for eight weeks (200 cm<sup>2</sup> per rat, 5 rats per cage). Control rats were kept 4 rats per cage (480 cm<sup>2</sup> per rat). Systolic blood pressure (sBP) was increased in the order Wis<BHR<SHR. Heart weight (HW), left ventricular weight (LVW) as well as both indexes HW/BW, and LVW/BW (BW=body weight) were increased with hypertension. Parameters of LV, like height, width, and thickness of the free wall demonstrated developed myocardial hypertrophy in BHR and that was more pronounced in SHR. Crowding stress increased values of BP and heart frequency more in BHR, and SHR and accentuated signs of left ventricular hypertrophy. Stress worsened the mechanical function of spontaneously beating isolated heart. Lowering of coronary flow in the order Wis>BHR>SHR was more emphasized in the crowded rats. Spontaneous frequency of the hearts isolated from SHR was slower than the heart frequency recorded from BHR and Wis being in accord with diminishing effect of sympathetic activation. Heart rate analysis showed approximately 2- and 15-fold higher incidence of rhythm disturbances in BHR and SHR, respectively compared to Wis. In the arrhythmias dominated incidence of ventricular ectopic beats. Crowding induced increased incidence of arrhythmias in Wis and BHR, and the short episode of ventricular tachycardia was found in 25 % of SHR. It could be concluded that both pre-hypertensive and hypertensive rats demonstrate left ventricular hypertrophy and increased heart frequency. All these symptoms were potentiated by crowding. Moreover, crowding weakened contractile ability of the myocardium, worsened perfusion of coronary vessels and increased incidence of ventricular arrhythmias.

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# RHYTHMIC CHANGES OF MELATONIN LEVELS IN PLASMA, PINEAL GLAND AND PERIPHERAL TISSUES OF RATS WITH STREPTOZOTOCIN-INDUCED DIABETES

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Diabetes mellitus (DM) belongs to metabolic diseases characterized by high glucose levels, which result from defects in insulin secretion or action. Streptozotocin (STZ) is a chemical compound used to induce experimental diabetes by disruption of the insulin-producing  $\beta$ -cells of the endocrine pancreas. In addition to different pathophysiological changes DM induces desynchronisation in the circadian system. Hormone melatonin is involved in regulation of circadian rhythms by influencing peripheral oscillators. Melatonin is synthesized primarily in the pineal gland exhibiting high levels during the dark part of the day. The aim of our study was to investigate rhythmic changes in melatonin production in the pineal gland, plasma and peripheral tissues - pancreas and duodenum in diabetic and control Wistar rats. Rats were kept on L: D cycle 12:12. Diabetes was induced by intraperitoneal injection of STZ (65 mg/kg) dissolved in 0.1 M citrate buffer. On days 17 and 18 after diabetes induction animals were sacrificed in 4-hour intervals during 24-hour cycle. Hormone concentration was determined by radioimmunoassay directly in plasma, after methanol extraction in pineal glands and after chloroform extraction in other tissues. Rhythmic daily pattern of melatonin concentrations was found in both control and diabetic rats. The amplitude of the pineal melatonin rhythm was lower in diabetic than in control rats. This difference was reflected in melatonin rhythms detected in duodenum and pancreas but not in circulation. Our data suggest that the damped amplitude of rhythmic melatonin production can contribute to desynchronisation of circadian rhythms in diabetic rats.

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# REGULATION OF DIPEPTIDYL PEPTIDASE-IV ACTIVITY AND/OR STRUCTURE HOMOLOGUES (DASH) IN HUMAN BRAIN TUMORS: AN ASSOCIATION WITH WHO GRADE?

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Post-translational modification is an important regulatory event of numerous biologically active peptides. Proteolysis of such peptides could be limited by the presence of an evolutionary conserved proline residue. Proline-specific "Dipeptidyl peptidase-IV Activity and/or Structure Homologues" (DASH) have been shown to modify quantitatively and also qualitatively some cellular signaling events. Therefore, their dysregulation is speculated to participate in the pathogenesis of multiple diseases, including cancer [1]. In our study, expression (real time RT-PCR and immunohistochemistry) and enzymatic properties (histochemistry, fluorimetric biochemical assays and inhibition studies) of dipeptidyl peptidase-IV (DPP-IV), fibroblast activation protein- $\alpha$  (FAP) and attractin were analyzed in biopsies from human brain tumors of different origin and malignancy classified by WHO grade. Significantly lower DPP-IV enzymatic activity has been associated with low-grade tumors (Grade I – II) compared to their high-grade counterparts (Grade III – IV). The results of inhibition studies suggested that the majority of DPP-IV-like activity at least in high-grade tumors could be attributed to the canonical DPP-IV. Moreover, the expression of attractin mRNA and presence of its protein probably lacking enzymatic activity was observed. Varying DASH pattern in particular tumor type and the correlation of DASH enzymatic activity with tumor WHO grade support hypothesis of DASH

participation in the pathogenesis of human brain neoplasias.

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# THE ROLE OF MATRIX METALLOPROTEINASES IN ADAPTIVE RESPONSES INDUCED BY ISCHEMIC PRECONDITIONING.

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Matrix metalloproteinases (MMPs) are family of enzymes that degrade extracellular matrix in both physiological and pathological conditions. Myocardial ischemia and ischemia/reperfusion (I/R) induce cell damage that leads to apoptosis, necrosis and cardiac remodelling. Ischemic preconditioning (IP) is a potent endogenous mechanism of cardioprotection that protects the heart against all major manifestations of acute I/R. Therefore, this study was focused on the investigation of changes in the expression and activities of MMPs during ischemia and reperfusion and in the IP. Isolated Langendorff-perfused hearts were subjected to test ischemia challenge induced by 30min global ischemia and 30min reperfusion. The tissue samples were taken at the beginning (control C), after 5 and 30 min of ischemia (I5, I30), after 10 min of reperfusion (R10) and at the end of I/R injury. IP was induced by 2 episodes of global I and R (5min each). Total contents or activities of MMPs were determined by Western blot analysis using specific antibodies or by zymography. Zymographic analysis of cytosolic metalloproteinases revealed some changes in their activities, mainly MMP-2, during ischemia and reperfusion and after IP when compared to control hearts. We found marked increase in gelatinolytic activity of MMP-2 after short-lasting ischemia (I5), however, prolonged ischemia lead to reduction of MMP-2 activation and this was further reduced during reperfusion. Preconditioned hearts showed partial down-regulation of MMP-2 activity, whereas application of PI3K/Akt kinase inhibitor, LY 294002, which was found to abrogate IP-induced cardioprotection, returned activation of MMP-2, in part, to the control level. Analysis with antibody specific against MMP-2, performed in order to reveal the nature of changes in MMP activities, did not show any differences in the levels of cytosolic MMP-2 between control and preconditioned hearts. These results point to the potential role of matrix metalloproteinases in I/R injury and in the adaptive response induced by IP.

