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RAMADAN FASTING AND THE CIRCADIAN RHYTHM OF BLOOD PRESSURE, HEART RATE AND ROBINSON INDEX

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In the lunar month *Ramadan*, more than one billion people worldwide fast from dawn to sunset (They do not eat, drink or smoke and avoid sexual intercourse). Our aim was to investigate the effect of Ramadan fasting (from 4 a.m. ±1 hr to 4:30 p.m. ±30min in the Winter) on the circadian rhythms of arterial blood pressure (BP):systolic (SBP), diastolic (DBP) and mean (MBP); heart rate (HR) and Robinson Index (= the rate pressure product) (RI) in normotensives. We examined 14 young men, age 28 ±7 years once during Ramadan (dR) and once after Ramadan (aR) by using an ambulatory blood pressure monitor (Accutracker II). The measuring lasted 3 days dR and 3 days aR continuously for each one. The diurnal measured values (mean ±SD) of SBP, DBP, MBP, HR and RI during Ramadan were slightly lower than those after Ramadan with significant differences at 9a.m.-4p.m. For instance at 11 a.m.: SBP 113.3 ±13.9 mmHg versus (vs) 125.2 ±19.9 mmHg, P<0.001; DBP 72.7 ±11.8 mmHg vs. 77.8 ±12.0 mmHg, P<0.03; MBP 85.8 ±11.6 mmHg vs. 93.1 ±13.2 mmHg, P<0.01; HR 72.6 ±15.9 beat/min vs. 80.8 ±15.1 beat/min, P<0.01; RI 82.6 ±23.8 (mmHg *beat/min)/100 vs. 101.9 ±30.3 (mmHg *beat/min)/100, P<0.001. Inverse differences were found at 4p.m.-6p.m. For example at 5p.m.: SBP, DBP and MBP were higher dR than aR but nonsignificant; HR 86.1 ±17.2 beat/min vs. 79.9 ±14.8 beat/min, P<0.05; RI 112 ±33.7 (mmHg *beat/min)/100 vs. 99.9 ±23.1 (mmHg *beat/min)/100, P<0.02. The nocturnal measured values dR and aR were the same or slightly higher in Ramadan reaching their peak at 3a.m. (SBP 109.3 ±15.3 mmHg vs. 100.5 ±13.3 mmHg, P<0.03; DBP 65.4 ±12.7 mmHg vs. 59.0 ±10.6 mmHg, P<0.05; MBP 79.6 ±12.9 mmHg vs.72.6 ±10.9 mmHg, P<0.05; HR P=0.20 nonsignificant. and RI 71.5 ±13.4 (mmHg *beat/min)/100 vs. 63.3 ±11.8 (mmHg *beat/min)/100, P<0.02). We confirm that fasting during Ramadan has advantages in lowering BP and HR during the day and disadvantages in the evening and early morning (at the peak moments) which could increase the risk for patients with uncontrolled hypertension, unstable angina pectoris, myocardial infarction or heart failure.

NARGHILE SMOKING, IS IT SAFER THAN CIGARETTE SMOKING? THE EFFECT OF SMOKING ON BLOOD PRESSURE AND ITS REGULATION

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Narghile (Water-pipe, Shisha) smoking is an old method of tobacco smoking which is now increasing again worldwide. In this method, the smoke passes through water before being inhaled. The aim of this study is to investigate the acute effect of Narghile smoking on heart rate (HR), blood pressure (BP) and the baroreflex control of BP. In 14 healthy volunteers (male), habitual Narghile smokers (HNS) age 27 years and 7 healthy volunteers (male), habitual cigarette smokers (HCS) age 30 years (served as control group), we used non-invasive methods (Finapres Ohmeda) for investigating inter-beat interval (IBI; ms), systolic, diastolic and mean blood pressure (SBP, DBP and MBP; mmHg). The baroreflex sensitivity in ms/mmHg (BRS) was determined by spectral analysis. The measurements were taken twice for each group. The first measurement was taken before the Narghile or cigarette smoking session (after >12 hours of smoking cessation with a complete stopping of alcohol, coffee or tea drinking). The second measurement was taken during a 5 minute period immediately after the smoking session. The session lasted for 45 minutes, during which the volunteer smoked 5g Maassel (fruit flavoured tobacco) (HNS) or 5 cigarettes (HCS). In the HNS group: the IBI decreased (822 ±103 to 664 ±82 ms, *P*<0.001), SBP increased (107 ±10 to 122 ±10 mmHg, *P*<0.001), DBP increased (68 ±12 to 82 ±11 mmHg, *P*<0.01), MBP increased (81 ±11 to 96 ±10 mmHg, *P*<0.001), and BRS decreased (8.38 ±3.4 to 5.17 ±2.4 ms/mmHg, *P*<0.01). In the HCS group: the IBI decreased (843 ±45 to 731 ±84 ms, *P*<0.01), SBP increased (100 ±9 to 111 ±12 mmHg, *P*<0.07, nonsignificant), DBP increased (59 ±7 to 72 ±10 mmHg, *P*<0.05), MBP increased (73 ±7 to 86 ±11 mmHg, *P*<0.05), and BRS decreased nonsignificantly. When comparing both groups before smoking, all measured values show no significant differences. After smoking, the IBI of HNS became less than that of HCS; P=0.097 (nonsignificant). The SBP, DBP and MBP became higher in HNS; P<0.05, P<0.05 and P<0.05; but the difference in BRS was not significant. Narghile smoking induced a higher increase in SBP, DBP, MBP and higher decrease in IBI and in BRS than did cigarette smoking. We conclude that Narghile smoking is not safer than cigarette smoking, with regard to cardiovascular risk factors.

ADHESION AND GROWTH OF ENDOTHELIAL CELLS ON ARTIFICIAL MATERIALS FOR CONSTRUCTION OF VASCULAR REPLACEMENTS

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Clinically used vascular prostheses are usually made of highly hydrophobic polymers not suitable for formation of confluent well-functioning endothelial cell layer on their inner surface. In addition, the roughness of the luminal surface of woven or knitted prostheses is often too high for cell spreading necessary for further proliferation and differentiation of anchorage-dependent cells. In the first set of experiments, the surface hydrophobicity of polytetrafluoroethylene foils was lowered by their irradiation with ultraviolet light in an ammonia atmosphere (1). This treatment resulted in the formation of oxygen- and amine- containing chemical functional groups, and improved the attachment, spreading and growth of human umbilical vein endothelial cells as well as the formation of confluent endothelial cell layer in cultures on these surfaces. The improved cell colonization was probably due to the preferential adsorption of cell adhesion-mediating extracellular matrix molecules from the serum of the culture media to the modified polymer and a higher accessibility of their specific amino-acid sequences (e.g., RGD, REDV) to adhesion receptors on cells (2). In the second set of experiments, selected cell adhesion-mediating proteins (collagen, fibrin, laminin) were attached in a controllable manner to the inner surface of knitted polyethylene terephtalate vascular prostheses produced in the company VÚP a. s., Brno. This treatment lowered the surface roughness, increased the adhesion and growth of human saphenous vein endothelial cells and in case of fibrin, it also improved the cell resistance to the detachment by shear stress in a perfusion system simulating blood flow. Therefore, modification of physicochemical properties of the inner surface of polymeric vascular prostheses and/or coating this surface with autologous proteins, which could be derived from the patient's blood (e.g. fibrin), could be suitable approaches to the endothelialization of these grafts.

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CHRONOPHYSIOLOGICAL DEPENDENCE CHANGES OF ECG PARAMETERS DURING APNOE AND REOXYGENATION IN WISTAR RATS

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The aim of study was to evaluate the effect of apnoe and reoxygenation on some electrophysiological parameters of the ECG in dependence on the light – dark cycle (LD cycle). The experiments were performed in ketamine/xylazine anaesthesia in female Wistar rats

(100mg/kg+15mg/kg, i.m., open chest experiments). The effect of the light period was followed after adaptation to the LD cycle of 12:12 hours, with the dark part from 18.00 to 06.00h and of the dark period after inverse setting of LD cycle, with the dark period from 06.00 to 18.00h. The animals were artificial ventilated by respirator at ventilatory parameters: 1 ml/100g of body weight and respiratory rate 40 – 50 breaths/min. The apnoic episodes was simulated by the swich off respirator for 2 minutes. PQ and QT intervals were evaluated from the single steps of experiments (intact animal before surgical interventions, after tracheotomy, artery preparation, thoracotomy, at the end of 5 min. stabilization, after 30., 60., 90., 120 sec. of apnoic episode and after 5., 10., 15. and 20 min. reoxygenation during the light and the dark periods). The experiments were performed during the whole year and results were averaged independently of seasons. The significant LD differences in duration of PQ intervals were found ($p<0,001$) after 30 and 60 sec. of apnoic episode, but this significance was not determined after 90 and 120 sec. Reoxygenation shortened the PQ intervals and recovered significant LD differences ($p<0,001$). LD differences in the durations of QT interval were not found in the control measurements. The significant differences were found ($p<0,001$) only after 90 and 120 sec. of apnoic episode with the longer durations in the dark period. After 15 and 20 min. of reoxygenation, prolongations of QT interval were marked in the dark period. It is concluded that the predisposition of the myocardium for the ventricular arrhythmias result from disorders of the production and impulse conduction is significantly influenced by LD cycle not only in the intact animals but also during the apnoic episode and reoxygenation. Dispersion of the refractory periods, presented by duration of QT interval, is independent on LD cycle. Probably, LD dependence in the dispersion of the refractory periods arises only after serious apnoic and reoxygenation injures.

THE ROLE OF PROTEIN KINASES IN RESPONSES TO CHRONIC SOCIAL STRESS IN RAT HEARTS

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Cascades of mitogen-activated protein kinases (MAPKs) and PI3K/Akt kinase cascade were shown to play an important role in responses of rat hearts to stress situations. The present study was focused on the investigation of the role of MAPKs and Akt kinase in responses of rat hearts to chronic social stress produced by crowding. Experiments were performed on normotensive rats (Wistar Kyoto) and spontaneous hypertensive rats (SHR). Adult males, 15 weeks old, were exposed to 8-week crowding stress (5 rats/cage, 200cm^2/rat). Control rats were kept 4 rats/cage (480cm^2/rat). The heart tissue samples were taken from all experimental groups. Total contents of MAPKs, Akt kinase and aFGF were determined by Western blot analysis using specific antibodies. Specific phosphorylation (activation) of kinases was detected using phospho-specific antibodies. The levels of total MAPKs and Akt kinase in all tested groups were not influenced by crowding stress. On the other hand, study of activation of protein kinases showed that crowding stress increased content of dual phosphorylated (Thr202/Tyr204) extracellular-signal activated protein kinases (ERK) in both normotensive and hypertensive rat hearts. This specific phosphorylation reflects the activation of ERK. For another protein kinases we did not observe significant changes in their activation by crowding stress. We observed also differences in protein levels of aFGF (acidic fibroblast growth factor), a potential activator of ERK cascade, when compared normotesive and hypertensive rat hearts. In conclusion, the results demonstrate that adaptation of rat hearts to chronic chronic social stress produced by crowding is associated with changes in the activation of cascade of ERKs. These changes could be involved in adaptive responses induced by chronic social stress.

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INFLUENCE OF REPETITIVE MOTOR TRAINING ON THE SPATIAL NAVIGATION ABILITY AND HIPPOCAMPAL LTP IN CEREBELLAR DEGENERATION MOUSE MODEL

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Possible interactions between cerebellar and hippocampal structures in Lurcher mutant mice (LMM) were evaluated in this study. LMM represent a model of genetically determined olivocerebellar degeneration which is primarily caused by the mutation of delta-2 glutamate receptor gene and results in excitotoxic apoptosis of Purkinje cell. Loss of granule cells and inferior olivary neurons is secondary. We compared a hippocampal long-term potentiation (LTP) and spatial learning ability in Morris water maze (MWM) between trained and untrained group. Adult Lurcher mutant mice (strain C57Bl/7) were used. One group of mice was exposed to repetitive motor training (rotarod 2 min.). Experiment was repeated four times in 15 min intervals. Total duration was 5 days weekly, 6 weeks and 2 days in the 7th week. 5 days after training the animals were exposed to tests of spatial learning in MWM during consecutive 10 days. Latencies, swimming distance and swimming speed were measured. LTP was performed under urethane anesthesia, 2g/kg i.p. (stimulation of perforant path and registration in the dentate gyrus, biphasic pulses, basal low frequency 0.1 Hz, duration 0.1 ms, high-frequency stimulation 100Hz, 10 bursts from 16 stimuli each 10 s). Differences in the magnitude of population spike was evaluated. Mice exposed to the repetitive motor training exhibited shorter latencies resp. distances in MWM in comparison with untrained animals whereas swimming speed in trained mice was gently lower than the speed in untrained controls. Shorter latencies and swimming distance in trained mice show their higher spatial learning ability what indicates that motor training did not advantage of animals movement. Trained group of mice revealed higher LTP ability than untrained animals, but results were not statistically significant. Evaluation of LTP ability shows only gentle similarity between higher LTP production on the one hand and better spatial navigation ability resulting from intensive motor training on the other hand. Our findings support recent results (including ours) that spatial learning tasks involve a number of nonspatial components (motor control or stress factors).

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CARDIOPROTECTIVE EFFECT OF POMIFERIN ON ISCHEMIA-REPERFUSION OF LABORATORY RAT

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The study was undertaken to evaluate the cardioprotective potential of flavonoid pomiferin isolated from the infructences of *Maclura pomifera*, Moraceae, against ischemia-reperfusion induced injury in rat hearts as a model of antioxidant-based composite therapy. Study was performed with isolated, modified Langendorff-perfused rat hearts and ischemia of heart was initiated by stopping coronary flow for 30 min followed by 60 min of reperfusion (14 ml.min^{-1}). Wistar rats were divided into three groups. Treated group received pomiferin (5 mg/kg/day in 0.5% Avicel); placebo group received only 0.5% Avicel; intact group was left without any applications. Biochemical indicators of oxidative damage, lipid peroxidation product malondialdehyde, antioxidant enzymes (superoxide dismutase, glutathione peroxidase), total antioxidant activity in serum and myocardium has been evaluated. We also examined the effect of pomiferin on cardiac function (left ventricular end-diastolic pressure, left ventricular pressure, peak positive +dP/dt (rate of pressure development) after ischemia and reperfusion.
Our results demonstrate that pomiferin attenuates the myocardial dysfunction provoked by ischemia-reperfusion. This was confirmed by the increase in both the antioxidant enzyme values and the total antioxidant activity. The cardio-protection provided by pomiferin

treatment results from the suppression of oxidative stress and correlates with the improved ventricular function.

Key words: Maclura pomifera; flavonoid pomiferin; heart ischemia-reperfusion; reactive oxygen species

K557E MUTATION IN C-TERMINUS OF KCNQ1 GENE AS A CAUSE OF LONG QT SYNDROME

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Long QT syndrome (LQTS) type 1, related to a mutation in the KCNQ1 gene coding the α-subunit of I_{Ks} channel, forms about 50% of all hereditary LQTS. It stresses the importance of I_{Ks} channel in the proper course of myocardial repolarization. Here we report biophysical characteristics of a K557E mutation located in the C-terminus of KCNQ1 gene. Heterozygous mutation K557E was detected in a family with LQTS [1]. Functional properties of I_{Ks}-channels (KCNQ1/KCNE1) were studied by whole-cell patch-clamp technique at room temperature on transfected CHO cells. The tail current density in K557E channels was decreased by about 95% in comparison with wild-type channels (p<0.01). K557E channels were characterized by an enormous rightward shift in the voltage dependence of activation (voltage of half-maximal activation: 121.3±1.3 mV *vs.* 10.5±1.3 mV in wild type, p<0.001), deceleration of activation (time constant at full activation: 3415±320 ms at +180 mV *vs.* 1038±79 ms at +80 mV in wild type, p<0.001) and acceleration of deactivation (time constant at -80 mV: 202±15 ms *vs.* 410±23 ms in wild type, p<0.001). To mimic situation in heterozygous carriers, we cotransfected wild-type and mutant KCNQ1 subunits (ratio 1:1, in presense of KCNE1). The functional defect was much milder. Decrease of the tail current density just by about 40% was accompanied by a 23-mV rightward shift of the voltage dependence of activation. The time course of activation was decelerated to a less extent and the deactivation proceeded faster only at voltages between –120 and -60 mV. We conclude that pattern of the observed biophysical properties in K557E channels is in an agreement with data published in other C-terminal KCNQ1 mutations [e.g. 2, 3]. In heterozygous setting, availability of the I_{Ks} current decreased to about 60% of the wild-type current with gating characteristics situated between characteristics in entirely wild-type and K557E channels. Thus, both wild-type and mutant KCNQ1 subunits probably constitute I_{Ks} channels in heterozygous carriers of the K557E mutation.

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C-TERMINAL SCN5A MUTATION, F2004L, IN BRUGADA SYNDROME: NEW ARRHYTHMOGENIC CONCEPTS

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Two arrhythmogenic mechanisms are generally considered in Brugada syndrome (BrS): (1) Heterogeneous loss of the epicardial action-potential (AP) dome in the right ventricle (RV), which generates transmural dispersion of repolarization resulting in phase-2 reentry; (2) RV outflow tract conduction delay due to reduced depolarization, also favoring reentry. Here we report biophysical properties and arrhythmogenic consequences of a new SCN5A mutation F2004L. The F2004L mutation was detected in a Dutch kindred. The proband, a 26-year old male, experienced syncope and had coved-type ST elevations in ECG leads V1 and V2. QRS width was 135 ms. Functional consequences of the mutation were studied in transfected CHO cells by whole-cell patch clamping. Peak Na^+ current (I_{Na}) was reduced to 54% compared to wild type (WT; V_{hold} −120 mV). Persistent tetrodotoxin-sensitive I_{Na} was reduced to 56%. F2004L channels showed increased closed-state and slow inactivation. Recovery from inactivation was also slowed (e.g., fast tau 49±8 ms in F2004L vs. 20±4 ms in WT at V_{hold} − 80 mV, p<0.05). Electrophysiological characteristics of F2004L and WT currents were introduced in a mathematical model of the transmural RV wedge. In the mutant model, the excitation wave was decremental from endo- to epicardium, and eventually died out at cycle length (CL) 1000 ms (but not at CL 300 ms). Propagation continued, however, by phase-2 conduction causing long delays of excitation and slow upstrokes at the epicardium. We hypothesize that the observed transmural decrement and epicardial loss of excitation in the RV was caused by a gradual decrease of I_{Na} (slow depolarization → increased closed-state inactivation of I_{Na} → decreased availability of I_{Na} channels) and the physiological increase of transient outward K^+ current towards the epicardium. Our data may explain ST-segment elevation on the basis of massive transmural voltage gradients during early repolarization. Reentry-based tachycardia could be evoked during conditions that further exaggerate conduction block in this BrS phenotype.

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ADAPTATION MECHANISMS TO C-FOS GENE KNOCK-OUT

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C-fos is considered as one of the most important players in the intracellular signalization. The hypothesis that has been tested postulated that c-fos gene disruption would not influence the receptor density in the central nervous system (CNS: brain cortex and cerebellum) and in the periphery (lung, heart). The role of c-fos gene disruption on binding characteristics of selected G protein-coupled receptors has been investigated. The following receptors were studied: muscarinic receptors (MR),□$_1$-adrenoceptors (AAR), □-adrenoceptors (BAR), D_1-like dopamine receptors (D_1R), D_2-like dopamine receptors (D_2R). Binding to muscarinic receptors, dopamine receptors and adrenoceptors (□-adrenoceptors,□-adrenoceptors) was investigated using radioligand binding with ^{3}H-QNB (muscarinic receptors), ^{3}H-SCH23390 (D_1-like dopamine receptors), ^{3}H-spiperon (D_2-like dopamine receptors), ^{3}H-prazosin (□$_1$-adrenoceptors) and ^{3}H-CGP 12177 (□-adrenoceptors). Phospholipase C activity was measured by the enzymatic assay procedures using phosphatidylinositol bisphosphate as a substrate.
Surprisingly to our hypothesis, there were important changes in receptor density both in the periphery and in the CNS. Both MR and BAR were increased in the lung and heart. The effects of c-fos gene disruption in the CNS were more selective. In general, the receptors that activate Gq-phospholipase C-proteinkinase C pathway (AAR, MR) were affected, while the others (that activate/or inhibit adenylylcyclase: BAR, D_1R, D_2R) were not. On the contrary, phospholipase C activity was decreased in the cerebellum. This is important in context that IP3 receptor mRNA is increased in c-fos KO animals in the heart (1). These results suggest that disruption of c-fos gene could significantly change the expression of G protein-coupled receptors. Moreover, these changes could be comprehended as one of the adaptive mechanisms that help the organism to cope with c-fos gene disruption.

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ASTROCYTE VOLUME CHANGES DURING ISCHEMIA/ REPERFUSION IN SITU: CONFOCAL 3D MORPHOMETRY

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Volume changes of eGFP-labeled astrocytes during ischemia/reperfusion *in situ* were studied in the cortex of GFAP/EGFP transgenic mice using confocal microscopy combined with 3D reconstruction (1). Brain slices of 30-40-day-old mice were exposed for 20 min to solutions modeling ischemia: glucose-free ACSF saturated with 5%O_2 / 15%CO_2 (OGDpH, pH 6.8) or glucose-free ACSF saturated with 5%O_2 / 5%CO_2 (OGD, pH 7.4). Inhibitors of chloride channels (NPPB, DIDS, Cd^{2+}) and the Na-K-Cl cotransporter (bumetanide) were used to study the contribution of chloride movement to cell volume changes and regulatory volume processes.
The application of either OGD or OGDpH revealed two populations of astrocytes: high response (HRA) and low response astrocytes (LRA). In LRA, the application of OGD led to a small volume increase of 5%, while OGDpH application caused a volume decrease of 8%. Subsequent perfusion with ACSF (reperfusion) for 40 min led to complete volume recovery. In HRA, the application of OGD evoked a marked volume increase of 41%, while OGDpH application caused at first a volume decrease followed by a volume increase of 15%. Reperfusion after OGD or OGDpH led within the first 20 min to an additional volume increase of 15% or 12%, respectively, with no apparent volume recovery. OGD application together with NPPB, DIDS or Cd^{2+} led to a smaller volume increase in HRA than seen with OGD alone, while bumetanide had no effect on the volume changes evoked by OGD. The application of Cd^{2+}, NPPB, DIDS or bumetanide during reperfusion evoked a significant volume decrease. Our data suggest the presence of two populations of astrocytes in the cortex of GFAP/EGFP mice that respond differently to ischemia and also an essential role for Cl^- movement, carried by the Na-K-Cl cotransporter and Cl channels, in volume regulation in HRA.

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OCCURRENCE OF RISK FACTORS OF CARDIOVASCULAR DISEASES IN STUDENTS

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Based upon the results of long-term prospective and intervention studies in a lot of countries of the world, early distinguishing the main risk factors and reduction of their occurrence in population leads to an apparent decrease in the cardiovascular mortality. The risk of the onset of cardiovascular diseases is significantly higher if the main risk factors are present – obesity, increased cholesterol level in blood, arterial hypertension, dyslipidemia, diabetes mellitus of type 2,and unfit life style. Methods: 482 students (320 women and 162 men) of the Medical Faculty of Safarik University at the age of 18-23 years formed a set. We observed their anthropometric parameters, body weight and height from which the body mass index - BMI (kg/m^2) was calculated. Of the biochemical parameters the serum concentration of total cholesterol (TCH), triacylglycerol (TAG) were determined by commercial sets of Fy Biola Lachema (Czech republic) and concentrations of vitamin C were determined using the spectrophotometric method of Roe and Kuether.
Results: the TAG concentration in men ranges from 0.37 to 3.94 mmol/L, and in women from 0.37 to 2.64 mmol/L. A statistically significant increase in the TAG values was found in men compared to women ($p<0.001$). The mean values of TCH were lower in men (4.33±0.81) than those in women (4.56±0.78), while this difference was significant ($p<0.001$). Women had higher percentage of the values of vitamin C in the physiological range (34–68 μmol/L) than men (72.81% *vs*. 68.51%). The mean value of BMI was in men and women 22.44±2.95 and 20.69±2.47, respectively.Underweight (BMI<18.5 kg/m^2) was found in 6.17% of men and 15.93% of women, normal weight (BMI 18.5–24.9 kg/m^2) had 75.75% of men and 79.37% of women, and overweight (BMI 25.0–29.9 kg/m^2) had 14.19% of men and 4.68% of women. Obesity of degree I was recorded in 3 men and 1 women, and obesity of degree II in one man. The aim of our work was to find intersexual differences in evaluation of some cardiovascular risks and to draw attention to the change of life style to reduce the risk of obesity as the most spread metabolic disease. Our resuls suggest a higher cardiovascular risk in the group of men (higher concentration of TAG and percentage of overweight, and lower concentration of vitamin C).

CELLULAR STRESS AND GENOTOXIC HAZARD IN PEDIATRIC PATIENTS TREATED FOR PSORIASIS WITH GOECKERMAN REGIMEN

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Objective: Goeckerman regimen (GR) of psoriasis includes combines dermal exposure to coal tar and UV-radiation which represent potent environmental mutagenic and carcinogenic agents. Aim: Evaluate the influence of GR on cellular stress reaction and induction of chromosomal abnormalities. Methods: We compared serum level of heat shock proteins (Hsp) and chromosomal aberrations in peripheral lymphocytes (CA) in a blood samples collected before and after GR in a group of 26 pediatric patients with psoriasis. Psoriasis Area and Severity Index (PASI score) evaluated the efficiency of GR. Results: We found significantly increased serum level of Hsp70 ($p<0.05$) and CA ($p<0.01$) after GR. The PASI score decreased significantly after GR ($p<0.001$). Discussion: Results are preliminary. Significantly decreased PASI score confirmed high efficiency of GR. On the other hand significantly increased Hsp and CA indicated high level of cellular stress and the presence of genotoxic hazard. Conclusion: GR is efficient therapy. Stratification by a unique risk of GR is still lacking.

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MODULATION OF NMDA RECEPTOR FUNCTION BY TEMPERATURE

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Temperature dependency of recombinant NR1/NR2B receptors expressed in HEK293 cells was studied by patch clamp technique and kinetic modeling.
Single channel activity and whole-cell responses induced by fast application of glutamate were recorded at temperatures 25 - 45°C. Responses were fit to a six-state kinetic model and the rate constants characterizing glutamate binding (k_B), unbinding (k_U), receptor desensitization (k_{D+}), resensitization (k_{D-}), Ca^{2+}-dependent inactivation (k_{I+}), reactivation (k_{I-}) and channel opening (k_O) and closing (k_C) were determined. Arrhenius plot of the rate constants k_{D+} and k_{D-} shows a high degree of temperature-dependency with the activation energy values 190 and 124 kJ/mol, respectively (Q_{10} = 10.1 and 4.6) in contrast to the rate constants k_B, k_U, k_{I+}, k_{I-}, k_O and k_C that are characterized by low values of activation energy. In accordance with the predictions made by the kinetic model, current responses induced by brief (2 ms) application of 1 mM glutamate exhibit double-exponential deactivation time course characterized by activation energy of the fast and slow component 106.1 and 79.4 kJ/mol, respectively (Q_{10} = 3.6 and 1.0).

Single NMDA receptor channel conductance temperature dependency is characterized by activation energy of 26.2 kJ / mol (Q_{10} = 1.4).
The results of our experiments characterize temperature dependency of conformational transitions of NR1/NR2B receptor channels. The data indicate that desensitization accelerates the early component and decelerates the late component of deactivation of currents evoked by brief application of glutamate, used as a model of synaptic transmission.

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INFRADIAN DYNAMICS OF SALIVARY TESTOSTERONE

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Background. The dynamics of testosterone levels exhibits several cyclic patterns with various period lengths. Circadian and circannual rhythms of testosterone are known in both genders. Among infradian rhythms only the circalunar cycle in women is widely accepted. In our previous studies we have found a circatrigintan (30 days) and a circavigintan (20 days) cycle in men. Whether cyclic patterns with higher frequency are present in the dynamics of testosterone levels in men or in women is unknown.
Aim. To analyze the infradian dynamics of salivary testosterone in both genders for the presence of cyclic patterns.
Subjects & Methods. Seventeen young and healthy women and fifteen men were asked to collect saliva samples during 30 consecutive days. Testosterone levels were determined using radioimmunoassay, Analysis of Rhythmic Variance II (ANORVA II) was used for statistical analysis. Potential period lengths of 3-15 days were evaluated.
Results. The dynamics of salivary testosterone showed high intraindividual variability in both genders (coefficient of variation – 28% in women and 26% in men). ANORVA II analysis showed no significant rhythms, although a weak circaseptan cyclic pattern has been found in women.
Discussion. Our results showed no significant infradian cyclic variation with a period between 3 and 15 days. Further studies should concentrate on potential longer periods. Described intraindividual variability of testosterone levels in both genders should be considered in endocrine research.

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SURVIVAL AND FUNCTIONAL MANIFESTATION OF THE EMBRYONIC CEREBELLAR GRAFT IN ADULT C57BL/7 LURCHER MUTANT MICE

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Lurcher mutant mice represent a model of olivocerebellar degeneration, which results in cerebellar ataxia and spatial learning deterioration (1, 2). The aim of the study was to assess survival of the solid embryonic cerebellar graft and its influence on motor coordination and spatial learning ability in adult Lurchers.
Embryonic cerebellar tissue obtained from wild type mice embryos was applied into the cerebellum of adult C57Bl/7 Lurcher mutant mice. To sham-operated controls only vehiculum was administered. One part of animals was subjected to forced swimming (training) for 6 weeks 8 minutes daily. Motor coordination was examined using a bar, ladder and rotarod method before and 4, 8 and 10 weeks after the surgery. Spatial learning was tested in the Morris water maze 9 weeks after the surgery. Mean latencies, swimming distance and velocity were recorded. Presence of the graft was detected histologically in some individuals 3 or 6 weeks after the transplantation without fulfilment of the functional tests, in other mice after finishing the experiments 10 weeks after the surgery.
The graft survived 3 weeks in 100 % and 6 weeks in 78 % of mice. After 10 weeks the graft was present in 83 % of trained and in 73 % of untrained mice. Motor coordination was significantly improved by the training. In untrained mice there was no beneficial effect of the transplantation. The best results were found in trained mice in which the graft survived. These mice also showed the highest swimming velocity during the spatial learning test. In untrained animals the capability of spatial learning was higher in mice after transplantation, both with surviving and dissolved graft, as compared with sham-operated controls. In trained mice the effect of transplantation was not observed.
The cerebellar graft survived at least for 3 weeks. Transplantation improved capability of spatial learning in Lurchers. This impact was not dependent on long-term graft survival and it should be attributed rather to temporary trophic effect of the graft than to cell substitution. Motor coordination was improved thanks to forced swimming but not due to the transplantation alone. Forced motor activity increased the graft survival only insignificantly.

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THE INFLUENCE OF OBESITY ON THE EXPRESION OF ADIPONECTIN AND ITS RECEPTORS IN SUBCUTANEOUS ADIPOSITE TISSUE

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Adiponectin is a protein hormone produced predominantly by adipocytes. Experimental studies have shown that it exerts potent anti-diabetic and anti-inflammatory properties. Tissue effects of adiponectin are mediated through its receptors AdipoR1 and AdipoR2. The aim of our study was to measure serum adiponectin levels, its mRNA expression and mRNA expression of AdipoR1 and AdipoR2 in subcutaneous adipose tissue (SC) in patients with different degrees of obesity and lean control subjects. We further aimed to characterize the influence of degree of obesity as measured by body mass index (BMI) and its modulation by very low calorie diet (VLCD) on the above mentioned parameters.
74 patients were included into the study. They were stratified into subgroups according to BMI: morbidly obese group (BMI>40 kg/m^2, n=25), moderately obese group (BMI 30 – 40 kg/m^2, n=15), overweight group (BMI 25 -30 kg/m^2, n=12) and lean group (BMI 20 – 25 kg/m^2, n=22). Subcutaneous adipose tissue was obtained by needle biopsy and mRNA was isolated using MagNA Pure Compact RNA Isolation Kit (Tissue) (Roche, SRN). Adiponectin, AdipoR1 and AdipoR2 mRNA expression was measured by real-time PCR using ABI PRISM 7500 instrument (Applied Biosystems, USA) and specific TaqMan® Gene Expression Assays. β-2-microglobulin was used as an endogenous reference and results were normalized to these values. Serum adiponectin levels were measured by commercial kit (LINCO Research, USA).
Serum adiponectin levels as well as adiponectin and AdipoR1 mRNA expression were inversely related to BMI (r=-0,549, p<0,001; r=-0,482, p<0,001; p=-0,333, p<0,05 respectively), while no relationship between AdipoR2 and BMI was found. 3 weeks of VLCD did not significantly influence serum adiponectin, SC adiponectin, AdipoR2 and AdipoR2 mRNA expression despite 9% reduction of body weight.
We conclude that both adiponectin and AdipoR1 mRNA expression in SC are markedly reduced in patients with obesity and may contribute to some of its metabolic complications.

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EXPRESSION OF CALCIUM BINDING PROTEINS (CaBPs) IN SKELETAL MUSCLES OF RATS WITH ALTERED THYROID STATUS

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Many hormones exert a strong systematic influence on skeletal muscle during development as well as in the adult stage. For the thyroid hormones, it has been shown in rats that both hypo- and hyperthyroidism were paralleled by modifications in the fiber type composition. Hypothyroidism causes fast-to-slow transitions and hyperthyroidism causes slow-to-fast transitions of the fibre type phenotype (1-4). Calcium binding protein (CaBPs) are a group of proteins that function either as Ca^{2+} effectors or buffers. When Ca^{2+} enters the cytosol, only a very small proportion ends up being free, because most of it is rapidly bound to the buffers and, to a lesser extent, to the effectors (5). We are investigating whether the effect of the thyroid hormones might alter six members of this group of proteins: parvalbumin, calreticulin, calsequestrin, calmodulin, calcineurin and S100A1 in the slow soleus (SOL) and the fast extensor digitorum longus (EDL) muscles of 8 months-old female inbred Lewis strain rats. Proteins are determined by SDS-PAGE and verified by western blot analysis. Gene expression will be assessed using reverse transcription and subsequent polymerase chain reaction (RT-PCR). Our results already have confirmed that parvalbumin is largely unaffected by thyroid hormones in both SOL and EDL muscles, while preliminary results with calsequestrin and calreticulin suggest the real influence of thyroid hormones reflected by the upregulation and downregulation of these proteins in altered thyroid states. We can thus conclude that thyroid hormones may alter not only the muscle phenotype, but also the calcium homeostasis and therefore the excitation-contraction coupling.

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PROTECTIVE EFFECT OF ANTIOXIDANT IN MYCOTOXIN TOXICITY OF BROILER CHICKENS

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Deoxynivalenol (DON), a mycotoxin produced by *Fusarium spp.,* is a frequent contaminant of cereals. Toxic effects of DON on animals have been well documented and concern protein synthesis and the gastrointestinal tract (1). The sensitivity to DON varies considerably between species (2, 3). Poultry are more sensitive than ruminants but less sensitive than pigs (4). The aim of this study was to evaluate effect of DON on plasma indices and efficacy of dietary selenium to counteract toxicity of DON in growing broiler chicks. Three groups of broiler chicks were formed with 14 birds in each group. Three diets included control (0.2 ppm DON, 0.4 mg selenium/kg diet), contaminated (3 ppm DON, 0.4 mg selenium/kg diet) and contaminated (3 ppm DON) plus Sep-plex® (1.4 mg selenium/kg diet). Chicks were fed the diets from the day of hatch to 42 d of age. Then all birds were sacrified and blood samples for chemical analyses were collected. Plasma calcium and alanine aminotransferase activity were significantly elevated and magnesium, total proteins, triglycerides and free glycerol were decreased in chicks fed DON-contaminated diet compared with those fed the control diet. Supplementation of Sel-plex® to the diet decreased plasma alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activities and reversed plasma levels of magnesium in chicks induced by dietary DON. Chloride and phosphorus levels were not affected by diets. The inclusion of selenium to DON-contaminated diet, however, did not completely alleviate toxic effect on protein and lipid metabolism by the liver. Supplementation of selenium enriched yeast product (Sel-plex®) counteracted most of the plasma parameter alterations caused by DON-contaminated diet in chicks.