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# APOPTOSIS IN MOLAR TOOTH DEVELOPMENT – WITH AND WITHOUT CASPASE-3

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Tooth development is underscored by reciprocal epithelial-mesenchymal communication during embryogenesis. Apoptosis represents one of the morphogenetic mechanisms. Caspase machinery acts in most types of developmental programmed cell death. Caspase-3 is the central caspase in both, receptor-mediated and intrinsic apoptotic pathways and becomes activated by cleavage by upstream caspases. Pro-caspase-3 and activated caspase-3 were localized in the tooth germs using specific biotinylated antibodies and cryopreserved embryos from embryonic

days E13.5 to 15.5. At this stage the signalling centre of the primary enamel knot develops and becomes eliminated apoptotically. Activation of caspase-3 was shown to correspond strongly with this event. To investigate the role of caspase-3 activation in dental apoptosis and molar tooth morphogenesis, specific *ex vivo* inhibition and mutant analyses were performed. Mandibular explants were dissected from mouse embryos at E13.5, cultured for 4 days and analyzed in 24 h intervals to follow the tooth germ morphology and alterations in apoptosis. Inhibition of activated caspase-3 in explant cultures was achieved by adding a specific caspase-3 fluoromethylketone inhibitor in the culture medium. To detect any impact of caspase-3 deficiency on *in vivo* growth of the tooth germs, caspase-3 mutants were analyzed. Altered molar tooth germ morphology was found at E 15.5 when the molar tooth germs reach the bell stage. The most affected part was the original primary enamel knot area. However, the final molar teeth were not altered, showing proper shape, cusps and correct enamel formation as revealed by scanning electron microscopy. Caspase-3 was shown to be activated during dental apoptosis, however, not to be essential for proper final molar tooth formation and mineralisation. Possible compensation by other effector caspases, such as caspase-4 and caspase-7 must be considered and the exact role of apoptosis in tooth shaping and patterning still needs to be elucidated.

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#### THE EFFECTS OF DIAZOXIDE ON ISCHEMIA/REPERFUSION-INDUCED ALTERATIONS IN RAT MYOCARDIUM

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One of the way of increased myocardial tolerance against ischemia/reperfusion (I/R) injury can be pharmacological treatment. Pretreatment with diazoxide (D), mitochondrial K(ATP) channel opener, triggers protection of the heart against I/R injury. Our aim was to characterize the effects of D on the alterations of regulatory myocardial proteins. We also investigated the effect of ischemia and D-pretreatment on mitochondrial ultrastructure and integrity as well as induction of apoptotic responses. Isolated Langendorff-perfused hearts were subjected to 25 min global ischemia followed by 35 min reperfusion (index ischemia-II). To test the role of diazoxide, the [K(ATP) opener was applied in concentration 50µmol/l 15 min before II. The ultrastructure of mitochondria was investigated by electron microscopy of ultrathin sections of mitochondrial fractions embedded in Epon812. The levels and activation state of specific proteins were determined by Western blot assay with specific antibodies. The activities of matrix metalloproteinases were determined by zymography using gelatine as a substrate. It was found that hearts pretreated with D showed better recovery of contractile function after II. Electron microscopy studies revealed that application of D was connected with better preservation of integrity of mitochondria at basal conditions and after II as compared to controls. 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 II-induced cytosolic levels of pro-apoptotic Bax protein. D-treatment increased activation of extracellular-signal regulated protein kinases (ERK) and we found also moderate increase in Raf-1 activities in D-treated hearts after II. The results suggest that the cardioprotection mediated by D in rats is associated with preservation of mitochondria integrity and function. The effects of D on enzyme systems of ERK pathway and Bax protein suggest the role of these protein systems in D-mediated adaptive responses of myocardium to ischemia and point also to possible modulation of ischemia-induced apoptotic responses by diazoxide.

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#### METHAMPHETAMINE ADMINISTERED TO RAT DAMS DURING GESTATION AND LACTATION AFFECTS MATERNAL BEHAVIOR OF THEIR OFFSPRINGS

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Previous studies have demonstrated that stimulant drugs, such as amphetamine, methamphetamine (MA) and cocaine, administered during gestation and/or lactation attenuates maternal behavior of rats. Additionally, our previous work demonstrated that drugs, specifically morphine, when administered during gestation affects maternal behavior of female offsprings. The aim of the present study was to investigate effect of MA administered during prenatal and early postnatal period on maternal behavior of rats. Adult females exposed during prenatal and preweaning periods to 5 mg/kg MA daily, were examined for regularity of estrous cycle and mated with stimulus, unexposed males. Maternal behavior was examined by using two tests: Observation test (without disturbance of the mother and pups) and Retrieval test (with short separation of pups from the mother). In Observation test, eleven types of activities and three types of nursing positions of mothers were recorded ten times during each 50-minute session for the 22-day lactation period. In Retrieval test, mothers were tested for pup retrieval from postpartum days 1 through 12. Our data demonstrate that MA-exposed mothers displayed more nursing, were more often in the nest and in contact with their pups, and that they were faster in retrieving their pups than saline-exposed and/or control mothers. Thus, the present study demonstrates that MA administration to dams during gestation and lactation periods affects maternal behavior of their female offsprings.

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#### BEHAVIORAL CHANGES IN RATS IRRADIATED WITH $\gamma$ -RAYS ON THE HEAD.

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The impairment of certain brain functions, resulting in behavioral changes belongs to the possible adverse effects of the ionizing radiation, e.g. as it is seen after radiotherapy of brain tumors in humans (1). In this work, the effects of irradiation of the head with sublethal doses of gamma-rays on some innate forms of behavior were studied in a rat model. Twenty male Sprague-Dowley rats were tested daily in the open field test during a 5-day control period. The parameters of innate behavior in a new environment were recorded. Two weeks after finishing the control period 14 animals were irradiated on the head only with a dose of 10 Gy of gamma-rays from a  $^{60}\text{Co}$  radiation source and tested again in open field for 5 consecutive days. The radiation caused statistically significant suppression of exploratory activities (horizontal and vertical locomotion, crossings of the center of the field) and of comfortably behavior (washing) up to 3rd day after irradiation; freezing behavior appeared only after irradiation. The score of defecation, as a measure of anxiety was significantly lower one day after irradiation compared with the control period. These findings seem to support the hypothesis about direct effects of relatively low doses of radiation on brain centers controlling innate forms of behavior.

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# INTERACTION OF NITRIC OXIDE AND REACTIVE OXYGEN SPECIES PRODUCTION IN PULMONARY VASOCONSTRICTION

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Hypoxic pulmonary vasoconstriction (HPV) is modulated by interaction of nitric oxide (NO) production and release of reactive oxygen species (ROS). Based on our previous experiments we know that antioxidant Tempol (intracellularly acting SOD mimetic) inhibits HPV. This can be caused by two possible ways: 1. As reaction of superoxide and NO produces potent vasoconstrictor – peroxynitrite, direct inhibition of superoxide production by Tempol reduces vascular tonus. 2. Inhibition of superoxide production decreases consumption of NO for peroxynitrite production. Increase in NO concentration diminishes HPV. We compared pulmonary vasoconstriction before and after administration of Tempol (50 mg/kg) into perfusate (group I, n=6). To investigate the effect of NO on the intensity of hypoxic pulmonary vasoconstriction, we inhibited the NO production by adding L-NAME ( $5 \times 10^{-5}$  mmol/l) into the perfusate (group II, n=6). We used isolated lungs of adult male rats (Wistar) perfused with salt solution with albumin (4 g/100 ml) and Meclofenamate by constant flow (4 ml/min/100 g). The lungs were ventilated with normoxic (21 % O<sub>2</sub> + 5 % CO<sub>2</sub>) gas mixture. Pulmonary vasoconstriction was induced by arterial injection of bolus of Angiotensin II (0.2 µg) or by hypoxic challenge (0 % O<sub>2</sub> + 5 % CO<sub>2</sub>). We were continually monitoring the perfusion pressure. We found significant decrease of vasoconstriction response induced by hypoxia and angiotensin II after administration of Tempol. We did not find any differences in HPV inhibition caused by L-NAME administration. Conclusion: SOD mimetic Tempol inhibits Angiotensin II and acute hypoxia induced vasoconstriction by reduction of ROS concentration. This effect does not depend on actual release of NO.