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APOPTOSIS AND PROLIFERATION IN BOVINE OVIDUCTAL CELLS: EFFECT OF LEPTIN AND IGF-I

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Apoptotic cell death is important for normal development and homeostasis. Apoptosis plays a key role both in normal and pathological processes, including cancerogenesis, because it acts against uncontrolled cell proliferation and eliminates abnormal, damaged or excessive cells. The aim of this study was to examine effects of leptin on apoptosis and proliferation of cultured fragments of bovine oviducts and to compare it with the effects of well-known mitogen – insulin-like growth factor I (IGF-I). Effects of leptin on the expression of pro-apoptotic peptide (Bax, caspase-3), anti-apoptotic peptide (Bcl 2) and proliferation peptide (PCNA) were analyzed using Western-blotting and on the release of IGF-I by cultured oviductal fragments using RIA procedure.
Leptin stimulated IGF-I at all doses added. At low dose leptin, similarly to IGF-I, stimulated the expression of anti-apoptotic peptide Bcl2. Higher leptin concentrations inhibited proliferation (PCNA) and stimulated the expression of pro-apoptotic peptides – Bax and caspase-3, whilst IGF-I has an opposite effect on these substances. These opposite effects of leptin and IGF-I on apoptosis and proliferation assume an existence of feedback interrelationships between these substances. It is not to be excluded, that leptin and IGF-I are antagonistic in their effects on oviduct functions. Since the effects of both these substances on the oviduct are different, we can assume that leptin and IGF-I may have different biological roles within the oviduct.

Key words: oviduct, proliferation, apoptosis, growth factors, leptin, IGF-I

MOLECULAR ASPECTS OF HYPODONTIA

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Numeric dental anomalies are the most common craniofacial congenital malformations in humans. More than 20 % of human population miss one or more third molars. Approximately 5 % of population display agenesis of another tooth, second premolars and upper lateral incisors are predominantly affected. Despite of the high frequency of hypodontia in population, the molecular basis is not yet well understood in many cases.
Teeth develop as complex epithelio-mesenchymal organs on basis of reciprocal signalling cascades between the oral ectoderm and its underlying neural crest derived mesenchyme. Molecular networks regulating tooth formation are mostly studied in the mouse. Biomedical research takes advantage from the fact that the mouse genome is known and methods for targeted manipulation of particular genes are well established. More than 200 genes have been found so far to contribute to proper tooth formation, among them particularly genes from the fgf,

bmp, wnt and shh pathways. All of these genes are candidate molecules for human dental disorders.
Hypodontia refers to both aberration in number, size and shape of the teeth and abnormalities of dental development and time of eruption. Hypodontia may occur as an isolated feature or as a part of a complex syndrome. Tooth number is reduced in patients with Rieger syndrome, anhidrotic ectodermal dysplasia or Witkop tooth-and-nail syndrome. These syndromes show disorganised development of more ectodermal organs including nail, hair and tooth. Key regulatory genes (pitx2, ectodysplasin A or msx1) mediating common pathways controlling epithelio-mesenchymal signalling are mutated in these disorders.
Until now, mutations in only three genes (pax9, msx1, axin2) have been associated with isolated forms of hypodontia. Interestingly, mutations in different genes result in different teeth missing. When pax9 is mutated, molars are predominantly affected, whereas, msx1 mutations cause loss of second premolars and third molars and may contribute to non-syndromic forms of cleft lip and/or palate. Identification of other genes involved in hypodontia is a great challenge for molecular biology.

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THE INFLUENCE OF CADMIUM AND COPPER ON CATALASE ACTIVITY IN POND SNAIL (LYMNAEA STAGNALIS)

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Snails as good bioindicators are often used in ecotoxicological, and ecophysiological studies. Snails living in aquatic ecosystems such as the pond snail are often exposed to heavy metals, which are extremely harmful to animals (1). Thus there is a need to study the heavy metals accumulation and their influence on physiological parameters in bioindicatory animals such as the pond snail. In the present study pond snails after 2 weeks of adaptation were exposed to Cd (1 mg l^{-1}) or Cu (0.025 mg l^{-1}) for 24 h. Then the protein level, catalase (CAT) activity, and Cd, Cu contents were measured. CAT activities were 2.0±0.7 (control), 168±116 (Cd), 23±30 (Cu) units×mg^{-1} protein×10^4. The differences were statistically significant. The protein level was 0.56±0.24 (control), 0.10±0.06 (Cd) and 0.28±0.13 (Cu) mg protein×g^{-1} body weight. The differences were significant. In control snails, Cd and Cu contents were 0.005±0.002 and 0.07±0.02 mg×g^{-1} dry body weight (d.b.w.) respectively. Animals exposed to metals contained 0.09±0.01 mg of Cd ×g^{-1} d.b.w. and 0.14±0.03 mg of Cu ×g^{-1} d.b.w. The differences in comparison to control were significant. Accumulated ions significantly decreased protein level and increased CAT activity. CAT activity is thus useful parameter in ecotoxicological studies, although it does not respond specifically and should be combined with other parameters.

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EFFECT OF BACLOFEN ON PAIN THRESHOLD DURING EARLY POSTNATAL PERIOD

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Baclofen – specific GABA-B receptor agonist – is used in clinical practice for the treatment of skeletal muscle spasticity. Off-label uses of baclofen include treatment of neuropathic pain. The aim of the study was to describe its effect on acute nociception in rat pups. Plantar test and tail-flick test allowing partially distinguish spinal and supraspinal actions were used to measure paw and tail withdrawal reaction to the noxious heat stimulation, respectively. Pain thresholds were measured in three age groups of male pups (7, 16 and 21 postnatal days) before and 20 min after the baclofen administration. Two doses of subcutaneously administered baclofen were tested – 1 mg/kg and 5 mg/kg. In order to evaluate possible motor-deficit action of baclofen, age-relevant motor-deficit tests were employed in all groups (surface righting test, horizontal bar holding test, rotarod). Baclofen significantly increased tail-flick latencies in a dose-dependent manner, however, no changes in plantar-test latencies were observed. Baclofen (1 mg/kg and 5 mg/kg) did not impaired motor function in any groups, except the higher dose (5 mg/kg) induced sedation in the youngest pups. It is concluded that baclofen has antinociceptive effect also in the developing nervous system and that this effect is expressed particularly at the spinal level.

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THE EFFECT OF LUMINAL CALCIUM ON THE STABILITY OF COUPLED GATING OF TWO RYANODINE RECEPTORS

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The intracellular signal that triggers the contraction of muscle cell is a transient rise in intracellular Ca^{2+} released from lumen of the sarcoplasmic reticulum (SR) through a major intracellular Ca^{2+} release channel - ryanodine receptor (RYR). Two or more RYR channels reconstituted into bilayer lipid membrane (BLM) can open and close independently (single gating) or simultaneously (coupled gating) (1, 2). Observed phenomenon of coupled gating might advance our current understanding of processes involved in excitation - contraction coupling in muscle cells, mainly termination of Ca^{2+} release from the SR.
We systematically examine functional properties of coupled RYR channels reconstituted into BLM. In this study, we investigated the potential effect of luminal Ca^{2+} on stability of coupled gating of two RYR channels isolated from the rat heart. In contrast to single channel most of coupled RYR channels exhibited noisy open-channel current level. This behavior likely reflects thermodynamic stability of channel complex. Using noise analysis of open-channel current level we determined a parameter of stability for each detected simultaneous opening and further averaged for experiments performed under identical conditions. Absence of luminal Ca^{2+} was mimicked by luminal Ba^{2+}.
We observed, that high concentration of luminal Ca^{2+} (53mM) noticeable destabilized functional coupling between RYR channels in contrast to lower concentrations (8mM -10mM). When luminal Ca^{2+} was replaced by luminal Ba^{2+}, stability of coupled gating was decreased to the comparable level as was found for 53mM luminal Ca^{2+}.
We can conclude, that luminal Ca^{2+} is not essential for appearance of coupled gating of two RYR channels, however presence of Ca^{2+} on the luminal side (<10mM) strengthen stability of functional channel coupling.

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DISTRIBUTION OF LANGERHANS CELLS IN CAT EMBRYONIC SKIN

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Langerhans cells (LCs), keratinocytes, epidermotrophic T-lymphocytes and peripheral lymphonodes belong to an integrated tissue system mediating skin immune protection and forming so called skin-associated lymphoid tissue. LC stem cells originate from pluripotent hematopoietic progenitor cells CD34+ in the bone marrow. As immature cells, LCs migrate to non-lymphatic tissues, such as the skin, where they finish their maturation by expression of specific molecules (ATPase, CD45, MHC II, langerin/CD207) and formation of the Birbeck granula.
Distribution of LCs may correlate with skin immune defense function, moreover, intradermal vaccination and dermatitis development may depend on presence and density of LCs. To find out more about LCs distribution and formation in the skin, a preliminary study focused on embryonic cat skin was designed.
Results of this investigation based on CD1 receptor detection showed spatial-dependent distribution of LCs in embryonic cat skin, with the

highest density corresponding with dorsal part of the embryo, particularly *regio parietalis*, *vertebralis thoracis*, *sacralis* and *regio caudalis*. All these regions contain thick epidermis and hair follicles, which seem to be related to distribution of LCs. LCs were present scattered or clustered in interfollicular epidermis among keratinocytes in *stratum germinativum* (*stratum basale* and *stratum spinosum*). They were found also among cells of outer epithelial sheath in region of hair follicle infundibulum close to sebaceous gland and among epidermal cells around the hair canal. Some isolated LCs were detected in dermis among hair follicles.
Further research may reveal other important roles of Langerhans cells in body surface protection and immune reactions. Moreover, modulations of Langerhans cells can be helpful in cancer therapy in both human and veterinary medicine.

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LONG-TERM TREATMENT WITH ACE INHIBITOR RAMIPRIL IMPROVES INSULIN SENSITIVITY IN MICE WITH DIET-INDUCED OBESITY

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ACE (angiotensin converting enzyme) inhibitors are used clinically in the treatment of arterial hypertension. Recent studies have suggested that it may also exert insulin-sensitizing effects possibly through its action in adipose tissue. The aim of our study was to assess the effect of 3-months treatment with ACE inhibitor ramipril on the development of obesity and insulin resistance in C57BL/6J mice fed high fat (HF) or control (C) diet with special respect to its possible modulation of endocrine function of adipose tissue.
3 months old male mice were fed HF or C diet for 3 months. Ramipril was administered in the drinking water from the beginning of HF feeding. The mice were assigned into following groups (n=8/group): C, HF, C+Ramipril, HF+Ramipril. Serum biochemical and hormonal parameters were measured at the end of experiment using colorimetric, RIA and Luminex kits. mRNA was isolated from gonadal adipose tissue using MagNA Pure Compact RNA Isolation Kit (Tissue) (Roche, SRN). mRNA expression of adipose tissue-derived hormones was measured by real-time PCR using ABI PRISM 7500 instrument (Applied Biosystems, USA) and specific TaqMan® Gene Expression Assays.
3-months HF feeding induced obesity, liver steatosis and insulin resistance as measured by increased fat pad weights, mild hyperglycemia and marked hyperinsulinemia in HF-fed animals. All of these changes were completely blunted by ramipril treatment while no effect of ramipril on these parameters was observed in mice fed C diet. HF diet markedly increased gonadal fat mRNA expression of leptin, proinflammatory monocyte chemoattractant protein-1, macrophage infiltration marker Emr 1 and decreased expression of anti-inflammatory and insulin-sensitizing hormone adiponectin and its receptors AdipoR1 and AdipoR2 indicating that endocrine dysfunction of adipose tissue markedly contributed to the overall insulin resistance phenotype. Ramipril treatment fully prevented proinflammatory changes in adipose tissue.
We conclude that treatment with ACE-inhibitor ramipril prevented the development of HF diet-induced obesity and insulin resistance at least in part by modulation of endocrine function of adipose tissue.

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SODIUM AZIDE IN ANIMAL MODELS OF ALZHEIMER DISEASE

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Mitochondrial dysfunction is often linked with accumulation of amyloid beta peptides that could mediate pathological cascade leading to Alzheimer Disease (AD). From this reason, the mitochondrial dysfunction is recently in the centre of interest in the neurodegeneration research. Sodium azide, a selective inhibitor of mitochondrial cytochrome c oxidase, is often used in modelling of the neurodegeneration. Our study shows a short summary of alterations mediated by sodium azide in the case of its subcutaneous application (22.5 – 45.0 mg/kg/day for one month) to adult male Long Evans rats. Subcutaneous infusions were applied in various ages and subsequent behavioral and neurochemical analyzes were done in various intervals after application. Behavioral tasks tested were the Morris water maze, Active Allothetic Place Avoidance, Passive Avoidance, Elevated Plus Maze. In biochemical way following parameters were analyzed: activity of cholinergic system in the brain, activities of nitric oxide synthases and concentrations of soluble amyloid beta peptide 1-40. The experiments are still in progress, but our actual results show only limited validity of this animal model of AD. This could be caused by different effects of various doses of sodium azide, or/and by reparative abilities of the damaged brain. The results will be completed and discussed.

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RENIN-ANGIOTENSIN SYSTEM IN SOME BRAIN NUCLEI, HEART AND KIDNEY IN TGR(MREN-2)27 RATS

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TGR(mREN-2)27 rats with changed function on renin-angiotenisin system (RAS) are characterised by fulminant hypertension and inverted blood pressure profile (1,2). To elucidate reasons for disturbed blood pressure profile in TGR rats, expression of angiotensin II receptor type 1 (AT1) and angiotensin converting enzyme (ACE) mRNA was investigated in our study over 24hr cycle. Heterozygous male TGR and control Sprague Dawley rats were synchronized to the light:dark cycle 12:12. Daily profile of AT1 and ACE mRNA expression was determined in the suprachiasmatic nucleus (SCN), nucleus of the solitary tract (NTS), area postrema (AP), heart and kidney. Samples were taken in regular 4hr intervals. Gene expression was measured after total RNA isolation and reverse transcription by real time PCR ABI PRISM® 7900HT (Applied Biosystems, USA). In agreement with previously published data, expression of AT1 mRNA was down regulated in kidney of TGR rats (3) in comparison with control. Expression of AT1 mRNA showed significant rhythm in SCN and AP of TGR rats and a trend to increased levels was observed in NTS of TGR rats in comparison with control. Expression of ACE mRNA was mostly arrhythmic in studied tissues and down regulated in kidney. Expression of ACE mRNA in the heart displayed a daily rhythm only in TGR rats with increased levels during the night-time. Presence of RAS in the SCN, cirkumventricular organ area postrema as well as peripheral tissues implicates functional pathway regulating final pattern of daily blood pressure profile in TGR rats.

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ZINC DISTRIBUTION IN ROMANY CHILDREN

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Zinc is an essential element in human and animal nutrition with multiple biological functions. Zinc plays catalytic, structural or regulatory roles in the metalloenzymes that have been identified in biological systems and zinc fingers. Zinc fingers are exploited by transcription factors for interacting with DNA and regulating the activity of genes. Another structural role of zinc is in the maintenance of the integrity of biological membranes resulting in their protection against oxidative injury. Zinc is an integral component of antioxidant enzymes that protect organism against free radical damage.
In selected group of hospitalized Romany children (25 boys and 22 girls) aged from 2 month to 6 years we examined selected parameters - zinc, alpha-2-macroglobulin, Cu/Zn superoxidddismutase and their relationship. The serum concentration of zinc (Zn) was measured with commercial kit fy AMP Diagnostics (Austria) and serum concentration of alpha-2-macroglobulin (α2M) radial immunoassay fy Sevapharma (CR). The enzymatic activity of Cu/Zn superoxiddismutase (Cu/Zn SOD) was measured spectrophotometric method with RANSOD kit (Randox Lab., U.K.).
The mean concentration of zinc was 11.78 ± 3.54μmol/L. The zinc deficiency, it means the serum concentration of Zn lower than 9 μmol/L had 23.4% children and zinc concentration more than 16 μmol/L had 12.8% children. Significant correlation ($r=0.326$; $p\leq0.05$) was between zinc and α2M only in the physiological range of zinc. The activity of Cu/Zn SOD was increased with mean concentration of zinc and α2M.
Results of our study showed on decreased saturation of organism with zinc in Romany children caused by insufficient exogenous intake of zinc or as a secondary consequence of respiratory system infection. Zinc supplementation may be an effective public health intervention means to improve the zinc status of the population.

TIME-DEPENDENT CHANGES IN EPITHELIAL SECRETION DURING MURINE EXPERIMENTAL COLITIS

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The typical symptom of inflammatory bowel disease is diarrhoea that may relate to a reduction in electrolyte absorption or an increase in electrolyte secretion. This study evaluated the time course of disturbances of colonic chloride secretion during experimental colitis with changes in the severity of inflammation. Colitis was induced by treating Balb/c mice with 2 % dextran sodium sulphate (DSS) in drinking water for 5 days followed of normal tap water. At time points 1, 2, 3, 4, 14, and 28 days after induction of colitis the effect of histamine (5.10^{-4} M) , carbachol (10^{-4} M) and 5-hydroxytryptamine (5HT; 10^{-4} M) was studied on colonic chloride secretion under short-circuit conditions (voltage-clamp, Ussing chambers) in the absence or presence of neuronal sodium channel blocker tetrodotoxin (10^{-6} M). In addition, histology and transcript levels of indexes of inflammation, inducible NO synthase, cyclooxygenase-2, and proinflammatory cytokines, were measured using real-time quantitative RT-PCR. Whereas, the indexes of inflammation were elevated, we have not observed increased pro-secretory tonus. The cholinergic agonist carbachol has either no effect on chloride secretion or even decreases it. In contrast, pre-incubation with histamine, a mediator whose level is increased during inflammation, was associated with stimulatory effect of carbachol on secretion. In healthy animals, the stimulatory effect of carbachol was higher in the absence of TTX but at 1 and 2 days after DSS, carbachol triggered a higher secretory effect in the presence of TTX whereas this effect was independent of TTX in next days. The effect of 5-HT was higher in the presence of TTX in mice exposed to DSS for 1-4 days but 2 and 4 weeks later the response to 5HT was independent of TTX similar to control animals. These data suggest changes in the gut nervous system early in the experimental colitis.

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PRODUCTION OF NO DURING CHRONIC VENTILATORY HYPOXIA

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The lung production of NO increases in hypoxic pulmonary hypertension. In developed pulmonary hypertension NO – induced vasodilatation prevents the excessive increase of pulmonary arterial blood pressure. At the beginning of hypoxic exposure, however, NO in the presence of free oxygen radical – superoxide – readily creates peroxynitrite with a vasoconstrictive effect. Because it seems that the key point is in the dynamics of NO production, the aim of the presented study was to determine the levels of NO production in early and late phases of chronic ventilatory hypoxia.
The Wistar male rats were divided into 3 experimental and 1 control groups. Experimental groups stayed in isobaric hypoxic chamber (FiO_2 = 0,1) for 1 day (H1; n = 8), 4 days (H4; n = 7) and 21 days (H21; n = 8). Controls were kept in normoxic conditions (N; n = 8). Immediately after removing from the chamber we measured production of NO in expired air and concentration of oxidative products of NO (nitrite - NO_2^- and nitrate – NO_3^-) in plasma. The NO production was measured in the perfusate drained form isolated ventilated and perfused lungs. For all the NO analyses we used a chemiluminiscence analyser.
In expired air we found the highest NO production after 1 and 4 days in hypoxia (H1: 699,9, H4: 656,6, H21: 412,4, N: 265,7 [$pg.min^{-1}.100g^{-1}$], $p<0,001$).
The plasmatic concentration of NO oxidative products (nitrite - NO_2^- and nitrate – NO_3^-) was significantly increased after 1, 4 and 21 days in chronic hypoxia compared to controls. (H1: 29,8, H4: 37,1, H21: 28,4, N: 20,0 [μM], $p<0,05$)
In the perfusate we found the highest NO production after 1 day (H1: 5,1, H4: 2,7, H21: 1,5, N: -0,14 [$\mu M.\ g^{-1}$], $p<0, 01$)
Our finding shows that the onset of increased NO production appears very early, at the first day in chronic hypoxia and – when put together with superoxide concentration measurement (results presented on 83. FD – E. Johnson) - precedes the increase of superoxide production.

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THE ROLE OF NITRIC OXIDE SYNTHASE IN SPONTANEOUS AND SALT HYPERTENSION

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There are at least three different isoforms of nitric oxide synthase (neuronal - nNOS, endothelial - eNOS, inducible - iNOS) which are involved in cardiovascular homeostasis. A centrally generated NO participates in decrease of sympathetic activity in contrast to central angiotensin II which contributes to enhanced sympathetic tone. We demonstrated that elevated blood pressure of spontaneously hypertensive rats (SHR) and salt-sensitive Dahl rats on high salt diet (DS 8 % NaCl) is mainly caused by increased activity of sympathetic nervous system which is driven by abnormal central nervous mechanisms. The aim of our study was to elucidate how NO system participates in spontaneous and salt hypertension and if there are any differences between these two hypertensive models. Therefore we determined NO synthase activity as well as nNOS, eNOS and iNOS protein expression in two central regions important for cardiovascular regulation (brainstem and diencephalon) and in the kidney by Western blot analysis. These tissues were obtained from 12-week-old SHR (MAP 143±5 mm Hg) and age matched control Wistar-Kyoto rats (WKY) (102±4 mm Hg) and from 8-week-old Dahl salt-sensitive rats on high salt diet (156±4 mm Hg) which were compared with Dahl salt-resistant rats on high salt diet (DR 8 % NaCl) (116±6 mm Hg). The only change of NOS activity was a decrease in brainstem of SHR rats compared to WKY. This was due to reduction of nNOS and iNOS expression in this brain region. We obtained the similar data on protein expression in brainstem of DS 8 % NaCl rats, but it was not accompanied by any changes of NOS activity. Neuronal and inducible NOS isoforms in diencephalon were characterized by lower expression

in DS 8 % NaCl compared to DR 8 % NaCl rats as well as by reduction of eNOS and iNOS protein expression in the kidney. On the other hand in SHR rats there were no significant changes of expression of any NOS isoform in diencephalon and kidney except a reduction of endothelial NOS expression in the kidney of SHR compared to WKY. Our results show that centrally generated NO plays a more important role in regulation of blood pressure (BP) in Dahl salt-sensitive rats than in SHR rats. The lower synthesis of endothelial NO in the kidney contributes to lower excretion of Na^+ and thereby to elevation of BP in both forms of hypertension. We can conclude that alterations in NO system participate more in salt hypertension (Dahl) than in genetic hypertension (SHR).

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POLYMORFISMS OF GENES RELATED TO TESTOSTERONE IN RELATION TO SPATIAL ABILITIES IN GIFTED CHILDREN

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Background. Spatial abilities are known to be related to testosterone levels in men and women. Gifted children were reported to have lower testosterone levels [1]. Although genetic factors of intelligence are thoroughly studied, specific cognitive performance associated with spatial abilities has not been yet analyzed in connection with polymorphisms of androgen metabolism genes. Aim. To analyze genetic polymorphisms of androgen receptor (*AR*), aromatase (*CYP19*) and 5α-reductase (*SRD5A2*) in relation to mental rotation and spatial visualization in prepubertal intellectually gifted children. Subjects & Methods. DNA samples of 36 boys (10,0±0,4 years) and 11 girls (9,7±0,5 years) with IQ higher than 130 were isolated from buccal cells [2]. DNA was amplificated by PCR. The *CYP19* C^{1558}-T polymorphism and *SRD5A2* A49T polymorphism were determined by RFLP analysis and the *AR* $(CAG)_n$ polymorphism by fragment analysis. Salivary testosterone levels were measured with radioimmunoassay. Spatial abilities (mental rotation and spatial visualization) were assessed using standard psychometric tests. Results. No relationship between testosterone levels and studied genetic polymorphisms was found in boys. *AR* and *CYP19* gene polymorphisms were not associated with spatial abilities. Heterozygotes in A49T polymorphisms (AT) had significantly better results in both spatial abilities tests in comparison to AA homozygotes. TT homozygotes were not found. No relationship between studied parameters was found in girls. Discussion. The T allele of A49T polymorphism was reported to have a 5-fold increased enzyme activity in comparison to the A allele [3]. AT heterozygotes outscored AA homozygotes in tests of spatial performance. As dihydrotestosterone has a higher affinity to the androgen receptor, this might indicate a potential molecular mechanism for the influence of 5α-reductase gene polymorphism on spatial abilities in intellectually gifted prepubertal boys.

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INTERNATIONAL EDUCATION IN PHYSIOLOGY USING MULTIMEDIAL APPROACHES

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The progress in international education thanks to the European Union platform opens many opportunities and challenges. As many textbooks are generally available as extra source of knowledge accomplishing lecture-based data, practical education is usually not only country but also university or even faculty specific. Therefore, protocols as guidance for physiological training in English are more than welcome. Moreover, the current level of computer sciences allows not only text and figure based but also multimedial presentations.
University of Veterinary and Pharmaceutical Sciences in Brno has been running international education courses at the Faculty of Veterinary Medicine for the last three years and the number of students has been gradually increasing. Therefore, a textbook for physiology students – Physiology I - has been recently prepared in English as a trial version to be tested and improved before the final issue. This publication starts with blood physiology and immunology including up-to-date technologies and methods in hematological and immunological laboratories, followed by cardiovasculary and respiratory systems. The book has 83 pages and includes more than 50 figures and schematic displays. Moreover, the interactive version of this text book has been made to present the topics during practical courses and allows students to continue and review experiments during self-study. The interactive version on a CD can be run using any internet explorer software, net-connection is not requested. The CD presents the text in independent chapters, with hypertext cross-references to figures, tables, schemes, animations and movies.
Some parts of this publication may be used for education at other faculties of the University of Veterinary and Pharmaceutical Sciences in Brno but also at other universities with an English curriculum. Moreover, it can help also Czech students in preparing for research and study stays abroad. The interactive version, moreover, is very flexible and can be gradually improved and modified according to current needs and requests of curriculum, students and teachers.

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DIETARY INTAKE OF IRON, ASCORBIC ACID AND FIBER IN THE INTERRELATIONSHIP WITH NUTRITIONAL, BIOCHEMICAL AND BIRTH PARAMETERS OF PREGNANT WOMEN: LONGITUDINAL STUDY

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Pregnancy is a time of increased need for iron and ascorbic acid.
Iron deficiency anemia early in pregnancy is associated with a > 2-fold increase in the risk of preterm delivery. On the other hand, iron supplements and increased iron stores have recently been linked to maternal complications (eg, gestational diabetes) and increased oxidative stress during pregnancy (1).
Vitamin C is involved in the synthesis and degradation of collagen and is important for maintenance of the chorioamniotic membranes. Inadequate availability of ascorbic acid during pregnancy has been proposed as a risk factor for premature rupture of the chorioamniotic membranes (2).
This longitudinal study evaluated dietary iron, ascorbic acid and fiber intake in the interrelationship between nutritional, biochemical and gynecological parameters. 692 pregnant women (in age of 26±4 years) in the third trimester were studied. For determination of nutritional parameters was used program Nutricon. Iron, calcium, magnesium, phosphorus, LDL and HDL lipoproteins were measured in serum. Weight gain in pregnancy, duration of pregnancy, extent of blood loss during delivery, and weight of a newborn were observed. General evaluation demonstrates low dietary iron intake (16.68 mg/day, 60 % RDA). Consumption of ascorbic acid was 75.91 mg/day (64 % RDA) and fiber 7.16 mg/day (28 % RDA). Concentration of calcium, phosphorus and iron (19.06 mmol/l) in serum were within normal range. A significant negative correlations ($p<0.05$) were observed between dietary iron intake and preconceptive weight and BMI, birth weight. Other positive correlations were evaluated between dietary fiber intake and age, negative correlation between dietary fiber intake and preconceptive and weight before delivery.
These results suggest low dietary iron and ascorbic acid intakes of Czech pregnant women that correspondent with anemia as typical symptom in pregnancy with other pathophysiological consequences. Low intake of dietary fiber participates frequent constipation and decrease the detoxication activity in gastrointestinal tract.

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MODULATION OF EXCITATORY SYNAPTIC TRANSMISSION BY K_{Ca} AND $GABA_B$ RECEPTORS ON CENTRAL TYPE SYNAPSES

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Calcium-dependent potassium channels (K_{Ca}) are activated by low concentrations of Ca^{2+} and hyperpolarize a wide range of neuronal cells of the central nervous system. $GABA_B$ receptors are the G-protein coupled receptors that are inhibitory to voltage-gated Ca^{2+} channels (VGCC). The tight coupling between intracellular Ca^{2+} and the activity of K_{Ca} suggests that the inhibition of VGCC by $GABA_B$ could subsequently result in a reduction of K_{Ca} currents and K_{Ca}-induced hyperpolarizations. Principal cells (PCs) of the medial nucleus of the trapezoid body (MNTB) accurately convert excitatory signals originating in the cochlear nucleus to glycinergic inhibitory signals directed to other brainstem auditory nuclei. The precise action of the MNTB synapses is important for sound source localization in mammals. The firing pattern of PCs is profoundly affected by the afterhyperpolarization phase (AHP) following a postsynaptic action potential. The aim of this study was to investigate the indirect modulatory effects of $GABA_B$ on K_{Ca} and K_{Ca}-mediated AHP in PCs using MNTB-containing live brainstem slices isolated from P18-25 mice. Whole cell membrane potentials were recorded by the patch-clamp technique. Postsynaptic action potentials were evoked by current injections (0.5-1 nA/0.5 ms) into PCs. The potentials were followed by AHP consisting of a fast and a slow component. The fast component was blocked by TEA^+, suggesting that it was mediated by high threshold activated K^+ channels. The slow component was sensitive to apamin, bicuculline and intracellular BAPTA and was enhanced after a high frequency (200 Hz) train of action potentials. This indicated that it was mediated by small conductance K_{Ca} channels (SK). Baclofen (100 μM), a $GABA_B$ agonist, reduced the slow component while it had no effect on the fast one. The slow component was also blocked by Cd^{2+}, ω-conotoxin GVIA and ω-agatoxin IVA, and it was insensitive to nimodipin. This indicates that the activity of K_{Ca} and slow AHP was induced by the opening of N- and P/Q-type VGCC. Trains of 20 current injections delivered to PCs at 200 Hz evoked both action potentials and frequent failures. Baclofen significantly reduced the number of failures, indicating that $GABA_B$ modulates the firing of PCs by inhibiting K_{Ca}-mediated AHP. Our results show the complex interactive effects of postsynaptic VGCC, K_{Ca} and $GABA_B$ on the reliability of excitatory transmission at auditory synaptic relay stations.

SUBSTANCE P RECEPTOR IN NORMAL AND DIABETIC RAT HEART

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Substance P (SP) is an 11 amino acid peptide amide that has been associated with many physiological processes in the cardiovascular system. In the periphery, SP is co-stored with calcitonin gene-related peptide (CGRP) in a special class of nociceptive neurons that has both afferent and efferent functions. SP/CGRP-containing sensory nerve fibers within the heart derive from cell bodies located in the dorsal root ganglia. Peripheral processes of these neurones innervate intrinsic cardiac ganglia and coronary arteries. Their actions are mediated through G protein-coupled receptors denoted NK_1-NK_3. The actions of SP in the heart are extensive and include indirect negative chronotropic and inotropic effects by stimulating cholinergic neurons. This innervation has been shown to be affected by diabetes (1, 2).
Here, we investigated tissue distribution of NK_1 receptor in the rat heart by means of immunofluorescence. Additionally, we studied the involvement of this system in the events underlying development of diabetic cardiomyopathy in the rat model of streptozotocin (STZ)-induced diabetes by real-time RT-PCR, and immunohistochemistry. Wistar rats were sacrificed by decapitation 6 and 12 months after application of STZ. The quantitative RT-PCR reactions were carried out in an iCycler (BioRad). Relative gene expression was expressed as a ratio of target gene concentration to housekeeping gene.
Indirect immunofluorescence showed that NK_1 receptors are located on the surface of smooth muscle cells of coronary vessels in the heart, and that neither distribution nor quantity of the receptors visibly changed in the course of diabetes. The results of RT-PCR indicate that mRNA for NK_1 receptor is expressed in each heart chamber, and it was significantly lower in the right atrium 12 months after induction of the diabetes, and significantly higher in the right ventricle at the same time point.

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INTERACTION OF NO AND REACTIVE OXYGEN SPECIES DURING DEVELOPMENT OF THE HYPOXIC PULMONARY HYPERTENSION

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Exposure to chronic hypoxia causes hypoxic pulmonary hypertension (HPH). At the begining of exposure to hypoxia. Release of free radical species induces damage of walls of periferal pulmonary vessels (1). Interaction of vasodilatatory NO and vasoconstrictory reactive oxygen specieces (ROS) plays important pathogenetic role (2).
In our experiments we treated adult rats with SOD mimetic-Tempol (80mg/kg, b.w. in drinking water) and with NO/superoxide donor-Molsidomine (15mg/kg, b.w. in drinking water) alone and in a combination during first 7 days of exposure to chronic hypoxia. Than the lungs were isolated and perfused with salt solution with albumine to study hemodynamic changes of pulmonary circulation by analysis of perfusion pressure increments induced by stepwise increase flow (P/Q relationship). We compared groups of rats treated with Tempol (T), Molsidomine (M), combination Tempol and Molsidomine (M+T) against not treated normoxic control group (C) and not treated rats exposed to hypoxia (H). The P/Q relationship was linear in all rats.The value of Intercept of P/Q relationship was significantly reduced in all groups (T, M, M+T, C) compared to group H. Thus, in all groups the basal tonus of pulmonary vessels was significantly lower than in group H. The value of slope of P/Q relationship in groups treated with Molsidomine (M, M+T) was significantly higher than group H. Thus, adding Molsidomine in first week during exposition to chronic hypoxia increased upstream pressure to perfusate flow. This can explained as an increase in wall stiffness due to potentiation of structural reconstruction of vascular walls induced by ROS release after Molsidomine treatment.

Group	n	Intercept P/Q [torr]	Slope P/Q [torr/ml.s^{-1}]
N	8	7,03 ± 0,42*	0,37± 0,03*
H	6	11,46 ± 1,59	0,57 ± 0,08
T	8	6,8 ± 0,57*	0,47 ± 0,07
M	7	8,24 ± 1,18*	0,92 ± 0,04*
M+T	8	7,95 ± 0,33*	0,99 ± 0,01*

* - significantly different against group H

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EFFECT OF CHRONIC SOCIAL STRESS ON LEVELS AND ACTIVITY OF MATRIX METALLOPROTEINASES IN RAT HEARTS

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Matrix metalloproteinases (MMPs) are family of enzymes which are involved in remodelling of the myocardial extracellular matrix (ECM) in both physiological and pathological conditions. This study was focused on the investigation of changes in the expression and activities of MMPs in rat hearts exposed to chronic social stress produced by crowding. In this model we also investigated protein expression of some "heat shock" proteins (Hsp), a group of proteins involved in stress response. Experiments were performed on normotensive rats (Wistar Kyoto) and spontaneous hypertensive rats (SHR). Adult males, 15 weeks old, were exposed to 8-week crowding stress (5rats/cage, 200cm^2/rat). Control rats were kept 4rats/cage (480cm^2/rat). The heart tissue and serum from all experimental groups were sampled. Total contents of MMPs, TIMP-2 (tissue inhibitor of MMPs) and Hsps were determined by Western blot analysis using specific antibodies. Measurements of MMP activities were performed by zymography in polyacrylamide gels containing gelatine as a substrate. Zymographic analysis of serum metalloproteinases revealed strong gelatinolytic activity in ≈30kDa area; however, there were no significant changes in their activities between unstressed and stressed experimental groups. Likewise, a total content of MMP-2 (gelatinase A) in serum was not different between the groups. In heart tissue, MMP-2 and MMP-9 (gelatinase B) have marked abundance. However, we did not observe significant changes in total content MMP-2 or MMP-9 in the hearts of normotensive or hypertensive rats with/without crowding. Also the protein levels of TIMP-2 were similar between all groups. Immunoblot assays of cytosolic Hsp60, Hsp70 did not show any differences in their levels between the control and hearts from the stressed groups. Analysis with monoclonal antibody specific against Hsp90 showed reactions in various areas with molecular weights of proteins approximately 80kDa, 62kDa, 50kDa and 26kDa. The amount of Hsp90 in all tested groups was not influenced by crowding stress. On the other hand, we found some differences between normotensive and hypertensive rat hearts when total content of cytosolic Hsp90 with molecular weight ≈80kDa was up-regulated in SHR compared with WKY group and down-regulated in ≈26kDa area. In conclusion, further experiments are required to elucidate our findings.