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# INSULIN MODULATES AMPA RECEPTORS MEDIATED SENSORY SYNAPTIC TRANSMISSION IN SPINAL CORD NEURONS

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Trafficking of AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors between the postsynaptic cell membrane and cytoplasm regulate the number of receptors at the synapse and can lead to long term modulation of synaptic efficacy. Recent studies on hippocampus have shown that insulin can induce internalization of AMPA receptors containing GluR2 subunits, which is subsequently followed by long term depression of synaptic transmission. Insulin receptors (IRs) are expressed on spinal cord dorsal horn (DH) neurons, but their possible role in modulation of sensory transmission is not known. Using patch-clamp recordings from superficial DH neurons in acute spinal cord slices, the effect of insulin on fast excitatory AMPA receptors mediated postsynaptic currents (AMPA-EPSCs) was studied. The EPSCs were evoked by electrical stimulation of dorsal rootlets in spinal cord slices prepared from 6-10 days old mice in the presence of bicuculline (10 µM), strychnine (5 µM) and MK-801 (15 µM). Under control conditions there was no change in the AMPA-EPSCs for the duration of the recording (20 min, n=15) and the evoked EPSCs were completely blocked by application

of CNQX (10 µM). AMPA mediated EPSCs were reduced to 65 % in 18 out of 24 recorded neurons following insulin (0.5 or 10 µM) application. There was no significant change of the EPSCs size in the other 6 neurons. The process of insulin induced AMPA receptors endocytosis is dependent on phosphorylation of the GluR2 subunit. There was no change in the EPSC size when protein-tyrosine kinase inhibitor Lavendustin A (10 µM) was applied before the application of insulin (n=9). Our results suggest that insulin could play an important role in the modulation of sensory synaptic transmission including nociception in the spinal cord dorsal horn. Its possible role in the long term changes in synaptic efficacy that is thought to underlie several forms of pathological pain syndromes such as hyperalgesia and allodynia needs to be further evaluated.

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# ECG BODY SURFACE MAPPING (BSPM) IN DIABETIC PATIENTS (TYPE 1)

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Diabetes mellitus is a risk factor of cardiovascular diseases. ECG of diabetic patients type 1 (DM1) shows tachycardia (block of parasympathetic innervation) and abnormal repolarization (increased QT interval and QT dispersion) indicating a risk of ventricular tachycardia and sudden death in young people with DM1. The aim of the present work was to measure 145 parameters of heart electric field (ECG, VCG, BSPM) in 22 outpatients (14 men, 8 women) with DM1 without complications (mean age  $32 \pm 11.4$  years) and in 22 controls (11 men, 11 women), mean age  $30 \pm 3.4$  years. The parameters were registered by diagnostic system Cardiag 112.2 (1) and statistically evaluated by Student t-test and test of Mann-Whitney. Tachycardia (86 beats per min), shortening of QRS (79.9 ms) and QT (349 ms) were observed in DM1 patients when compared with controls (75 beats per min, QRS 89.9 ms, QT 374 ms), ( $p < 0.01$ ). The maximum (extremum) in depolarization isopotential maps (DIPM) were higher in DM1 patients from the beginning of Q wave until 30<sup>th</sup> ms of depolarization, and then less positive than in controls. The minimum in DIPM were less negative in DM1 patients than in controls during QRS complex, similarly as the minimum in depolarization isointegral maps (DIIM). The maximum in repolarization isopotential maps (RIPM) were lower in DM1 patients than in controls. The depolarization isoarea maps maximum and minimum (DIAM) confirmed the findings in DIPM maps. The amplitude of Q wave was more negative in DM1 than in controls. The spread of activation (depolarization) was more pronounced in DM1 than in controls (activation time). The duration of QT in 96 surface thoracic leads was shortened in DM1 patients than in controls. Our results confirmed the block of parasympathetic innervation (tachycardia, shortening in activation time), different depolarization and repolarization rate in DM1 patients. The differences in heart field parameters measured by the BSPM method in DM patients and in controls show the importance of ECG examination of DM1 patients in the prevention of cardiovascular diseases.

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## TWO-PHASE ENDOCRINE RESPONSE DURING ADJUVANT ARTHRITIS IN RATS.

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Leptin and ghrelin are two hormones with opposite effects on the regulation of feeding behaviour by modulating the expression of orexigenic peptides in the hypothalamus. Leptin as a cytokine-like adipokine has also marked pro-inflammatory effects via activation of T<sub>H</sub>1-cell proliferation and can promote the onset or progression of experimental autoimmune responses in several animal models (1). The objective of our study was to find out the relation between anorexigenic leptin, and orexigenic ghrelin, food intake, and body weight in the development of adjuvant arthritis (AA) in rats. AA was induced by a single intradermal injection of complete Freund's adjuvant (cFA) at the base of the tail in male Lewis rats. Arthritic rats along with age matched intact controls were sacrificed on day 2, 4, 6, 12, 15 and 18 following the cFA inoculation. Hind paw edema became significantly manifest from day 12 of AA onwards, as did the enlargement of the spleen. Thymus weight was initially reduced on day 4, thereafter it recovered to the values of intact controls, and it dropped again on day 15 and 18 of AA. Food intake was reduced in the very first phase (days 1-5), then it normalized by day 9 and was decreased again from day 10 during the whole clinical phase of AA. This course was not reflected by the body weight changes: Body weight retardation was observed from the very beginning of the disease until the end of the experiment in arthritic rats, as compared to controls with normal progressive body weight gain. Plasma leptin levels did not differ from healthy animals by day 9 of AA, thereafter they were significantly lowered, and correlated with the loss of epididymal fat. Plasma ghrelin levels were found to be decreased in the second, clinical phase of AA as well. Our results have demonstrated that in the chronic phase of the inflammatory process there is a reduction of leptin levels which correlates with the fat loss and anorexia. Intriguingly a concomitant rise of ghrelin does not occur. Pro-inflammatory effects of leptin may play its main role in the early stage of the disease when T<sub>H</sub>1-cells are involved, and not yet clinical signs of AA are visible.

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## CAN WE SAY NO TO SOME PATHOPHYSIOLOGICAL SKELETAL MUSCLE STATES?

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Denervation of skeletal muscle is associated with some substantial functional and pharmacological changes in the sarcolemma. It is now well established that in adult innervated skeletal muscle, voltage-gated sodium channels 1.4 (Na<sub>v</sub> 1.4 IKs) isoforms are dominant whereas 1.5 isoforms (Na<sub>v</sub> 1.5 IKs) become highly abundant after denervation. Expression of Na<sub>v</sub> 1.4 IKs mRNA is unchanged at the neuromuscular junction after denervation (1) although the endplate Na<sub>v</sub> 1.4 IKs density is reduced by 40-50 % (2). On the contrary, Na<sub>v</sub> 1.5 IKs mRNA level increases up to 50-fold after denervation, with channel endplate density of about 43 %. Na<sub>v</sub> 1.5 expression can be down-regulated by direct muscle stimulation, thus simulating normal motor nerve activity (2). It is also established that first typical attribute of denervation, early postdenervation depolarization, is controlled via nitric oxide synthase activity and production of nitric oxide, which can diminish this phenomenon (3). Here we test the hypothesis that increased NO synthases activity mimicked by nitric oxide donor SNP (sodium nitroprusside) addition can revert Na<sub>v</sub> 1.4/Na<sub>v</sub> 1.5 IKs ratio after

denervation. Sciatic nerves of male Wistar rats (body weight 120-150 g) were dissected and animals were denervated for 7 days. Denervated animals were daily treated with i.p. injections of sodium nitroprusside (SNP; 1.5 mg/kg). Then, extensor digitorum longus (EDL) muscles of control, denervated and denervated/SNP-treated animals were removed and crude membranes for Na<sub>v</sub> 1.4/1.5 IKs immunodetection were prepared. We did not separate junctional and extra-junctional membranes. In a slight contrast with literary evidence, we were able to detect very low but not completely insignificant levels of Na<sub>v</sub> 1.5 IKs in control innervated muscles, too, with Na<sub>v</sub> 1.5 IKs level 4.9-fold higher in denervated than in control muscles. After SNP treatment, Na<sub>v</sub> 1.5 IKs level reached 54 % of Na<sub>v</sub> 1.5 IKs level in denervated muscles. Ratio of Na<sub>v</sub> 1.4 IKs level remained constant in control/denervated and SNP-treated/denervated animals, respectively. We conclude that NO-donor supply diminished Na<sub>v</sub> 1.5 IKs expression in denervated muscles.

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## CARDIAC CONTRACTION IN THE INTACT AND SYMPATHECTOMIZED ALBINO RATS

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The present study was designed to get better insight into the relationship between the developing cardiac sympathetic innervation and myocardial effector function. We studied contractile properties in control and sympathectomized albino rats from birth till adulthood. We evaluated concentrations of noradrenaline (NA) and neuropeptide Y (NPY) in the cardiac tissue. The chemical sympathectomy was performed by repeated injections of 6-hydroxydopamine (6-OHDA) immediately after birth. Experiments were carried out in 10, 20, 40, 60 and 90-day-old animals. The contraction force (CF) of the right ventricular papillary muscle was recorded in the modified Tyrode solution without and with tyramine (concentrations from 10<sup>-7</sup> to 10<sup>-4</sup> mol/l). Atrial and ventricular concentrations of NA and NPY were measured by radioimmunoassay diagnostic kits. Sympathectomy significantly lowered CF in all followed groups except 60 and 90-day-old animals where no difference in CF between the intact and sympathectomized rats was observed. Time course of contraction and relaxation was unchanged by denervation. No inotropic effect of tyramine and significant decrease in NA concentrations in all age groups of sympathectomized rats reflect the long-termed destructive effect of 6-OHDA on the cardiac sympathetic nerves. NPY is known to be co-secreted with NA from sympathetic postganglionic fibers. Moreover NPY is located in the intracardiac ganglia (1). It was found that NPY contributes to the increase in L-type calcium current density in the rat ventricle (2). In our experiments, NPY concentration in the cardiac ventricles of the younger (40 days and less) denervated rats was significantly lower than in control ones. In older 6-OHDA rats (60 and 90 days), NPY concentrations increased and were comparable with control values. Our results suggested that although sympathetic postganglionic fibers are destructed by 6-OHDA, NPY from preserved intracardiac ganglia might be able to improve contractile performance of the papillary muscle probably by influence on calcium metabolism in ventricular myocytes.

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# EFFECT OF THE PULMONARY VENTILATION ON HEART RATE VARIABILITY IN WISTAR RATS

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**Objectives:** The heart rate variability (HRV) belongs between non-invasive method for the determination of the regulatory interventions of the single sections of the autonomous nervous system. The aim of this study was evaluation of the HRV changes after surgical interventions and at the changes of pulmonary ventilation in anesthetized rats. **Methods:** Experiments were performed in female Wistar rats under ketamine/xylazine anesthesia (100 mg/kg + 15 mg/kg, i.m.) after adaptation on the light – dark cycle 12:12 hours, with the dark part from 6.00 to 18.00 h. The experiments were performed only in the active (dark) part of regime day. The animals were ventilated by artificial respirator at ventilatory parameters: 1 ml/100 g of body weight and respiratory rate 40-50 breaths/min. HRV was recorded in the single steps of experiment: in intact animal before surgical interventions (n=22), after surgical interventions (n=91) and after 5 min (n=54), 10 min. (n=43), 15 min. (n=28) and 20 min. normal pulmonary ventilation (n=14) following 2 min apnoic episode. **Results:** The obtained data showed large inter- and intraindividual distribution. Ketamine/xylazine anesthesia decreased sympathetic tone and increases parasympathetic tone in the active part of the light regime in female Wistar rats what was expressed by the relative low heart rate. The surgical interventions increased sympathetic tone (power VLF about 94 %), parasympathetic tone (power HF about 43 %) as well as baroreflex activity (power LF about 50 %) but with the different proportionality. Restoration of pulmonary ventilation after 2 min. of apnoic episode significantly decreased mainly sympathetic tone and baroreflex activity. Continuing reoxygenation did not restore sympathetic tone and baroreflex activity but step by step increased parasympathetic tone. The evaluation HRV changes from all steps of experiment by chi-quadrat test showed that significant changes were found only for power VLF ( $p<0.02$ ), power LF ( $p<0.001$ ), R-R interval ( $p<0.02$ ) and for relative power VLF ( $p<0.02$ ), relative power LF ( $p<0.03$ ) and relative power HF ( $p<0.007$ ). **Conclusions:** Although some described HRV changes were the statistically significant during experiment, it is concluded that rats under ketamine/xylazine anesthesia is not suitable animal model for the evaluation HRV changes in the aspect of the (i) large date distribution and (ii) non-perspicuousness of the changes and shifts in the single HRV parameters and (iii) persistent high parasympathetic tone during whole experiment.

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# NEONATAL INTRACEREBROVENTRICULAR INFUSION OF QUINOLINIC ACID INDUCES PROLONGED MORPHOLOGICAL AND BEHAVIORAL CHANGES: THE EFFECTS OF A SUBUNIT SELECTIVE NMDA RECEPTOR ANTAGONIST

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Retroviruses can participate on a range of psychiatric symptoms, including cognitive impairment and severe depression. The symptoms seem to be related to the overproduction of cytokines and related substances like quinolinic acid (QUIN) secreted by retrovirus-activated microglial cells. Our previous results suggested that both interleukin-1 $\beta$  and QUIN led to morphological changes after systemic and/or intracranial injection. In this study we used the experimental model of this diseases based on a perinatal exposure of rats to QUIN for examination of (a) late changes in behaviour and morphology of the brain and (b) possibility of protection of QUIN induced damage by a

neuroprotective substance RO 25-691 (Sigma), a selective antagonist of NMDA glutamate receptor containing the NR2B subunit. Twelve days old rat pups received QUIN (250 nmoles QUIN in 0.25  $\mu$ L saline/ventricle). Some animals received also RO 25-6981 (10 mg/kg i.p.) 0.5 h before and 22 h after infusion of QUIN. Behavior and brain morphology were studied on postnatal day (PD) 50 and 90. We found mild hyperlocomotion, declined habituation and a deficit in prepulse inhibition (PPI) in QUIN treated 50 day-old animals. On PD 90, there was longer social exploration time toward to rat pups. The PPI was similar to controls but there appeared higher acoustic startle reaction (ASR). In QUIN/RO treated 50 day-old animals the hyperlocomotion was still slightly higher, habituation and PPI was improved. On PD 90, the behaviour tests were rather similar to those observed in PD 50 group. Morphological examination of the brain of QUIN treated rats revealed massive reduction of the dorsal hippocampus and of the adjacent parasagittal cortex. In some animals small cellular post-necrotic loci appeared in the dorsal thalamus. The ventral parts of the hippocampus were relatively intact. In QUIN/ RO treated animals the extend of this lesions was reduced by about 25 %. The study showed that the schizophrenia-like behaviour of animals induced by QUIN was accompanied by a massive reduction of dorsal hippocampus and of the adjacent neocortical and brain stem regions. The protective effects of RO 25-6981 suggest that NR2B subunit of NMDA receptor plays an important role in the observed morphological and behavioral changes.