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BASIS OF XANOMELINE FUNCTIONAL SELECTIVITY TOWARDS M_1 VERSUS M_2 MUSACRINIC RECEPTORS

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Xanomeline is a functionally selective M_1/M_4 muscarinic acetylcholine receptor agonist that nevertheless binds with high affinity to all five subtypes of muscarinic receptors. A novel mode of interaction of this ligand with the muscarinic M_1 receptor characterized by persistent binding and receptor activation after extensive washout has been shown previously (1-5). Using human muscarinic receptors expressed in membranes of Chinese hamster ovary cells and [^{3}H]N-methylscopolamine as a tracer we show that persistent binding of xanomeline also occurs at the M_2 receptor with similar affinity as at the M_1 receptor. However, kinetics of formation of xanomeline wash-resistant binding to M_2 receptors was markedly slower than to M_1 receptors. Xanomeline was a potent fast-acting full agonist in stimulating guanosine 5-O-(3-[^{35}S]thio)triphosphate binding at M_1 receptors, whereas at M_2 receptors it behaved as a slow-acting potent partial agonist. We also demonstrate that xanomeline discriminates better between G- protein subtypes at M_1 than at M_2 receptors. Our data support the notion that xanomeline interacts with multiple sites on the muscarinic receptor, resulting in divergent conformations that exhibit differential effects on ligand binding and receptor activation. These conformations are both time- and concentration-dependent and vary between the M_1 and the M_2 receptor.

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INFLUENCE OF CALCIUM AND HYPOXIA ON NITRERGIC NEURONAL POPULATION OF THE RATS HIPPOCAMPUS

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We used NADPH-diaphorase staining to the study of the influence of calcium pre-treatment during long-lasting hypoxia on the brain structure of rats. NADPH-diaphorase is enzyme in neurons co-localized with NO-synthase that is responsible for NO synthesis. NO participates in hypoxic-ischaemic injury of the brain.
Hypoxia was induced in consecutive days since the day of birth till the 11th day of postnatal life in a hypobaric chamber (for 8 hours per day). Calcium was administered before each hypoxia exposition. 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 to the control animals, intermittent hypoxia brought about higher density of NADPH-diaphorase positive neurons in all studied areas of the hippocampal structure: in CA1 and CA3 areas of the hippocampus and in hilus, dorsal and ventral blades of the dentate gyrus. Calcium pre-treatment during hypoxia reduced number of NADPH-diaphorase positive neurons in all these areas.

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INDUCTION OF OESTRUS AND OVULATION IN ANOESTROUS EWES WITH GnRH, $PGF_{2}\alpha$ AND RAM IN THE FLOCK

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Tendency of rationalization and intensification in sheep breeding leads to innovation of biotechnological methods in oestrus cycle influencing. Aim of this work was to follow the effect of artificial oestrus induction and ram effect in anestrous ewes (1, 2). Blood samples (n=10) were taken to determine concentrations of progesterone (P4) and 17ß-estradiol (E2). Moreover, ewes were induced to oestrus with 0.5 ml GnRH per head, two applications in day. After 5 days, five ewes (group 1) were treated with 0.5 ml $PGF_{2\alpha}$ per head (3) and to other five ewes (group 2) ram was introduced. Forty-eight hours after treatment and ram introduction blood samples were taken again to determine concentrations of P4 and E2 using RIA methods. There was no significance in hormone concentrations determined in blood samples before GnRH treatment. Concentration of E2 was significantly increased in group 2 ($P<0.001$). From our results follow, that the ram pheromones can stimulate secretion of gonadotrophin and ovulation in anestrous ewes (1).

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ROLE OF ADRENERGIC RECEPTORS IN MEDIATING NONSHIVERING THERMOGENESIS IN HUMANS

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Effect of infusions of increasing concentrations of β1 and β2 adrenomimetics (Dobutamine, Bricanyl) on resting metabolic rate of young men (20 years) was followed. Dobutamine (maximal infusion rate 5 $\mu g.kg^{-1}.min^{-1}$) and Bricanyl (maximal infusion rate 0,4 $\mu g.kg^{-1}.min^{-1}$) increase metabolic rate by 30 %, or 20 %, respectively. This metabolic increase is about the same as the metabolic increase observed after administration of high doses of adrenaline or noradrenaline in non-cold adapted humans (24 %), (1) This may indicate that both types of adrenergic receptors may participate in mediating human nonshivering thermogenesis. The proportion of their participation cannot be established on the basis of the present knowledge, however. Experiments in vitro performed on isolated human leucocytes, using β1 and β2 adrenomimetics (Dobutamine, Bricanyl) and the β1 adrenolytic substance (Betaloc), confirmed participation of β1 and β2 adrenergic receptors in noradrenaline thermogenesis, measured as oxygen consumption by Clark electrode. Small change in metabolic response after administration of the α2 adrenomimetic substance clonidine indicates that the α adrenoceptors may also partially participate in leucocytes adrenergic thermogenesis. It is concluded that the adrenergic thermogenesis of humans is mediated by other types of adrenoceptors, than that in cold adapted mammals, which is located in brown fat and mediated mainly by β3 adrenoceptors.

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ANALYSIS OF THE EFFECT OF IVERMECTIN AT ATP-GATED P2X4 PURINERGIC RECEPTOR

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P2X4 receptor is ATP-gated cation channel. It is the only mammalian purinergic receptor which can be modulated by extracellularly applied ivermectin (IVM). IVM has several effects on electrophysiological characteristics of P2X4 receptor: it increases sensitivity to agonists, potentiates maximum current amplitude and prolongs the deactivation kinetics of the channel. In this study we focused on identification of the P2X4-specific residues responsible for the IVM effect on channel gating. To investigate this, we constructed several chimeric receptors of P2X4 and P2X2 receptors and several mutant P2X4 receptors with single point mutation. All receptors were expressed in HEK293 and the effect of IVM was determined using the patch-clamp technique. Experiments with chimeric receptors revealed that sequence Val49-Val61 but not the sequence Val64-Tyr315 is important for the effects of IVM on channel deactivation. P2X4 receptor-specific point mutations placed in sequences Gly29-Val61 and Asp338-Leu358 showed the importance of residues Trp50, Val61 and Val357 to IVM effect on channel deactivation but not to the effect on the maximum current amplitude. In conclusion, our results suggest the contribution of the first transmembrane domain, adjacent region and the second transmembrane domain to the effect of IVM on channel deactivation kinetics.

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BAROREFLEX SENSITIVITY AND A1166C POLYMORPHISM IN AT_1 RECEPTOR GENE

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Objective: Decreased baroreflex sensitivity (BRS in ms/mmHg; BRSf in mHz/mmHg) contributes to the development of hypertension and to the risk of sudden cardiac death in patients after myocardial infarction. It would be useful to know whether BRS is genetically determined. The aim of this study was to evaluate association of single nucleotide polymorphism (SNP) A1166C in angiotensin II type 1 receptor (AT_1R) gene with BRS and BRSf in man.
Methods: Systolic blood pressure (SBP) and interbeat interval (IBI), and instantaneous values of heart rate (HR) respectively, were recorded beat-to-beat 3 times in periods of one week (5 min, Finapres, breathing at 0.33 Hz) in 135 subjects (19-24 years). The power spectra of SBP and IBI (HR), cross-spectra and coherence were calculated. BRS was determined as a gain factor of the transfer function between variations in SBP and IBI (HR) at a frequency of 0.1 Hz. Genotypes were detected by means of polymerase chain reaction and restriction analysis using enzyme *DdeI*. We compared BRS and BRSf among genotypes of this SNP (Kruskal-Wallis, Mann-Whitney with Bonferroni-Holm correction).
Results: The frequency of genotypes of A1166C polymorphism was: 45.90 % (AA, n=62), 45.90 % (AC, n=62), 8.15 % (CC, n=11). Differences in BRS ($p<0.05$) and BRSf ($p<0.01$) among genotypes of this SNP were found (Kruskal-Wallis: BRS - AA: 7.9±3.3, AC: 8.6±3.6, CC: 5.9±2.3 ms/mmHg; BRSf - AA: 0.012±0.004, AC: 0.012±0.005, CC: 0.008±0.003 mHz/mmHg). Homozygotes in less frequent allele (CC) showed significantly smaller BRS and BRSf comparing carriers of other genotypes (Mann-Whitney: BRS - AA+AC: 8.2±3.5, CC: 5.9±2.5 ms/mmHg; $p=0.07$; BRSf - AA+CA: 0.012±0.004, CC: 0.008±0.003 mHz/mmHg; $p<0.01$).
Conclusion: We found significant association of A1166C polymorphism in AT_1 receptor gene with baroreflex sensitivity. Less frequent allele was associated with decreased BRS and BRSf.

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SUPEROXIDE PRODUCTION DURING CHRONIC VENTILATORY HYPOXIA

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The damage caused by increased production of reactive oxygen species is important factor in development of chronic hypoxia – induced pulmonary hypertension (1). The aim of the study was to determine the superoxide production during exposure of rats to chronic hypoxia.
We divided the Wistar male rats into 4 groups - 3 experimental groups and 1 group of controls. Experimental groups stayed in isobaric hypoxic chamber (FiO_2 = 0,1) for 1 day (H1;n = 8), 4 days (H4;n = 7) and 21 days (H21;n = 8). Controls were kept in normoxic conditions (N;n = 8). After removing from the chamber and after measurement of NO in expired air and plasma (results presented on 83.FD by D. Hodyc), we used the preparate of isolated ventilated and perfused rat lungs.
The concentration of superoxide was analyzed spectrophotometrically by reduction of cytochtome c in the perfusate. We have compared the spectral curves before and after administration of superoxiddismutase (SOD) and the difference indicated the amount of superoxide.
We found that the superoxide concentration started to increase after 4 days of hypoxia, with no change after 1 day of hypoxic exposure (H1: -0,06, H4: 2,64, H21: 0,545, N: 0,94[$\mu M.\ g^{-1}$], $p<0,05$). This result suggests that the free radical production caused by chronic hypoxia does not occur in the very early phases of hypoxia.

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MENTAL STRESS AND THE CARDIAC ELECTRIC FIELD

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Electrophysiological parameters characterizing ventricular repolarization have repeatedly been shown to carry information on the direct effect of sympathetic activation on the ventricular myocardium. The aim of the present study was to investigate the reflection of the psycho-emotional stress in the body surface potential distribution as documented by izointegral maps of cardiac activation and recovery. In 72 boys and men aged 18.3 ± 7.3 (SD) years with no history of cardiovascular diseases, body surface potential maps (BSPM) were recorded by the PC based ECG system CARDIAG 128.1 (METE Prague), sitting at rest and during the test of mental arithmetic (MA). The digitalized data for each of the 80 points of the QRS, STT and QRST integral maps, for each subject in both situations were evaluated by univariate and multivariate (spatial) statistical testing. The pattern of all integral maps at rest was dipolar, with negative values over the right superior anterior torso and right shoulder and positivity over the left precordium. The highest within group variability was found in the precordial region of positive potentials. The cardiovascular response to the forced MA (increase of BP by 10±1.5 / 8±1 mmHg and HR by 14 ± 2 beats/min significant at $p<0.001$), was accompanied by a marked decrease of the integral BSM values in the whole ECG cycle, at more than 2/3 in some individuals even in all of the lead-points. There was a highly significant decrease of the repolarization integral values mainly over the sternum and the right precordium, which contributed to analogically localized decrements also in the QRST BSM. This finding is in agreement with previous VCG studies documenting a significant diminution of the maximal spatial T-vector during the MA stress-test in healthy subjects (1). In conclusion the discriminative power of the difference STT and QRST integral maps was strong enough to discover in subjects with no cardiovascular symptomatology, the transient alterations in ventricular repolarization, due to the stress-test induced variations in sympathetic drive of ventricles.

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EFFECT OF DIFFERENT DOSES OF MYCOTOXIN ON METABOLISM IN BROILER CHICKENS

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Trichothecenes are a structurally diverse group of toxic secondary metabolites produced by *Fusarium* and related species of fungi which usually contaminate cereal grains in countries with temperate climates. Deoxynivalenol (DON) is the most prevalent trichothecene in crops used for food and feed production (1). Many studies describe the adverse effects of DON on animal and human health. Indeed, in domestic or laboratory animals, large doses of DON caused feed refusal, decreased weight gain, vomiting, gastrointestinal and dermal irritation and immunological alterations (2,3). Lower doses of DON have been shown to affect cell- mediated and humoral imunity (4). The aim of the study was to characterize the effect of different doses of DON on plasma variables in growing broiler chicks. Three groups of broiler chickens were formed with 14 birds in each group. Three diets included control (0.2 ppm DON) and experimental (1 ppm and 3 ppm DON). Chicks were fed the diets from the day of hatch to 42 d of age. Then all birds were sacrified and blood samples for biochemical analyses were collected. The mycotoxin doses were verified using gas chromatography-mass spectrometry. Plasma calcium and alanine aminotransferase activity were significantly elevated and potassium, magnesium, total protein, triglycerides and free glycerol were decreased in chicks fed 3 ppm DON in the diet. Plasma magnesium was significantly increased and potassium, total proteins, triglycerides and free glycerol were decreased in chicks fed 1 ppm DON in diet. No biochemical variable tested responded to increasing DON-concentration in the diet. Levels of DON used did not modify the 5 biochemical variables measured (including chlorides, alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, albumin). These results indicate that both levels of DON in the diet significantly affected protein and lipid metabolism in broiler chicks.

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INCREASED CELL SHORTENING OF ISOLATED CARDIOMYOCYTES IN ACUTE DAUNORUBICIN CARDIOMYOPATHY

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Usage of anthracyclines impairs cardiac function. We tested, whether the left ventricular performance is reduced due decreased function of isolated cardiomyocytes in acute phase of daunorubicine-induced cardiomyopathy. Wistar rats were treated for two weeks with daunorubicin (DAU, 3mg/kg, i.p., dosage in 48 h interval), control rats (CON) received vehiculum. Left ventricular function was studied using left ventricular catheterization. Left ventricular myocytes were enzymatically isolated and electrically paced at 0.5 Hz. Expression of the calcium releasing channel (ryanodine receptor, RyR) was determined in left ventricular homogenates using SDS-PAGE and Western blotting (n=8-10, per group). The hearts of DAU rats showed a loss of heart weight (see table) and this was associated with loss of cardiomyocytes on histological level. We observed an impaired contractility (dP/dt_{max}) and relaxation (dP/dt_{min}) of left ventricle. However, isolated cardiomyocytes of DAU exhibited augmented cell function and increased expression of RyR (100±26% vs 212±40%, $P<0.05$).

	CON	DAU
Gravimetry (n)	13	14
BW (g)	277±9	204±4*
Heart weight (mg)	857±20	600±14*
HW/BW (mg/g)	3.11±0.08	2.95±0.06
LV catheterization (n)	7	7
dP/dt_{max} (mmHg/s)	4976±503	2893±435*
dP/dt_{min} (mmHg/s)	-4144±304	-2868±297*
Isolated cells (animals/cells)	5/40	5/45
Cell shortening (%)	5.1±0.4	6.7±0.5*
Time to 50% relaxation (ms)	138.8±6.2	117.2±7.0*

We found contradictory findings in whole heart function and isolated cardiomyocytes during acute phase of daunorubicin-induced cardiomyopathy. We suppose, that the elevated function of single cells as adaptive response to loss of cardiomyocytes cannot rescue the decreased left ventricular function at this stage.

11β-HYDROXYSTEROID DEHYDROGENASE 1 IN THE LIVER OF THE HYPERTRIGLYCERIDEMIC RAT – GENDER DIFFERENCES

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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, hypertridyceridemia and obesity. Because not only insulin but also glucocorticoids strongly influence the metabolism of nutrients, we decided to study their local metabolism via 11□-hydroxysteroid dehydrogenase (11HSD) in liver that represent a glucocorticoid target organ. For our study we used female and male Prague hypertriglyceridemic rats (HHTg) and their normotriglyceridemic counterparts. HHTg rats were bred from Wistar rats at the Institute of Clinical and Experimental Medicine in Prague and serve as a model of metabolic syndrome which is not associated with obesity. We determined activities and mRNA levels of 11□-hydroxysteroid dehydrogenase type 1 (11HSD1). In addition, we measured mRNA levels of phosphoenolpyruvate carboxykinase (PEPCK) and peroxisome proliferator-activated receptor □(PPAR□, whose expression is stimulated by glucocorticoids. Activities were measured as a conversion of radioactively labeled substrate in tissue homogenates. The levels of mRNA were determined by RT „real-time“ PCR. The activities and mRNA levels of 11HSD1 were higher in females of HHTg rats than in healthy controls. In contrast, hypertriglyceridemia was not associated with increase of 11HSD1 activity and mRNA in males. Whereas healthy male rats are known to have higher liver 11HSD1 than healthy females, this difference is missing in HHTg rats. PPAR□ mRNA in females was also increased but PEPCK mRNA was decreased. Since there was no increase of 11HSD1 we didn´t measure PEPCK and PPAR□mRNA in males. The results indicate that the local concentration of glucocorticoids in the liver might be increased in females HHTg rats but not in males. We propose that increased glucocorticoid activation in the liver of HHTg females results in upregulation of PPAR□expression. In contrast, gluconeogenic pathway is suppressed in females.

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INCREASED EXPRESSION OF CONNEXIN-43 IN DAUNORUBICIN CARDIOMYOPATHY

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Background. Usage of anthracyclines causes changes of QRS complex. Aims: We tested, if the changes of QRS are linked with the expression of connexin-43. Methods: Male Wistar rats were treated for two weeks with daunorubicin (D, 3mg/kg, i.p., dosage in 48 h interval), control rats (C) received vehicle. Two days after last dose, 12-lead ECG was measured in rats under avertin anesthesia (10 ml/kg). Expression of connexin-43 was evaluated using Western blot analysis. Results: In daunorubicin-treated rats, the heart rate was significantly decreased comparing to controls (see table, $p<0.05$). Daunorubicin caused the prolongation of duration of QRS and QT resp. QTc interval. In daunorubicin-treated rats, the increase of QRS amplitude was present. Western blot analysis showed significant 84% increase of expression of connexin-43 in left ventricle.