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# GENE EXPRESSION OF THE PNMT IN CARDIOMYOCYTES AND ITS MODULATION BY 6-HYDROXYDOPAMINE

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Catecholamines norepinephrine (NE) and epinephrine (Epi) are physiologically important hormones and neurotransmitters in mammals with profound influence on the activity of cardiovascular system. In the heart they originate from different sources. NE is released from terminals of sympathetic postganglionic neurons. Epi is the principal hormone secreted by the adrenal medulla and is taken up from the circulation to the heart. However, there is some evidence that Epi can be synthesized also in the heart. Several authors have reported activity and gene expression of the phenylethanolamine N-methyltransferase (PNMT) in the rat heart. PNMT is the final enzyme in catecholamine synthesizing cascade that converts NE to Epi. Localization of PNMT mRNA in the heart is still under the investigation. In this work we have shown that besides cardiac neuronal cells (cardiac ganglionic cells) and intrinsic cardiac adrenergic (ICA) cells, PNMT mRNA is localized also in cardiomyocytes. To determine the origin of cardiac PNMT mRNA in normal and stressed conditions, we excluded PNMT production from neuronal cells by chemical sympathectomy by administration of the 6-hydroxydopamine (6-OHDA) which is highly selective neurotoxin for catecholaminergic neurons and determined PNMT mRNA levels in the left atria and ventricles of control and stressed rats. In the rats treated with 6-OHDA, PNMT mRNA levels were not changed under normal physiological conditions compared to control group of rats. Nevertheless, exposure to single, 2-hour immobilization stress significantly increased gene expression of the PNMT in atria and ventricles, but 6-OHDA prevents this increase and mRNA levels remained on control values. We have also observed a decrease of heart NE levels in 6-OHDA treated group of rats. In stressed rats after 6-OHDA treatments we have found a slight increase of Epi level but compared to vehicle treated group the increase was significantly lower. These results allow us to propose that in the heart, not only neuronal cells but at least also cardiomyocytes express the PNMT. Stress-induced

increase of PNMT is due to increased transcription in the neuronal cells in the heart. Thus, it seems that PNMT in cardiomyocytes synthesize Epi. Physiological relevance of the PNMT expression in the heart is not clear yet and must be elucidated.

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#### ISOLATED LUNG MAST CELLS DO NOT INCREASE ROS PRODUCTION IN HYPOXIA.

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A release of collagenolytic enzymes - tissue metalloproteinases (MMPs) from hypoxia-activated mast cells possibly plays an important role in structural remodelling of peripheral pulmonary vasculature during chronic hypoxia (1). Because the effect of hypoxia on MMPs release is attenuated by antioxidant N-acetylcysteine (NAC) (2), we hypothesized that mast cells activation is triggered by reactive oxygen species (ROS). Present study was designed to determine ROS formation in isolated lung mast cells exposed to hypoxia *in vitro*. Mixture of lung cells was isolated from rat lungs by enzymatic digestion of the lung tissue. The mixture was then divided into two groups and cultivated for 24 hours either in normoxia (21 % O<sub>2</sub>, 5 % CO<sub>2</sub>) or "*in vitro*" hypoxia (10 % O<sub>2</sub>, 5 % CO<sub>2</sub>). Light microscopy was used to examine presence of reaction products of ROS in macrophages and lung mast cells. While we have seen indications of ROS production in macrophages (exposed to normoxia or hypoxia), mast cells never showed any sign of such production. The results suggest that activation of lung mast cells in hypoxia depends on an external source of ROS.

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#### SYSTOLIC FORCE QUANTITATIVE MEASURING and HEART RATE VARIABILITY ANALYSIS

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This report deals with the quantitative measuring of systolic force and the analysis of heart rate variability (HRV) through the use of the quantitative seismocardiography (Q-SCG). The use of Q-SCG makes it possible to set up the characteristics as follows: the systolic force (F) and the minute cardiac force (MF), which is related to the body mass of each examinant so as to obtain comparable values. F represents response of force according to heart activity and is expressed in units of force (Newton). To obtain a picture of the total intensity of the heart activity, we use MF, which equals F multiplied by heart rate (HR) (F · HR) and is expressed in units of force per minute. When examining Q-SCG the sensing head directly entraps the signals according to heart activity without the necessity of applying the adhesive or tongue-electrodes to the body of the patient, and connecting him by cables to an apparatus. What we have is an absolutely non-invasive method. Up to present, the Q-SCG amplitude of the curves has been used as an indicator of the cardiac force. Whereas the impulses are recorded exactly action-timed, a new field is about to open for the use of Q-SCG in recording the heart rate as well as assessing the HRV. HRV in hypertension treatment was studied in 14 men and 9 women. Some

HRV parameters were measured. The first HRV measurement was performed in hypertonics which were either without or with insufficient treatment. The second one was performed after three months therapy which normalized blood pressure. Parameters defining relation between sympathetic and parasympathetic nervous system changed in the sense of lowering of sympathetic activity. The numeric value of the stress index (SI) which was in this connection till now not yet used was lowered. The importance of the central and autonomic mechanisms of blood pressure regulation was proved and also the importance of the HRV method in the study of these problems was shown. The exactitude and reliability of the above method will be discussed in relation to the current monitoring of persons during their conventional working activities. This absolutely non-invasive method can be used to monitor the operators either within their examination on the ground or in flight conditions.

#### INDUCTION OF LIVER BIOTRANSFORMATION SYSTEM IN PATIENTS WITH DIABETES MELLITUS

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Diabetes results from abnormality in the production or the use of insulin. It is associated with a lot of changes in intermediary metabolism. Liver plays a central role in regulation of metabolism of human body. It is not surprising that diabetes mellitus induces marked metabolic changes in liver tissue which would lead to disorders of liver function. Several authors reported on increased activity of liver biotransformation system in animals with experimental diabetes mellitus. The aim of our contribution was to study the activity of liver biotransformation system in patients with diabetes mellitus type I. Patients and methods: The study group consisted of 27 patients with diabetes mellitus aged 15-44 years. Activity of liver biotransformation was determined using antipyrine test. Glycated hemoglobin was determined as parameter for assessment of compensation of diabetes. Results: The results of our study showed significantly decreased half-life of antipyrine in patients with diabetes mellitus (controls vs. diabetes: 8.15 hours vs. 6.21 hours), which confirmed induction of liver biotransformation system. There was significant negative correlation between plasma glycated hemoglobin levels and antipyrine half-life ( $r = -0.812$ ,  $P < 0.001$ ).

	Controls	Compensated diabetes	Decompensated diabetes
Antipyrine half-life	8.15 hours	7.25 hours	4.5 hours
Glycated hemoglobin	5.2 %	7.8 %	13.5 %

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#### ADAPTATIONAL CHANGES OF SARCOMERE LENGTH IN OXIDATIVE MUSCLES OF MICE LACKING CREATINE KINASE

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Creatine kinase (CK) is a key enzyme of muscle energetics that efficiently links locations of energy production and utilization. Inactivation of both the mitochondrial and cytosolic isoforms of CK in different muscle cells triggers a tissue-specific adaptation of the mitochondrial function and reorganisation of their ultrastructure (1). Morphometric analysis of sarcomere length of slow and fast skeletal muscles of mice lacking CK (CK<sup>-/-</sup>) revealed adaptational changes at the level of contractile filaments (2). The lack of CK in skeletal muscle cells led to elongation of the sarcomere and the A band in comparison to

control mice. In fast skeletal muscle cells, the thickness of the Z line of CK<sup>-/-</sup> mice increased as well, in contrast to slow muscles. The aim of this study was to compare the resting length of the sarcomere and its constituents in two functionally different oxidative muscle types – the slow skeletal muscle (soleus) and the cardiac muscle of left ventricle of control and CK<sup>-/-</sup> mice. Morphometric analysis of electron microscopic images was performed using the program Image Tools v2. The results revealed, that in contrast to slow skeletal muscle fibres, in which the sarcomere length of CK<sup>-/-</sup> cells increased significantly by 15.8 % relative to controls (2189±454 nm, mean ± SD), and similarly, the A band length increased significantly by 7.1 % (from 1041±122 nm in controls), there was no significant change of the sarcomere length in cardiomyocytes of CK<sup>-/-</sup> mice. In control cardiomyocytes, the mean sarcomere length was 1770±189 nm, while in CK<sup>-/-</sup> cardiomyocytes the sarcomere length was 1810±172 nm. In control cardiomyocytes the mean A band length was 1203±79 nm while in CK<sup>-/-</sup> mice it was 1261±53 nm. In case of the Z line there were no significant differences in thickness between the control and the CK<sup>-/-</sup> cardiomyocytes (57.4±3.1 nm and 59.4±4.5 nm, respectively). We conclude that inactivation of CK in two different oxidative muscle types led to principally different regulatory mechanisms at different levels.

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#### GLUTATHIONE DETOXICATING SYSTEM IN LIVER OF ANIMALS WITH EXPERIMENTAL DIABETES MELLITUS

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Diabetes results from abnormality in the production or the use of insulin. It is associated with a lot of changes in intermediary metabolism. It is known that diabetes mellitus is a disorder connected with increased production of reactive oxygen radicals. Important role as antioxidant has reduced glutathione and glutathione related enzymes such as glutathione peroxidase. The aim of our study was to determine the status of liver glutathione antioxidant system in animals with experimental streptozotocin diabetes mellitus. Experimental conditions: Newborn male Wistar rats were used in the experiment. Diabetes was induced by repeated intraperitoneal administration of streptozotocin (45 mg/kg body weight in 0.1 mol.l<sup>-1</sup> citrate buffer, pH=4.5) on 2nd and 9th day after birth. Control group received only the same volume of citrate buffer at the same time. The level of reduced glutathione and the activities of glutathione peroxidase, glutathione reductase and glutathione transferase were determined in liver homogenates of diabetic and control animals. Results: The results of our study showed significantly decreased levels of reduced glutathione in diabetic animals (controls vs. diabetes: 8.0±0.6 µmol/g vs. 7.8±0.5 µmol/g, P<0.05). The activities of glutathione peroxidase and glutathione transferase were increased in diabetic animals in comparison to controls. There was no difference between the activities of glutathione reductase in control and diabetic animals.

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#### UCP1-INDEPENDENT THERMOGENESIS: POSSIBLE ROLE FOR ADIPOSE TISSUE

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The aim of this study was to investigate the possibility that UCP1-independent thermogenic mechanisms, resides in the adipose tissue. Ucp1<sup>+/+</sup> and cold-sensitive Ucp1<sup>-/-</sup> mice were gradually acclimated to cold environment (2 °C per day). Metabolic characteristics between the two genotypes were determined by indirect calorimetry and metabolic properties of adipose tissue were measured by Clarke electrode. Possible mechanistic links were further investigated by immunohistochemistry and qRT-PCR. Cold acclimation lowered the body weight (1.2±0.1 g, p<0.05) and body fat content (0.8±0.1 g, p>0.05) in both Ucp1<sup>-/-</sup> and Ucp1<sup>+/+</sup> mice, while the lean body mass remained unchanged. However, Ucp1<sup>-/-</sup> mice exhibited higher increase of the energy expenditure when acclimated to 4 °C. Respiratory exchange ratio was similar for both genotypes and remained unchanged with the declining temperature. Adipose tissue from cold acclimated Ucp1<sup>-/-</sup> mice had higher metabolic capacity. Distinctive changes in protein and gene expression for Ca<sup>2+</sup> ATPase and phospholamban suggested a role for Ca<sup>2+</sup>ATPase in the UCP1-independent thermogenic process. Cold acclimation-initiated UCP1-independent thermogenesis that was associated with higher metabolic capacity of adipose tissue and possibly with elevated Ca<sup>2+</sup> cycling.

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#### UNILATERAL FOREIGN MUSCLE TRANSPLANTATION HAS NO EFFECT ON PHENOTYPE OF UNOPERATED RAT MUSCLES IN LONG TERM EXPERIMENTS

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To reveal the effect of foreign innervation and altered thyroid status on the fiber type composition and myosin heavy chain (MyHC) isoform expression of transplanted rat slow soleus (SOL) and fast extensor digitorum longus (EDL) muscles, the method of heterochronous isotransplantation was developed (1, 2). In this experimental procedure the SOL or EDL muscles of young inbred Lewis rats are intramuscularly grafted either into the host EDL or SOL muscles of adult rats of the same strain with normal or experimentally altered thyroid status. To estimate the extent of fiber type transitions in the transplanted muscles, SOL and EDL muscles from the contralateral, unoperated leg and either SOL or EDL muscles from the ipsilateral, operated limb could be used as the controls in case that the experimental procedure does not affect these muscles. To verify this, in the present study the fiber type composition and MyHC isoform content of SOL and EDL muscles of naive and experimental 4- to 18-month-old rats was compared. We have demonstrated that the unilateral heterochronous isotransplantation procedure had no significant effect on the fiber type composition (determined by mATPase reaction and by immunocytochemical staining using monoclonal antibodies specifically recognizing MyHC1, 2a, 2x/d or 2b isoforms using a 2-D stereological method based on unbiased counting frame and point counting, C.A.S.T. Grid System, Olympus, Albertslund, Denmark, 3) and on MyHC isoform content (MyHC isoforms separated by SDS-PAGE have been densitometrically evaluated using AIDA 3.28 computer program, Advanced Image Data Analyzer, Germany) of the control unoperated muscles of the experimental animals when compared to the corresponding age groups of naive rats; hence these muscles can be used as controls in our current long term chronic grafting experiments.