	Controls	Daunorubicín
Heart rate (beats/min)	398±12	338±11*
QRS (ms)	22±1	26±1*
QT (ms)	79±2	94±3*
QTc (ms)	90±2	99±3*
QRSmax (mV)	0,87±0,08	1,14±0,08*

Conclusion: Application of daunorubicin caused the increase of QRS amplitude and duration. We suppose, that the increase of QRS voltage is associated with increased expression of connexin-43 in left ventricle of daunorubicin-treated rats.

INSECT ADIPOKINETIC HORMONES AND OXIDATIVE STRESS

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Intensive investigation of insect endocrine and nervous systems in the last few decades revealed structures of more than 200 neuropeptides that control practically all aspects of insect life. Stress adipokinetic hormones (AKHs) occupy an important position within this group: their function is to generally increase the concentration of energy substrates in insect haemolymph, inhibit synthetic reactions and control a number of corresponding biochemical and physiological actions on cell, organ and organismal levels (1). But recently, we have proven that the level of AKHs increases in insect body also in stress situations that are not apparently connected with direct mobilization of energy (insecticide treatment, photophase interruption, effect of constant darkness) (2, 3). Thus, AKHs operate as typical stress hormones activating reserves to help solve stressful situations that insects encounter during their life. This report links to those findings and proves that AKH actions also counter the incidence of oxidative stress – a situation that is characterized by over production of oxidative radicals and subsequent activation of antioxidant enzyme systems which eliminates these radicals and provide protection against their damaging effects in insect body (4). We found that AKH titre in insect body is enhanced after injection of a compound that evokes conditions of oxidative stress. On the other hand, an exogenous injection of AKH reduces oxidative stress markers in haemolymph. These facts indicate that there is a feed-back regulation between an oxidative stressor action and the level of AKH in insect body, and that AKHs might be involved in the activation of antioxidant protection mechanism. These results are to our knowledge the first documented evidence of a novel action of AKHs in response to oxidative stress, in addition to a plethora of other roles.

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IS THE OVERWEIGHT IN CHILDREN A POTENTIONAL CARDIOVASCULAR RISK FACTOR?

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Obesity is an important risk factor of premature atherosclerosis, because is accompanied with disorders in the metabolism of lipids and lipoproteins (1, 2). The aim of the pilot study was to find the risk of overweight on the basis of possible changes in the metabolism of lipids, lipoprotein (a), apoliprotein B_{100} and vitamin C in pupils of basic schools. 23 pupils with overweight (OW) – BMI=27±1 kg/m^2 and in the control group 21 pupils of normal weight (C) – BMI=21±2 kg/m^2 of comparable age average: 13±2 years were examined. Concentrations of Lp(a) and apo B_{100} were determined using the methods of radial immunodiffusion and electroimmunoprecipitation method, respectively. Antisera and standards were used from the production of IMMUNO-Vienna, ÚSOL-Prague, and BEHRINGWER-KE-Marburg. Concentration of the lipid parameters (TCH, TH, and HDL-CH) were determined using sets BIOLA-LACHEMA – Brno, and vitamin C concentration colorimetrically. Concentration of LDL-CH was calculated according to De Backer. Higher concentrations of Lp(a) in the group of pupils with overweight were found, but the differences compared to the control group were not statistically significant (p=n.s.).

Concentration of apo B_{100} was significantly increased in the OW group ($p<0.001$). Concentrations of TCH, TG, and non HDL-CH were statistically significantly increased in the OW group ($p< 0.001$; $p<0.05$; $p<0.001$), and concentrations of HDL-CH and vitamin C were significantly decreased ($p<0.05$; $p<0.001$). Based upon the parameters of apolipoproteins and lipids in pupils of basic schools, it can be stated that not only obese children are endangered, but also those with overweight, because disorders in the lipid and lipoprotein metabolism considerably increase the risk of premature development of atherosclerosis.

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MOTOR LEARNING IN ADULT AND YOUNG LURCHER MUTANT MICE

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Lurcher mutant mice represent a natural model of genetically determined olivocerebellar degeneration (1). They suffer from complete postnatal loss of cerebellar Purkinje cells and secondary decrease of granule cells and inferior olive neurons number. The degeneration results in cerebellar ataxia (2, 3). The aim of the study was to assess motor learning and the effect of repeated motor training on motor skills in adult and young Lurcher mutant mice.
Adult (older than 60 days) and young (25 days at the beginning of the experiment) Lurcher mutant mice of the C57Bl/7 strain were used. One part of them was trained on a rotarod for 6 weeks for 2 minutes four times a day. Mean latency of 1st fall and mean number of falls during one trial were evaluated. Motor skills were tested after 2 and 6 weeks of the training using a horizontal bar, slanting ladder and a rotarod with different diameter and rotation speed than that one used for the training. Mean latency of falls and percentage of successful trials were evaluated. The trial was considered as successful if the latency was longer than 60 s or if the animal left the apparatus actively.
At the beginning of the training on the rotarod young mice showed lower number of falls as compared with adult animals. Adult animals improved their performance so that within 2 weeks of the training they reached the same results as young individuals. Later, the results were stable till the end of the experiment in both types of animals. Tests on the bar and ladder did not show any significant effect of the training in either of them. In the rotarod test trained mice reached better results than untrained ones in both young and adult mice.
Adult mice needed several training sessions on the rotarod to learn to perform the task as successfully as young individuals, while young mice showed stable results for all courses of the training. The beneficial effect of repeated forced training was evident only on the rotarod - the test similar to the training method. Different motor skills were not significantly influenced by the training.

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EFFECT OF 3-HYDROXY-3-METHYLBUTYRATE TREATMENT ON PROTEIN METABOLISM IN SKELETAL MUSCLE OF INTACT RAT

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3-hydroxy-3-methylbutyrate (HMB) is the natural leucine metabolite, to whose described effects belong affecting of immunity system or protein metabolism in the skeletal muscle, especially in stress conditions. The aim of our study was to examine the effects of HMB on protein metabolism in the skeletal muscle of intact rats and possible differences in muscle responses depending on predominant type of myofibres.
Male rats weighing 40-60 g were administered continuously with HMB via osmotic pump in the dose of 0.5 g/kg/day (n=10); control animals were implanted with osmotic pump without HMB (n=10). After 24 hours extensor digitorum longus (EDL) and soleus (SOL) muscles were isolated and used for determination of total and myofibrillar proteolysis (PL), protein synthesis (PS), leucine oxidation (OL) and chymotrypsine-like activity (CHTLA) of proteasome. Results are expressed as means ± SEM. A difference was considered significant at $P < 0.05$.
In HMB treated animals we observed increased plasmatic concentrations of glucose and lipids and changes in concentrations of some amino acids. In EDL of HMB group we found decreased myofibrillar PL (74 ± 1 %) and increased protein turnover – increased total PL (113 ± 3 %) and PS (119 ± 5 %), compared to control. In SOL we found decreased OL (73 ± 5 %) and CHTLA of proteasome (72 ± 4 %), changes in other parameters were insignificant.
The results show that the anabolic effect of HMB treatment in muscles of intact rats is caused by changes both in protein synthesis and breakdown. Administration of HMB induced more pronounced changes in EDL than in SOL.

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IS INCREASED SUSCEPTIBILITY TO VENTRICULAR ARRHYTMIAS IN HYPERTENSIVE RATS?

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Summary: BHR–borderline hypertensive rats (BHR), are useful model for investigating the phenotypic traits of hypertension. The present study was designed to characterize the heart function in BHR (the first generation of offspring of a female SHR dams and a male WISTAR sires rats) and to compare it with the heart function of animals rats with NO-deficient hypertension induced by repeated doses of L-NAME (p.o,40mg/kg/day, during 4 weeks).
In BHR (n=6) the mean blood preasure was lower than that in L-NAME - treated rats (n=8) (135±2 vs. 155±2 mmHg, $p<0.05$) but only slightly higher than in control rats (127±1 mmHg, n=8). Development of high blood pressure in both strains was associated with left ventricular hypertrophy (index heart weight/body weight HW/BW for BHR 4,7±0,2; for L-NAME 5,6±0,1;for controls 4,0±0,2, $p<0.05$) and cardiac remodeling width of the free left ventricular free wall was in BHR, L-NAME and controls 4.2±0.2, 4.5± 0.2 and 3.5±0.1 mm, respectively.
Hemodynamic measurements revelead greater contraction and coronary vessel perfusion of spontaneously beating hearts isolated from BHR than that from L-NAME-treated rats but the obtained values were lower than the control ones rats.
ECG analysis from intact animals showed longer QT interval hypertensive animals (L-NAME 85.0 ± 4.00 ms, BHR 82.17 ±11.80 ms) in comparison to normotensive (74 ± 5 ms). Similar tendency of QT interval confirmed ECG recorded from isolated hearts. The susceptibility to arrhythmias was lower in BHR compared to L-NAME, while the s the proarrhythmogenesis was not detected in control hearts. We registered more episodes of ventricular extrasystoles, spontaneous terminated ventricular tachycardia and fibrilation in L-NAME than in BHR.
Data suggests that increased ventricular mass was not associated with improvement of myocardial contractility and resulted in increased incidence of arrhythmias. Thus, pressure-induced myocardial hypertrophy modifies heart function in both borderline as well as in L-NAME-induced hypertension.

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Keywords: L-NAME – BHR – hypertension – hypertrophy – ECG - arrhythmias

AN INCREASE IN EXPRESSION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE IN ISOPROTERENOL-INDUCED CARDIAC HYPER-TROPHY IN THE RAT IS ASSOCIATED WITH A DECREASED HEART RATE

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Background. Nitric oxide derived from endothelial nitric oxide synthase (eNOS) may possess cardioprotective properties. Aims: The aim of this work was to determine whether eNOS is involved in the regulation of heart rate in rats with isoproterenol-induced (ISO) cardiac hypertrophy. Methods: We induced cardiac hypertrophy in Wistar rats using ISO administration (5 mg/kg/d, i.p., during 8 days). Heart rate was measured in vivo using the tail cuff method. We used isolated right atria to study the effect of NO synthase inhibitor, L-NAME on basal and isoproterenol-stimulated spontaneous beating rate of the atria. Expression of eNOS was evaluated using Western blot analysis. Results: ISO treatment induced hypertrophy of the heart (+34%, $P<0.05$). Basal heart rate in vivo was decreased after 8 days of isoproterenol treatment (342 ± 8 min^{-1} vs 366 ± 6 min^{-1}, $P<0.05$). In isolated right atria ex vivo, we observed a trend towards a decreased beating rate (297±16 min^{-1} vs 332±13 min^{-1}). Blockade of eNOS using L-NAME caused a small, but significant increase in the beating rate of right atria isolated from Wistar-ISO (from 321±21 min^{-1} to 338±23 min^{-1}, $P<0.05$), but not from control rats. In isolated atria pre-stimulated with 10^{-7} M ISO *in vitro*, acetylcholine decreased heart rate more in ISO-hypertrophied atria (control: -8±4 %, ISO: -24±4 %; $P<0.05$). In control rats, L-NAME had no significant effect on the beating rate of isolated right atria stimulated by ISO *in vitro*, however, at high concentrations of ISO, it had a negative chronotropic effect in rats with ISO-induced cardiac hypertrophy. eNOS expression was increased in all parts of the heart approximately by 30 % ($P<0.05$). Conclusions: Decreased basal heart rate in ISO-induced cardiac hypertrophy is associated with an increased eNOS expression. This is supported by in *vitro studies* where NO synthase inhibition increased basal heart rate. However, NO synthase inhibition had a negative chronotropic effect in ISO-stimulated atria from ISO-treated rats, indicating that NO may have different effect on heart rate under basal conditions and catecholamine stimulation.

FUNCTIONAL COSEQUENCES OF STATUS EPILEPTICUS IN IMMATURE RATS

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It remains under dispute whether status epilepticus (SE) in the perinatal period or early childhood or the underlying neuropathology is the cause of functional impairment later in life. We examined whether SE induced by LiCl-pilocarpine in normal immature brain (at the age of 12 or 25 days - P12 or P25) causes impairment of motor development, cognitive decline and epileptogenesis. Early SE did not affect significantly normal body growth. The sensorimotor development (wire mesh test) was delayed in subpopulation of P12 animals with SE. Rats in the P12 group had delayed development of habituation (repeated exposure to open field paradigm) and they exhibited cognitive impairment in Morris water maze when assessed 3 mo after SE, although not as severe as in P25 group. Importantly, video-electroencephalographic monitoring 5 months after SE demonstrated that 65% of rats in the P12 and 90% in P25 group developed spontaneous seizures. Only nonconvulsive seizures (ictal activity in hippocampus accompanied by automatisms) were recorded in P12 group whereas rats in P25 group exhibited clonic convulsions. There was no difference in seizure frequency between the two age groups, but seizures were significantly longer in P25 than in P12 animals. There was no correlation between seizure frequency and cognitive impairment in neither age group. The present findings indicate that SE is harmful to the immature brain as early as in P12 group, which might be compared with early infancy in humans.
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TWINS GROWTH MODELLING FROM PARENT GROWTH POTENTIAL AND BODY HEIGHT AND WEIGHT DYNAMIC FENOTYPES

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The modelling method proposed by Novák (1) used in this study describes a human growth curve by three components which correspond with Karlberg's I, C, P phases (2) but are mathematically easily calculated and what is an advantage for a practical physician or biologist, they are each defined by biologically easily comprehensible constants. Each component is described by these constants: starting weight or height (G0, D0), genetic limit weight or height given by parent or genetic growth potential (GLi, DLi) and maximum weight or height gain (dGmax, dDmax). In our department we have been following a study set of over 5000 families and their children since their birth (and before) till present, i.e. 15 years of age of studied children. The study internationally follows over 40000 children, its nature is longitudinal, hence the name – European Longitudinal Study of Pregnancy and Childhood. The introduced bioversion of growth functions has been tested on the set of ELSPAC children and it's reliability in modelling child growth has been proven (3). ELSPAC study set also contains a unique set of twins and their longitudinal growth curves obtained from empiric data. These growth curves have been smoothed through the calculated curve using dynamic weight and height fenotypes allowing the confirmation of expected similarities and dissimilarities between uniovular and biovular twins. The model not only can exactly desribe the growth curve and it's derivations from the determined growth channel, with quality nutritional, socio-economic and health-status data it offers exact cause determination of such derivations. In the next research phase we plan to collect such nutritional data and in combination with the extensive range of background data already collected we hope to indicate the causes of individual growth derivations and establish general factors negative and beneficial for child development. Further twins studies using the bioversion of growth functions supported by quality nutritional data promises to contribute in the field of study of genetic and enviromental sides of human growth and development influence.

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THE IMPACT OF EXPERIMENTAL TYPE 2 DIABETES ON CATECHOLAMINES AND CGRP CONCENTRATIONS IN THE RAT HEART

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Diabetic patients are particularly prone to develop cardiovascular autonomic neuropathy (CAN) resulting from damage to both efferent and afferent innervation of the heart and blood vessels. The prevalence of CAN has been reported to be higher in type 2 than type 1 diabetics ranging from 22 to 34% depending on the criteria used (1). The purpose of this study was to evaluate how type 2 diabetes induced in 2-day-old rats by 100 mg/kg streptozotocin (STZ) affects the content of norepinephrine (NE) and sensory neurotransmitter calcitonin gene-related peptide (CGRP) in the heart compartments and selected arteries in relation to basal cardiovascular parameters, i.e. resting heart rate, systolic and diastolic blood pressures. At the age of 18 months, diabetic state closely resembling human type 2 diabetes was verified by determinations of blood glucose levels, glucose tolerance tests, plasma and pancreatic insulin concentrations, and immunohistochemical analysis of the pancreatic tissue. NE and CGRP concentrations were measured in the heart compartments, thoracic and abdominal aortae, mesenteric and femoral arteries using radioimmunoassay diagnostic kits. Although systolic pressure did not differ between the control and diabetic rats, diastolic pressure was significantly higher in diabetic animals of both sexes. NE concentrations were significantly elevated in the hearts and arteries of diabetic rats (~150% of control values) with no

difference between males and females. In contrast, CGRP tissue levels were significantly lower, reaching 40-70% of the control values. Interestingly, female diabetic hearts displayed significantly lower peptide concentrations than diabetic males. In conclusion, changes in NE and CGRP synthesis and/or turnover might be associated with the development of cardiovascular complications of type 2 diabetes, i.e. increased total peripheral resistance and decreased protection of the myocardium against ischemic injury. In addition, profound deficit in myocardial CGRP levels revealed in female diabetic rats might be implicated in the loss of "female advantage" in the prevalence of coronary heart disease frequently described in diabetic patients.

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UNRELIABLE PROPAGATION OF ACTION POTENTIALS THROUGH AXONAL BRANCHES – ESTIMATION OF CHANNEL CAPACITY

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Variable conduction of action potentials (spikes, APs) at branching points and other places of varying axonal geometry is supposed to be responsible for the spatiotemporal filtering of spike pattern propagating along the axonal tree. This not only changes the interspike intervals (representing the transmitted neuronal information), but also causes some APs fail to propagate further. This decreases the information relayed by neuronal spike trains, and causes the transmitted information is changed along the axon. To assess the way the information may be processed in axonal trees, we built a multicompartmental model of an arborised myelinated axon. We simulated propagation inhomogeneities at branching points and observed different pieces of input information (represented by spike pattern entering the axon, input pattern) may be selectively gated into different axonal terminals (endings of axonal branches, output pattern). This was analyzed using the Information theory (1) and the mutual information between spike trains at axonal beginning and axonal terminals and also between different axonal terminals was estimated.
Despite the effect of AP propagation failures, the net information represented by output patterns of all terminals was not significantly decreased due to the AP propagation - nearly 50% of the information available at the axonal beginning could be estimated from axonal terminals. The remaining 50% is probably not lost by AP propagation, but distributed into different axonal branches. Measuring the precision of AP propagation we found that the difference between the input pattern and its reconstruction (from all output patterns) differed only by few μs in axons of few μm in diameter and of length 10 cm. Supposing the information is encoded by interspike intervals, the 5 μs precision of reconstructed AP pattern indicates, that the axon with a refractory period of about 3 ms could transmit 10 bit/AP (2).

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THE INFLUENCE OF NIFEDIPINE ON ALPHA1- AND ALPHA2- ADRENERGIC VASOCONSTRICTION OF VESSELS ISOLATED FROM WKY AND SHR RATS

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In hypertensive rats, the augmented BP reduction after nifedipine injection results from the attenuation of elevated sympathetic vasoconstriction, which is characterized by enhanced Ca^{2+} influx through voltage-dependent Ca^{2+} channels. The aim of our study was to evaluate the role of nifedipine-sensitive Ca^{2+} influx in vasoconstriction elicited by α_1- or α_2-adrenergic agonists. We studied intact and deendothelized femoral arteries isolated from Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR), which were mounted to Mulvany-Halpern myograph to record isometric contractions in calcium-containing and calcium-free solutions. In calcium-free solution we could separate the phasic and tonic contractions. Norepinephrine (NE) and phenylephrine (PHE) caused transient phasic contraction in calcium-free medium. After addition of calcium to bathing solution the vessels responded by major tonic contraction, which was partially inhibited by nifedipine. Clonidine (CLO) did not cause phasic contraction, but its gradually increasing tonic contraction was completely abolished by nifedipine. In Ca^{2+}-containing solution NE caused contraction which was largely inhibited by the presence of endothelium in WKY but not in SHR. NE and PHE caused similar contractions in vessels with removed endothelium isolated from WKY and SHR. CLO caused the contraction of denuded vessels isolated from SHR but not from WKY. Nifedipine significantly reduced contractions induced by NE (36 % WKY and 45 % SHR) and PHE (48 % WKY and 40 % SHR) in Ca^{2+}-containing solution. CLO-induced contractions of femoral arteries from SHR are almost completely inhibited by nifedipine. We can conclude that NE and PHE elicit the same type of contraction, which involves the activation of intracellular Ca^{2+} stores and Ca^{2+} influx. CLO-induced contraction is based almost completely on Ca^{2+} influx through voltage-dependent Ca^{2+} channels. Nifedipine partially inhibited NE- and PHE-induced contractions but abolished fully clonidine-induced contraction.

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COMPARISON OF ANTICONVULSANT EFFECTS OF mGLUR5 AND mGLUR1 RECEPTOR ANTAGONISTS IN IMMATURE RATS

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Action of mGluR5 and mGluR1 antagonists - MTEP and AIDA - against cortical epileptic afterdischarges (ADs) was compared in immature rats. Cortical stimulation and registration electrodes were implanted to 12-, 18- and 25-day-old Wistar rats. Low frequency stimulation was applied six times with 20-min intervals. MTEP in doses of 2,5; 5; 10; 20 or 40 mg/kg and AIDA in doses of 5; 10 or 20 mg/kg were administered intraperitoneally. Control siblings received saline. Drugs were administered after the first AD. Behavior of animals was marked into the EEG recording. All groups were formed by 8-14 rats. ADs duration was measured and the duration of the first, predrug AD was taken as 100%.
Movements during stimulation as well as clonic jerks were not significantly influenced by either drug. Only a tendency to shortening of ADs duration was observed in all age groups with MTEP (up to the 20-mg/kg dose). The highest dose led to a significant difference in comparison of controls; duration of ADs was not progressively augmented with repeated stimulations. The action of AIDA was even less expressed –only the prolongation of ADs occuring in controls with repeated stimulations was blocked. The poor effect of the two antagonists indicates that group I of mGluR does not play an important role in generation of cortical ADs. It is in contrast to the results with generalized tonic-clonic seizures induced by pentetrazol – both antagonists are efficient against the tonic phase of these seizures in immature rats (data on file).

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CHANGES OF CHOLINERGIC MARKERS AND MUSCARINIC TRANSMISSION IN YOUNG AND AGED APP/PS1 DOUBLE TRANSGENIC MICE MODEL OF ALZHEIMER´S DISEASE

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Double transgenic APPswe/PS1dE9 mice mimick key fetures of Alzheimer´s disease (AD), i.e. early large increase of β-amyloid production with more marked rise of the 42 than the 40 amino acid fragments, formation of amyloid plaques in brain cortex and hippocampus (1,2), and impairement of episodic memory at the age of 12 month (3). We took advantage of this mouse model of AD to investigate in parietal cortex possible changes of selected markers of cholinergic synapses and functionality of signal transduction through muscarinic receptors in young (7 months) and aged (17 months) female transgenic animals and littermate controls. Immunohistochemistry confirmed only sporadic amyloid plaques in young transgenic animals and their dramatic increase in aged transgenic animals. We did not find changes of acetylcholinesterase staining. Biochemically, we found only age-dependent decrease of choline acetyltransferase and acetylcholinesterase activity but both age- and transgene-dependent decline of muscarinic receptors number, vesicular acetylcholine transporter density, and butyrylcholinesterase activity. Carbachol stimulated binding of GTPγS in membranes used as a functional indicator of muscarinic receptor coupling to G-proteins revealed age-dependent decline of carbachol efficacy in controls and transgene-dependent decline of carbachol efficacy in young animals and in addition its potency in aged animals. Our results demonstrate in this mouse model of AD an early decline of both pre- and postsynaptic cholinergic markers and the beginning of progressive impairement of muscarinic signal transmission that precedes acceleration of amyloid plaque formation and manifestation of cognitive deficit. These findings thus support the cholinergic hypothesis of AD (4) and offer theoretical basis for future direction of cholinergic therapy development.

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INFLUENCE OF ANTIOXIDANTS ON LEARNING IN WATER MAZE

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We developed a model of learning impairment caused by a single epileptic seizure elicited by Flurothyl in young male Wistar albino rats. This seizure lasted maximally 3 minutes. We did not observe any morphological changes elicited by the seizure. The impaired learning ability can be prevented by hypoxic preconditioning (3 days prior the seizure), by application of melatonin (100mg/kg i.p.) and partly also by glutathione (50 mg/kg i.p.) and tempol (50 mg/kg i.p) applied shortly before the seizure. We also tested the influence of these substances without the seizure. The substances were applied 24 hours before the start of learning in water maze.
Application of each of these substances resulted in prolongation of time necessary for the finding of islet. This effect was mostly prominent since the third day of learning. The same doses of mentioned substances which improved learning after epileptic seizure impaired learning in water maze in naïve rats. We suppose that both effects –the positive after the seizure and the negative in rats without the seizure – are related to the reactive oxygen species homeostasis.

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CHANGES OF ANTICONVULSANT ACTION OF IFENPRODIL, A SPECIFIC NR2B RECEPTOR ANTAGONIST, DURING ONTOGENY

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Nonselective NMDA receptor antagonists exhibit marked anticonvulsant action but also serious side effects. Therefore the research is now focused on subunit-specific antagonists. and we tested a possible anticonvulsant action of ifenprodil, a selective antagonist of NMDA receptors containing NR2B subunit, in two models of seizures in immature rats. Two types of motor seizures (minimal clonic and generalized tonic-clonic) were elicited by pentetrazol (100 mg/kg s.c.) in 7-, 12-, 18- and 25-day-old rats. Ifenprodil did not influence minimal clonic seizures but it specifically suppressed tonic phase of generalized seizures in the three younger groups. This action was reflected in decreased seizure severity expressed as a mean score (1). Ifenprodil (up to 80 mg/kg i.p.) failed to exhibit this action in 25-day-old rats. In contrast, only a proconvulsant effect was observed after the 20- and 40-mg/kg doses of ifenprodil in cortical afterdischarges (ADs) in 18- and 25-day-old rats - a threshold for transition of spike-and wave ADs into the limbic type was decreased and duration of ADs was increased). Ifenprodil failed to influence ADs in 12-day-old animals. Possible anticonvulsant action of ifenprodil is age- and model-specific. The qualitative developmental changes observed in our experiments are in agreement with ontogeny of NR2B subunit which is predominantly represented during the first two postnatal weeks in rats.

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INFLUENCE OF MELATONIN ON THE EXCITABILITY OF CORTICAL NEURONES IN RATS EXPOSED TO HYPOXIA

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Melatonin (N-acetyl-5-methoxytryptamine) has many functions in the central nervous system including regulation of biological rhythms and seasonal reproduction. In experiments, it acts as an antioxidant and it has a neuroprotective effect (antioxidant and antiepileptic). The aim of this study was to test the influence of melatonin on evoked epileptic seizures in rats exposed to hypobaric hypoxia.
Melatonin (50 mg/kg i.p.) or the solvent only (ethanol 96,5 %, 0,2 ml/100 g i.p.) were applied to the 11, 24 and 34-day-old rats. Immediately after this procedure, rats were placed in specialised hypobaric chamber (1h, simulated altitude of 9 000 m). Experiments were performed 24 hours after the exposition to hypoxia on freely moving rats with implanted stimulation (on the right sensorimotor area) and registration electrodes (on the left sensorimotor and on both visual areas). Epileptic seizures were elicited by the repeated (5 times) stimulation of sensorimotor cortex (bipolar pulses, frequency 8 Hz, interval between the end of the evoked epileptic seizure and the next stimulation was 1 min, intensity necessary for eliciting the seizure activity was 3 – 5 mA, duration of stimulation was 15 s). The durations of evoked epileptic seizures were measured and analysed by ANOVA and t-test.
The exposition to hypoxia decreased the duration of evoked seizures in 12-day-old rats in comparison to controls (hypoxia not exposed rats). Melatonin but not ethanol pre-treatment brought about further decrease of excitability ($p< 0.001$). Exposition of 25-day-old rats to hypoxia had only minimal effect on excitability of cortical neurones. Melatonin pre-treatment brought about shortening of epileptic seizures only after the 1st and 5th stimulation. Ethanol did not influence evoked seizures. High mortality (18 from 23 animals, 78%) in 35-day-old animals was observed after the exposition to simulated altitude of 9000 m. The pre-treatment with melatonin or ethanol decreased the mortality (33% or 7%) in those experimental groups, but there was no difference in the cortical excitability between the ethanol or melatonin pre-treated rats.

Our study demonstrates that melatonin in the dose of 50 mg/kg i.p. significantly decreases the excitability of cortical neurones in 12-day-old rats exposed to hypobaric hypoxia and in older animals it can increase the resistance to hypoxia.

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CONCENTRATION OF SELECTED SERUM PARAMETERS AFTER CADMIUM ADMINISTRATION

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Cadmium, as a toxic metal, still attracts an attention because it is often detected in the food products extending the maximum allowable limits. Kidney and liver are considered to be the major organs, which accumulate cadmium and probably the most susceptible organs to the cadmium effects (1–3). The aim of this study was to determine cadmium influence on some serum parameters after an experimental administration. Experiments were conducted on rabbits (n=24), divided into three groups. Animals in group A (n=8) received intraperitoneally 1.5 mg Cd per kg of body mass and were killed after 48 hours. In group B animals (n=8) received 1.0 mg Cd per kg of body mass per os for five month in palletized food. Group K (n=8) was the control. Blood for vena jugularis was used for evaluation of selected parameters of blood biochemistry. In mineral profile significant decrease of Ca and Ca:P and increase of P in group A was detected. Concentration of Mg, K, Na, Cl^-, and Na:K ratio were not affected significantly. Level of glucose and cholesterol was significantly increased in group A and AST and ALT decreased in group B in comparison with control. Concentration of total proteins and total lipid was not affected. From this study it can be concluded that experimental cadmium administration results in some significant serum alterations suggesting metabolic changes in the body.

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MODULATION OF INTRACELLULAR CASPASE MACHINERY USING ORGAN CULTURE APPROACH

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Organ explant cultures eliminate animal suffering during experiments but simultaneously allow investigations of intact organ, tissue and cell systems with preserved interactions corresponding to the situation *in vivo*. Explant cultures refer to *ex vivo* systems and a mouse model is usually used. The explants can be manipulated in several ways such as implantation of beads soaked in purified signalling proteins or separation of epithelium and mesenchyme which allows heterotypic/chronic recombinations. Moreover, cells can be transplanted into specific regions of the explants and their fates followed. DNA (gene constructs) can be electroporated into specific areas of the tissue to misexpress genes, inhibit protein function using dominant negatives or inhibit translation using morpholino antisense oligonucleotides.
To modulate intracellular caspase machinery during embryogenesis, cultures of several tissues and organs, such as limbs, teeth and middle ear have been established. Inhibition of activated caspases in explant cultures was achieved by adding fluoromethylketone inhibitors into the culture medium. Cultures were analyzed in 24 h intervals to follow the morphology and alterations in apoptosis. Penetration of the inhibitor throughout the tissue was confirmed using a biotinylated pan caspase inhibitor followed by tracing of biotin in the histological sections by alkaline phosphatase colour reaction.
Inhibition of caspases in the tooth germs leads to lack of apoptotic bodies and specific DNA fragmentation (TUNEL) in the primary enamel knot, a signalling centre of developing teeth and shows dental apoptotis as caspase dependent. Whereas, separation of middle ear ossicles (malleus and incus) seems to be caspase and apoptosis independent. Thus, transdifferentiation is likely to be involved in malleal-incudo joint formation. Inhibition of caspases in developing limbs inhibits digitalisation, involvement of particular caspases is under study.

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INVOLVEMENT OF MITO K(ATP) CHANNELS AND REACTIVE OXYGEN SPECIES IN ANTIARRHYTHMIC EFFECT OF ISCHAEMIC AND PHARMACOLOGICAL PRECONDITIONING IN THE RAT HEART

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Ischemic preconditioning (I-PC) induced by brief episodes of ischemia and reperfusion (I/R) protects the heart against sustained I/R (1). K(ATP) channels localized in mitochondria are suggested as key players in this process. Interaction with reactive oxygen species (ROS) has been proposed to play a role in the mechanisms of cardioprotection conferred by mitoK(ATP) opening (2).
This study was designed: i. to compare the effect of I-PC (1cycle of I/R, 5-min each) and pharmacological PC induced by mitoK(ATP) opener diazoxide (D) on ventricular arrhythmias during test ischemia (TI, 30-min LAD coronary artery occlusion) in Langendorff -perfused rat hearts, ii. to characterize the effect of both forms of cardioprotection on myocardial reactive oxygen species production and endogenous antioxidant systems. Production of ROS was determined with two methods: i. determination of concentration of conjugated dienes (CD-an indicator of increased ROS), ii. TBARS (thiobarbituric acid reactive substances). Levels of coenzyme Q (CoQ9, CoQ10) and alpha-tocopherol (α-toc) were determined (HPLC) in non-ischemic hearts, in the non- adapted controls after TI, as well as in the hearts exposed to I-PC or 15 min-pretreatment with D (50μM) prior to TI. I-PC significantly reduced a total number of ventricular premature beats (VBP) and episodes of ventricular tachycardia (VT) to 195 ± 40 and 0,2 ± 0,1 (from 518 ± 71 and 12,1 ± 2,4 in non-adapted controls; $P<0,05$). D exerted similar antiarrhythmic effect (VPB 168 ± 22, VT 2,3 ± 0,6; $P<0,05$ vs. controls). I-PC suppressed production of ROS after prolonged ischemia. Moreover, both I-PC and D stimulated a marked elevation in CoQ9, CoQ10 and in particular α-toc levels (increased by 55% and 47% respectively). Conclusions: mitoK(ATP) opening confers an efficient antiarrhythmic protection in the rat heart. Potential mechanisms of cardioprotection induced by PC might be related to the changes in the pro/antioxidant state of the myocardium.

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EFFECT OF N-ACETYLCYSTEINE ON NITRIC OXIDE PRODUCTION IN EARLY PHASE OF CHRONIC HYPOXIA

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Pathogenesis of hypoxic pulmonary hypertension (HPH) is initiated by oxidative injury to the pulmonary vasculature associated with increased of NO in expired air (1). Because early application of antioxidant N-

acetylcysteine attenuates oxidative stress and inhibits development of HPH (2) we decide to test its influence on pulmonary NO production. Three groups of male Wistar rats were used. Experimental groups H and NAC+H were exposed for 4 days to isobaric hypoxia (F_IO_2 = 0.1). Antioxidant N-acetylcysteine (20g/l in drinking water) was given 7 days before and during exposition of hypoxia in group NAC+H. Control normoxic group (N) was kept in air. The amount of exhaled NO was measured in unanesthetized rats by the chemiluminiscence analyser (Sievers). ANOVA with Fisher's PLSD test was used for statistical evaluation. We found that 4 days in hypoxia increased NO production (pmol/min/100g BW, mean ± SE) in both experimental groups, however less in rat drinking NAC (H: 1754 ± 81; NAC+H: 1203 ± 43; N: 865 ± 121). Administration of NAC did not affect the NO production during air breathing (N: 865 ± 121; NAC+H: 699 ± 35). We conclude that early application of N-acetylcysteine significantly reduces NO production in rats exposed 4 days of hypoxia.

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ACTIVATION OF NON-PREFERENTIAL $G_{Q/11}$ AND G_S G-PROTEINS BY MUSCARINIC M_2 RECEPTORS IS AGONIST-DEPENDENT

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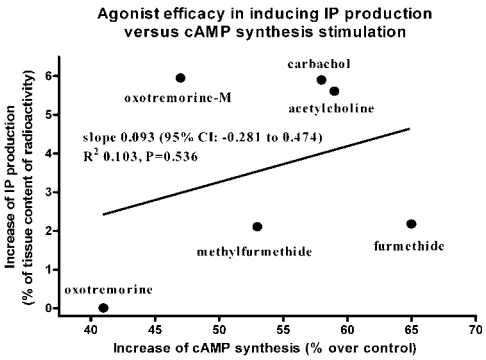

We demonstrated previously in M_2 muscrinic receptors expressed in Chinese hamster ovary (CHO-M_2) cells that at concentrations higher than needed for standard inhibition of forskolin-stimulated cAMP synthesis, muscarinic agonists reduce the extent of this inhibition. Inactivation of inhibitory $G_{i/o}$ G-proteins by pertussis toxin or higher expression level of M_2 receptors reversed the inhibition to net stimulation (1). In the present experiments we investigated whether stimulation of CHO-M_2 cells also increases phosphatidylinositol breakdown and whether non-preferential G_s and $G_{q/11}$ G-proteins are responsible for stimulation of cAMP synthesis and presumed increase of inositolphosphates (IP) accumulation, respectively (2). Knockdown of the $G_s\alpha$ subunit using RNA interference abolished stimulation of cAMP synthesis induced by 1 mM carbachol in both control and pertussis toxin-treated CHO-M_2 cells but had no effect on the inhibition of forskolin-stimulated cAMP synthesis. Carbachol increased IP accumulation in CHO-M_2 cells with EC_{50} of 79 μM. Knockdown of G_q, G_{11}, or both α subunits reduced this response by 78%, 54%, and 92%, respectively, while knockdown of the $G_s\alpha$ subunit had no effect. A series of full agonists with respect to preferential response, i.e. inhibition of adenylyl cyclase displayed different efficacies with respect to both stimulation of cAMP synthesis and increase in IP accumulation (see Figure). These results demonstrate direct coupling of M_2 receptor with the α subunits of G_s and $G_{q/11}$ G-proteins. In addition, they support the concept of multiple receptor conformational states that depend on both the concentration and the nature of used agonist.