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#### THE CHANGES OF FUNCTIONAL FEATURES OF ISOLATED PULMONARY VESSELS AFTER EXPOSURE TO EARLY PHASE OF CHRONIC HYPOXIA

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Exposure to chronic hypoxia results in hypoxic pulmonary hypertension (HPH). Two mechanisms are involved: structural remodeling of the vessel wall and vasoconstriction. We centered our interest to changes of pulmonary vascular smooth muscle reactivity in to early phase of exposure to chronic hypoxia. We studied isolated pulmonary vessels from adult male rats exposed for 4 days to isobaric hypoxia ( $F_{iO_2} = 0.1$ ) and compared them with normoxic controls. The bath in the chamber of small vessel myograph was saturated by gas mixture containing 21 % or 95 % of  $O_2$  with 5 %  $CO_2$ , balanced with  $N_2$  and we measured reactions of vessels to  $PGF_{2\alpha}$  and acute hypoxic challenge with 0 %  $O_2$ . We didn't observe any difference among the groups when the normoxic conditions were settled in bath. When the bath oxygenation increased to 95 %  $O_2$ , the contraction induced by hypoxic challenge decreased in rats exposed to hypoxia (for the first phase of hypoxic response from  $0.6 \pm 0.0$  % to  $0.4 \pm 0.4$  % of maximal contraction,  $P \leq 0.1$ , for the second phase from  $0.1 \pm 0.2$  % to  $-0.1 \pm 0.4$  %,  $P \leq 0.3$ ). It did not change in normoxic controls (for the first phase  $0.5 \pm 0.8$  % in bath saturated by 21 %  $O_2$ ,  $0.9 \pm 0.4$  % in bath gassed by 95 %  $O_2$ ,  $P \leq 0.9$ , for the second phase  $0.9 \pm 0.2$  % in bath with 21 %  $O_2$ ,  $0.5 \pm 0.3$  % in bath with 95 %  $O_2$ ,  $P \leq 0.7$ ). We hypothesized that reduced reactivity of vessels from hypoxic rats in hyperoxia results from depletion of antioxidant systems due to increased free radical production during exposure to chronic hypoxia.

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#### PATHOPHYSIOLOGY OF THE ISCHAEMIC-REPERFUSION SYNDROME OF LOWER LIMBS

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Ischaemic-reperfusion damage (IRD) of the tissue in patients with ischaemia of lower limbs (LL) has been an actual problem. Sudden occlusion or reduction in the arterial blood inflow to tissues or organs will cause ischaemia that could be of various degree. Ischaemia alone causes morphological and pathophysiological changes leading to subsequent clinical status, manifesting according to this which tissue or organ is affected by ischaemia. Patients were hospitalised at the 1st Surgical clinic in Košice. There were 35 patients of the mean age of 50 y./22 patients operated on the non-ischaemic disease of LL-represented CG and 13 patients with ischaemic disease of LL (ICHLL). In the patients with ICHLL two pilot groups were formed: the group 1 without CLI (critical limb ischaemia) without revascularising operation, with limb saving in all of them and the group 2 with CLI. The examination of the peripheral limb congestion was performed (angiography, Doppler USG and malleolus-brachial indices). In a link with these examinations the concentrations of some biochemical parameters were found out: activity of antioxidant enzymes – CAT, SOD, GPX, concentration of TBARS, TAS. Of the low-molecular antioxidants: vitamins A, E, C, ceruloplasmin (Cpl), transferrin (Trf) in serum. Of the lipid parameters: apo B, and cytokine IL-6, TNF-alpha.

Concentrations of vitamins A, E, C was shown to be decreased at the initial sampling, and in non-enzymatic Trf and CPI the values were on the upper limit of the physiological values in the patients with ICHLL in comparison with their concentrations in the CG. Regarding the reduced serum values of the parameters we suppose that their insufficient saturation also contributes to the development of diabetic angiopathy. We assayed the concentrations of IL-6 (in CG they ranged from 7.62 to 73.34 pg/ml and in patients with ICHLL from 10.9 to 61.05 pg/ml), TNF-alpha (in CG the values ranged from 3.4 to 109.14 pg/ml and in patients with ICHLL from 2.8 to 28.84 pg/ml). At the comparison of our values with other studies, the lower concentrations of antioxidant enzymes, TAS in the group of patients with ICHLL versus CG were recorded. The difference in the values of – CPL and Trf was also recorded between the group of patients with and without ICHLL, where the decreased values were in the patients with ICHLL. Contrariwise, the concentrations of apoB were increased on the significance level  $p < 0.05$ . In the values of IL-6, TNF-alpha the higher concentrations were found in the patients with ICHLL. Our goal remains to look for the most suitable and most specific markers that could help at the making diagnosis more accurate and to postpone the negative consequence of this diseases such as amputation.

#### SPONTANEOUS VESICULAR AND NON-VESICULAR ACETYLCHOLINE LEAKAGE IN NEUROMUSCLAR SYNAPSE DURING ONSET OF HYPOXIA

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Quantal and non-quantal spontaneous acetylcholine release expressed as miniature endplate potential frequency (MEPP) and the curare-induced endplate hyperpolarization (H-effect) respectively, increased during the first 30 min of hypoxia in oxygen-deficient superfusing solution when normal extracellular calcium ( $[Ca^{2+}]_0 = 2.0$  mM) was present. The about ten times increase of the MEPP frequency induced by hypoxia was almost absent in low calcium solution ( $[Ca^{2+}]_0 = 0.4$  mM) at 20 °C, whereas there was still significant increment of the non-quantal, non-vesicular release. The latter is apparently based on the vesicle associated transporters incorporated into the terminal membrane during vesicular transmitter release. Despite having common background, each of these two processes of release is influenced by different oxygen- and calcium sensitive mechanism(s) as indicated by present data. The rise of f-MEPPs during the onset of hypoxia apparently requires the  $Ca^{2+}$  entry into the nerve terminal, whereas the non-quantal release can be increased by another factors such as the lower level of the ATP (1).

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#### LOCAL CONTROL OF CALCIUM RELEASE-DEPENDENT INACTIVATION OF CALCIUM CURRENT.

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The L-type calcium current ( $I_{Ca}$ ) of mammalian cardiac myocytes displays a fast-inactivating component, induced by sarcoplasmic reticulum calcium release. To characterize the kinetics of this release-

dependent inactivation (RDI) quantitatively, we have developed two models that account for the local control of calcium release activation and for the subsequent rapid inactivation of  $I_{Ca}$ . Both models were based on the concept of independent excitation-contraction coupling units (couplons) and assumed that calcium current activation triggers activation of calcium release with a variable probability and that the subsequent inactivation of calcium channels by the released calcium is immediate. They differed in the description of the slow component of inactivation. In the CDI model, it was modeled as current-dependent inactivation affecting all calcium channels. In the DDI model, it was modeled as a process evoked by late calcium release events that affected only a fraction of channels. While both models provided good description of the kinetics of  $I_{Ca}$  and showed excellent convergence, the DDI model provided significantly lower  $\chi^2$  values. Both models provided the same values of parameters of  $I_{Ca}$  activation and of the fast component of RDI. The degree of RDI and the synchrony of calcium release had a bell-shaped dependence on membrane potential,  $V_m$ , while the delay between stimulus and maximum release had a U-shaped dependence. The probability of triggering calcium release decreased with  $V_m$ . The voltage dependence of RDI parameters suggests that triggering of release by  $I_{Ca}$  is controlled by the rate of calcium channel activation at negative membrane potentials while it is controlled by the unitary  $Ca^{2+}$  current amplitude at positive potentials. Once the release event in the couplon is triggered, all its adjoined calcium channels inactivate. The duration of delay between activation of calcium current and activation of calcium release suggests that the fidelity of coupling between DHPR and RyR channels is substantially less than one at all membrane potentials. The new kinetic model of calcium current provides means to track  $Ca^{2+}$  release in situ under physiological conditions.

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#### ALLOSTERIC REGULATION OF RYANODINE RECEPTOR ACTIVATION BY CALCIUM.

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Ryanodine receptors (RyRs) provide the cytoplasm with calcium ions required for contraction by the process of calcium-induced calcium release. In numerous RyR point mutants, implicated in the arrhythmogenic disease phenotype CPVT (catecholaminergic polymorphic ventricular tachycardia), the regulation by channel activation is defective, and in certain mutations channels show increased activity at low calcium concentrations (1). The mechanism by which the mutations affect activation of RyRs is not understood. Previously it was generally accepted that ryanodine receptors can open only after binding of (several) calcium ions. We have found that the calcium dependence of RyRs containing one or two monomers deficient in calcium-dependent activation (2) cannot be adequately described by such linear models, which require that channel opening follows calcium binding. However, other calcium-activated channels such as the high-conductance calcium-activated potassium channel have been shown to possess separate pathways for channel opening and for calcium binding, and their activation is understood as the result of allosteric coupling between the two processes. Therefore we analyzed the effects of allosteric regulation, modal gating, and structure of the Ca binding site on the calcium dependence of activation of RyR variants with variable number of mutant monomers. To adequately describe the effect of mutation on RyR activity, RyR models needed (i) four independent Ca binding sites, (ii) allosteric coupling between Ca binding and channel opening, and (iii) the presence of low-activity gating modes. Our results give a quantitative basis to the previously suggested relationships between conformational changes and gating transitions of the RyR. These results open new insights into the molecular mechanisms of RyR activation by calcium and provide important clues for understanding

2+2+2+

RyR regulation in health and disease.

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#### TWO TYPES OF CALCIUM RELEASE EVENTS AND THEIR RELATIONSHIP TO INACTIVATION OF CALCIUM CURRENT IN CARDIAC MYOCYTES

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Functionality of Ca-release units, important for efficiency of excitation-contraction coupling, was assessed by measuring the variability of the amplitude and the latency of Ca-spikes evoked by calcium currents in isolated rat ventricular myocytes. We have found two distinct categories of Ca-spikes. Latencies of early Ca-spikes were distributed normally near the peak of calcium current, while the late Ca-spikes were of significantly lower amplitudes and occurred when the calcium current was already substantially inactivated. Correlation analysis of calcium currents and Ca-spikes showed that the probability of late Ca-events was higher in cells with low Ca-current densities and with late peaks of Ca-current. Cells with relatively high occurrence of late Ca-events showed low fraction of release-dependent inactivation of Ca-current and slower release-dependent inactivation kinetics and had lower synchronization of early events. There was a pronounced correlation of Ca-release event synchronization with current density. The probability of late occurrence of Ca-events increased in cells, in which the calcium load of the sarcoplasmic reticulum was reduced by repeated release stimulation and was accompanied by the decrease of the amplitude of both the early and late events. These results suggest that the early Ca-release events are tightly coupled to calcium current activation, are responsible for the fast component of the release dependent inactivation of calcium current, and provide most of the intracellular calcium concentration increase. The presence of late Ca-spikes with low amplitude suggests the existence of a subpopulation of "lazy" RyR clusters or dyads, which may contribute to the slow component of the calcium current inactivation, and which prolong the intracellular calcium concentration increase and thus slow down the rate of relaxation of myocyte contraction.

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#### THE RELATIONSHIP BETWEEN PHYSICAL ACTIVITY AND BLOOD PRESSURE REGULATION IN ADOLESCENTS TREATED FOR MALIGNANT DISEASE.

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**Introduction:** The aim of the study was a comparison of body and circulatory parameters with respect to physical activity between healthy subjects and those who were previously treated for a malignant tumour. **Methods:** We examined 32 healthy children and adolescents (Co; 15.7±2.1 years) and 21 subjects after antitumour therapy (M; 15.5±3.2 years). In group M, 8 subjects had a non-cardiotoxic therapy (A0), and 13 children were treated with cardiotoxic anthracyclines (A+). In all children, we recorded non-invasively beat-to-beat systolic (SBP) and diastolic (DBP) blood pressure and inter-beat intervals (IBI). Baroreflex sensitivity was estimated by spectral analysis as BRS (ms/mmHg) and BRSf (mHz/mmHg). Physical activity was evaluated on the basis of questionnaires completed by all subjects for one week. **Results:** We found significantly lower SBP in group A+ vs. A0 and Co (A+: 101±11, A0: 123±4, Co: 116±4 mmHg; p<0.001). Significant differences

between group A+ and Co were found in the parameters followed: higher BRSf (A+:  $21 \pm 7$ , Co:  $15 \pm 7$ ,  $p < 0.001$ ; A0:  $19 \pm 10$  mHz/mmHg); lower weight (A+:  $52 \pm 13$ , Co:  $61 \pm 10$ ,  $p < 0.005$ ; A0:  $56 \pm 15$  kg) and height (A+:  $160 \pm 13$ , Co:  $172 \pm 8$ ,  $p < 0.05$ ; A0:  $165 \pm 10$  cm); lower total physical activity (A+:  $9546 \pm 4605$ , Co:  $12741 \pm 3377$ ,  $p < 0.01$ ; A0:  $12830 \pm 7832$  kJ/day) and physical activity related to 1 kg of body weight (A+:  $193 \pm 38$ , Co:  $208 \pm 38$ ,  $p < 0.01$ ; A0:  $212 \pm 69$  kJ/kg/day). Conclusion: The subjects treated for a malignant tumour by anthracyclines in comparison to healthy controls and subjects treated for a tumour without anthracyclines had increased parasympathetic and decreased sympathetic tonic activity as was documented by lower systolic blood pressure and higher baroreflex sensitivity. The increase of parasympathetic reflex control of the heart after antitumour therapy is not a sign of physical training as it is seen in healthy population, but rather could be a consequence of cardiotoxic and neurotoxic therapy or of a disease.

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#### DAY-NIGHT PLASMA UROTENSIN II CONCENTRATIONS IN HYPERTENSIVE TGR RATS

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Urotensin II is a vasoactive peptide involved in control of cardiovascular physiology. Urotensin was initially demonstrated in fish and its cyclic heptapeptide sequence is evolutionary highly preserved in all vertebrate classes. High evolutionary conservation and experimental evidence suggest urotensin II as effective regulator of blood pressure (BP) but possible control mechanisms are not known. TGR (mREN2) 27 rats exhibit high expression of renin-angiotensin system in several peripheral tissues and brain resulting in severe hypertension. Hypertension develops between weeks 5 and 8 of age and during this time a circadian rhythm in BP inverts, exhibiting higher values during the day than during the night. The aim of our study was to measure plasma urotensin II concentrations in the middle of the day and night in TGR rats to reveal if this peptide participates in control of high BP and the inverted profile of BP in this strain of rats. Mature male (11 week old) control (Sprague Dawley; SD) and hypertensive TGR rats were killed in the middle of the day (8 animals per each strain) and the night (8 SD and 9 TGR). Blood was collected into EDTA containing tubes and transferred to centrifuge tubes containing aprotinin to inhibit activity of proteinases. Plasma was stored at  $-20^{\circ}\text{C}$  for one month and assayed by radioimmunoassay kit (Phoenix Peptide, Belmont, USA). Samples were assayed both directly and after extraction using SEP columns and results were compared by t-test and ANOVA. Plasma urotensin II concentrations were significantly lower in hypertensive TGR rats ( $10.95 \pm 0.44$  pg/ml) than in control animals ( $13.32 \pm 0.74$  pg/ml). There were no significant differences between daytime and nighttime hormone concentrations either in control or hypertensive rats although the nighttime levels were significantly higher in control than in TGR rats. Plasma urotensin II concentrations probably do not play a key role in control of hypertension and the inverted circadian profile of BP in TGR rats.

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