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STEREOLOGIC ANALYSIS OF THE T-TUBULES AND THE TERMINAL CISTERNS OF SR OF OXIDATIVE MUSCLES IN MICE

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The main place of excitation-contraction coupling is at connections of the outer (T-tubules) and the inner (terminal cisterns of sarcoplasmic reticulum) membrane systems; these are the triads in skeletal muscles and the dyads in cardiac muscles. In spite of their different function, these tubulo-reticular complexes (TRC) are constructionally similar. Therefore we tried to compare quantitative characteristics of oxidative muscles - triads of slow skeletal muscle (*soleus*) and dyads of cardiac muscle (left ventricle), with the aim to gather quantitative data useful for understanding of mechanisms operating in this complexes. Electron microscopic images of muscle samples and the method of vertical sections (1) were used for analysis. From the ultrastructural aspect, longitudinal orientation of triads in skeletal muscle as well as of dyads in cardiac muscle was observed in the random sections. In both types of muscles, TRC isolated from myofibrils by mitochondria were observed, especially in cardiac myocytes that are characterized by clusters of mitochondria. Stereological analysis of the volume and surface parameters of TRC did not reveale statistically significant difference. The values of the surface-volume coefficient point to significantly larger diameter of T-tubules in cardiomyocytes, whereas the diameter of cisterns was not significantly different. Analysis of the neighbourhood of T-tubules revealed similar occurrence of various organelles in the vicinity of T-tubules in both muscle types. The environment of T-tubule is composed mostly of the membrane of cisterns (54% *soleus* and 57% ventricles), cytoplasm (20% and 26%), I-band (12 and 7%), mitochondria (8% and 6%) and A-band (4% and 2%). Less frequently were found membranes of SR (1% a 2%), lipid droplets (0.4% a 0.1%), and membranes of the T-system (0.2% a 0.0%). The environment of the cisterns revealed statistically significant difference in the communication with the T-tubules (31% *soleus* and 38% ventricles) and with A-band (8% and 1%). Other nearby organelles are cytoplasm (38% and 32%), I-band (12% and 10%), mitochondria (10% and 16%), membranes of SR (2% and 2%) and lipid droplets (0,1% and 0,1%). Results of the quantitative analysis revealed only slight difference among TRC in compared muscles, but it should be noted, that there is only one TRC per sarcomere in cardiac muscle, but two in soleus. *Supported by VEGA 2/6079/26 and APVT-51-31104*

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DEGENERATION OF THE HIPPOCAMPUS AFTER SUSTAINED EXPOSITION OF THE ETHANOL

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A neurotoxic effect of alcohol on the CNS of laboratory rats in the prenatal and postnatal period was studied. Next 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 10% alcohol, p.o. at libitum, every day , a) since the conception to the birth, b) 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, experimental group i) wasn not exposed to postnatal alcohol effect , experimental group ii) was exposed to postnatal alcohol effect (alcohol in breast milk). At the age of 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 rats aged 90 days was analysed under the light microscope. In CA1 and CA3 areas and in Gyrus dentatus of the hippocampus. In all offspring some cells with fine granulated karyons were identified, which were accompanied with high numbers of glial cells. The width of the pyramidal cell layer in the areas CA1, CA3 and the width of granule cell layer in both blades of the gyrus dentatus was smaller in experimental animals than in controls in the all age groups. We also.found significant differences between experimental groups i) and ii) in the areas CA1 and CA3. Our results demonstrate the neurotoxic effects of alcohol and the high vulnerability of the developing CNS. Presence of cells with fine granulated karyons could have been suggestion 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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SINGLE SUBCUTANEOUS ADMINISTRATION OF TURPENTINE OIL AS A MODEL OF PROTEIN CATABOLISM

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Objective: Turpentine oil (TO) administration is one of the simplest and most reproductive animal models of inflammation. It has been used to study metabolic response to injury and sepsis (hormonal changes, regeneration mechanisms, acute phase protein production, etc.). The aim of our study was to evaluate the influence of turpentine administration on skeletal muscle protein degradation.
Methods: Male rats (40-60g) received s.c. injection of 0.1, 0.2, and 0.5 ml TO/100g body weight or saline solution (control). After 24 or 48 hours the animals were killed by blood withdrawal under narcosis. M. soleus (SOL) and m. extensor digitorum longus (EDL) were dissected from both legs and incubated for 2 hours in modified Krebs-Heinseleit buffer. We estimated total (nmol of tyrosine released into the medium/g of muscle/hour) and myofibrillar (nmol of 3-methylhistidine released into the medium/g of muscle/hour) proteolysis (PL), proteasomal chymotrypsin-like activity (CHTLA) expressed as nmol of amino-methyl coumarine released from fluorogenic substrate/g of protein/hour, and plasmatic amino acid concentrations. Significance was accepted at $P<0.05$.
Results: The dose of 0.1 ml TO/100g body weight enhanced total proteolysis and CHTLA in EDL both after 24 and 48 hours. After administration of 0.2 ml TO/100g body weight these parameters increased in both types of isolated muscles, while the dose of 0.5 ml TO/100g body weight failed to increase them. Myofibrillar PL was not affected in any group against control. Total amino acid plasmatic concentration increased in 0.1 ml TO/100g body weight group after 48 hours.
Conclusion: The effect of turpentine administration was in our conditions dose and muscle type dependent. However, turpentine administration can not be recommended as a suitable model of muscle protein catabolism, because no affection of myofibrillar PL was observed.

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RECEPTOR DENSITY CHANGES AS ADAPTATION MEANS FOR COPING WITH NULL ACETYLCHOLINESTERASE ACTIVITY

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In order to ascertain the importance of receptor structures responsible for lung function regulation (i.e. α_1-adrenoceptors, β-adrenoceptors and muscarinic receptors) the animals lacking acetylcholinesterase were used. In these animals, the content of synaptic acetylcholine is extremely increased and therefore the number of muscarinic receptors is decreased.
Binding to muscarinic receptors and adrenoceptors (β-adrenoceptors, α-adrenoceptors) was investigated using radioligand binding with ^{3}H-QNB (muscarinic receptors), ^{3}H-CGP 12177 (β-adrenoceptors) and ^{3}H-prazosin (α_1-adrenoceptors). In order to discriminate between adrenoceptor subtypes, specific antagonists were used (CGP 20712A for β_1-adrenoceptors, ICI 118.551 for β_2-adrenoceptors, RS 17053 for α_{1A}-adrenoceptors, L-765,314 for α_{1B}-adrenoceptors and BMY7378 for α_{1D}-adrenoceptors). G protein coupling was assessed using pseudo-competitions with phenylephrine (α_1-adrenoceptors), isoprenaline (β-adrenoceptors) and carbachol (muscarinic receptors). cAMP contents was measured using EIA kit. Phospholipase C activity was measured by the enzymatic assay procedures using phosphatidylinositolbisphosphate as substrate.
We demonstrate here that not only muscarinic receptors, but also α_1-adrenoceptor subtypes (α_{1A}, α_{1B}, α_{1D}) and β-adrenoceptor subtypes (β_1 and β_2) are decreased. On the contrary, the signalization into the cell (coupling to G proteins, cAMP contents, PI-phospholipase C activity) was not changed in knockout animals in comparison to wild type controls suggesting that the receptor changes could be an effective tool that allow the animal to cope with high acetylcholine concentrations.

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CARDIOPROTECTIVE EFFECT OF OSAJIN ON ISCHEMIA-REPERFUSION OF LABORATORY RAT

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The study was undertaken to evaluate the cardioprotective potential of flavonoid osajin isolated from the infructences of *Maclura pomifera*, Moraceae, against ischemia-reperfusion induced injury in rat hearts as a model of antioxidant-based composite therapy. Study was performed with isolated, modified Langendorff-perfused rat hearts and ischemia of heart was initiated by stopping coronary flow for 30 min followed by 60 min of reperfusion (14 ml.min^{-1}). Wistar rats were divided into three groups. Treated group received pomiferin (5 mg/kg/day in 0.5% Avicel); placebo group received only 0.5% Avicel; intact group was left without any applications. Biochemical indicators of oxidative damage, lipid peroxidation product malondialdehyde, antioxidant enzymes (superoxide dismutase, glutathione peroxidase), total antioxidant activity in serum and myocardium has been evaluated. We also examined the effect of osajin on cardiac function (left ventricular end-diastolic pressure, left ventricular pressure, peak positive +dP/dt (rate of pressure development) after ischemia and reperfusion.
Our results demonstrate that osajin attenuates the myocardial dysfunction provoked by ischemia-reperfusion. This was confirmed by the increase in both the antioxidant enzyme values and the total antioxidant activity. The cardio-protection provided by osajin treatment results from the suppression of oxidative stress and correlates with the improved ventricular function.

Key words: Maclura pomifera; flavonoid osajin; heart ischemia-reperfusion; reactive oxygen species

MEMBRANE PROPERTIES OF HIPPOCAMPAL ASTROCYTES AND NG2 GLIA AFTER GLOBAL CEREBRAL ISCHEMIA IN RATS

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Changes in the expression of NeuN, GFAP, nestin, NG2, markers of apoptosis (cleaved caspase-3, cleaved poly ADP-ribose polymerase and

Fluoro-Jade B) and cell proliferation (BrdU incorporation) were studied in the hippocampus (CA1 region) of 8-week-old rats after global cerebral ischemia and correlated with time-dependent changes in astrocyte and NG2 glia membrane properties as determined by the whole-cell patch-clamp technique. Ischemia was induced by a bilateral, 15 min occlusion of the common carotids combined with hypoxic conditions (6% O_2 and 94% N_2), followed by reperfusion for 2, 6, 24 hours, 3, 7 and 35 days. Immunohistochemical analyses revealed that in the hippocampal CA1 region, ischemia leads to an increase in GFAP/nestin immunoreactivity and a decrease in NeuN staining, which was preceded by the appearance of apoptotic markers. In both sham-operated rats (control) and those after ischemia, astrocytes from the CA1 region displayed passive symmetrical non-decaying K^+ currents with the additional expression of delayed outward K^+ rectifier (K_{DR}) and/or inward K^+ rectifier (K_{IR}) currents. NG2 glia displayed a complex current pattern, i.e. $K_{DR,}$ $K_{IR,}$ A-type K^+ current and voltage dependent Na^+ currents (I_{Na}). Astrocyte depolarization and a shift of reversal potential to more positive values were observed starting 2 hours after ischemia. Membrane capacitance was decreased from the first day after ischemia. The K_{IR} current density was increased at the 6 hour time point after ischemia, when apoptotic markers appeared in neurons. After 5 weeks of reperfusion, apoptotic markers in CA1 astrocytes were detected, coinciding with an increase in the K_{DR} current density. In NG2 glia, increase in $K_{DR,}$ K_{IR} and I_{Na} currents was observed at the 6 hour to 1 day time point after ischemia. Our data show that global cerebral ischemia results in changes typical of astrogliosis and neuronal damage in the CA1 hippocampal region. Moreover, ischemia-reperfusion leads to transient changes in astrocyte passive membrane properties as well as the expression of K^+ voltage-dependent channels both in astrocytes and NG2 glia.

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ACUTE EFFECTS OF SIGMA RECEPTOR LIGAND HALOPERIDOL ON ISOLATED GUINEA PIG HEART

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Sigma receptor ligand haloperidol is a psychotropic drug used in treatment of various psychiatric disorders and agitation. Mechanisms of its life-threatening cardiovascular side effects (cardiac arrhythmias such as torsade de pointes, ventricular fibrillation or even cardiac arrest) are not elucidated yet. Therefore we examined the effects of haloperidol on 3-D electrogram of isolated guinea pig hearts.
Six adult male guinea pigs (body mass 653±75 gr) were sacrificed under deep ether anesthesia. The hearts were perfused according to Langendorff with Krebs-Henseleit solution (K-H) at constant pressure (85mmHg) and 37°C ($CaCl_2$, 1.2 mM). The experiment consists of four 30min periods: control, 10nM haloperidol, wash-out, 10nM haloperidol. Ten successive RR intervals were averaged at the end of control (steady state heart rate). This value was used for normalization of heart rate during the rest of experiment. The incidence of arrhythmias was evaluated according to Lambeth Conventions.
Normalized spontaneous heart rate showed a clear tendency to decrease during both haloperidol applications and this effect was partially reversible. In all hearts but one, typical arrhythmias were observed during both haloperidol periods (torsade de pointes, flutter and fibrillation), the hearts were mostly classified by number 4.
Since nanomolar concentration is close to binding constant of sigma receptors, we can conclude that these effects are mediated by binding of the drug to cardiac sigma receptor and not by direct effect on membrane ionic channels. However, the putative downregulation of sigma receptors (known from rat heart) has not been proven because heart rate changes as well as incidence of arrhythmias were not diminished by the second haloperidol administration.

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DYNAMIC FENOTYPE AND ITS SIGNIFICANCE FOR ANIMAL AND HUMAN GROWTH ASSESSMENT

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The growth of living organisms is a deterministic process. Weight genotype expression is dependent on adequate nutrition and an environment capable of absorbing all body heat produced by the physiological processes and biochemical reactions which cover the maintenance requirement and growth needs. Weight growth has a shape of sigmoid curve and can be derived from quadratic or exponential differential equation (1). Using an orriginal approach we do describe the growth curve course from birth till maturity by three directly measurable „dynamic fenotype“ constants. These are the genetically limited weight of the mature individual (GLi, kg), maximum weight gain in the growth curve inflexion point (dGmax) and the birth weight (G0, kg). The maximum weight gain depends on metablizable energy intake (PMEP, MJ/d) and adequate Environmental cooling effect (ECE, MJ/d) in the optimal case equal to the Total heat production (THP, MJ/d). The dynamic fenotype (G0, GLi, dG max) defines the classic growth functions constants (Table 1 and Table 2).

Table1

Function type	Differential equation	Weight growth
Logistic	$dG/dt = a.G-bG^2$	$G = GLi/(1+C.exp(-a.t))$
Exponential	$dG/dt = \alpha.G-B.G.lnG$	$G = GLi.exp(-C.exp(-B.t))$

Table 2

Function type	Anabolism coeff.	Catabolism coeff.	Integration constant
Logistic	$a = 4.dG\ max/GLi$	$b = 4.dG\ max /GLi^2$	$c = (a-b.G0)/(b.G0)$
Exponential	$\alpha = B.ln(GLi)$	$B = e.dG\ max/GLi$	$C = ln(GLi/G0)$

The derived relationships have been experimentally verifyed on Wistar rats (2), PIC pigs (3) and ROSS chickens (4). Animal growth has a shape of an exponential function. Child weight growth however is composed of three components, each component is determined by three autonomous „dynamic fenotype“ constants.

Component	Age, year	G0, kg	GLi, kg	dGmax, kg/year
Infancy	0 – 1,5	3,2	11,0	10,1
Childhood	1,5-10	10,69	60,0	2,68
Puberty	10-18	33,8	60,0	7,48

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IDENTIFICATION OF BETA-3 ADRENOCEPTORS IN THE HEART

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The existence of β_3-adrenoceptors in the heart is still matter of debate. Although the changes in the heart activity when β_3 agonist is applied were shown, there is no direct evidence of β_3-adrenoceptor ligand binding in the heart. The aim of our study was to answer following questions: (1) are there binding sites for β_3-adrenoceptor ligand in the human heart, (2) could be these receptors changed in cardiomyopathic heart, (3) are there changes in β_3-adrenoceptor gene expression in mice heart atria expossed to immobilization stress (IMO), (4) is there any

effect of corticotropin releasing hormone (CRH) gene disruption on that regulation.
The specific ligand for β_3-adrenoceptors, ^{3}H-SB206606 in saturation binding experiment in human heart ventricles membrane preparation was used. The Bmax in control heart ventricles was 923.7±131.3 fmol/mg protein and K_D was 32.9±14.4 nmol/l. In cardiomyopathic heart, there was no change in Bmax and K_D (837.6±216.6 fmol/mg protein, 29.4±11.2 nmol/l.
On the contrary, gene expression of β_3-adrenoceptors was affected significantly by immobilization stress. The effect of CRH KO as well as the effect of stress differed in right atria (RA) and left atria (LA). While in RA the amount of β_3-AR mRNA was dramatically decreased in CRH KO animals (to 52%), in LA there were no changes. Similarly, the IMO caused the decrease in the amount of β_3-AR mRNA in RA. On the other hand, there was about 2.5 fold increase in β_3-AR mRNA after 7 IMO in LA of WT animals. On the contrary, the CRH KO animals have not revealed the changes in β_3-AR mRNA during IMO neither in LA nor in RA.
These results indicate the important role of mice heart β_3-AR in the response to stress and also in coping with CRH insufficiency. Moreover, it could be hypothesized about the ineffective β_3-adrenoceptor regulation in human cardiomyopathy.

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AMBULATORY BLOOD PRESSURE MONITORING IN PATIENTS AFTER ANTHRACYCLINE THERAPY

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The anthracyclines have gained widespread use in the treatment of a variety of childhood haematological malignancies. The most important side effect of anthracycline chemotherapy is their cardiotoxicity and their interaction with the autonomous nervous system.
Ambulatory blood pressure monitoring (ABPM) in subjects after anthracycline treatment in a period between 4 to 16 years was the aim of the present study. We examined 110 subjects. Children and adolescents (A, number of subjects n=45) after anthracycline treatment for acute lymphoblastic leukaemia (the mean follow-up period – end of the treatment to ABPM: 9.7±3.1years; a total cumulative dose of anthracyclines 227±42mg/m^2) were compared with the healthy subjects of an appropriate age (H, n=65). The subjects were divided into 3 groups according to the age: 13-15 years (A: n=10; H: n=9), 16-18 years (A: n=20; H: n=33) and 19-21 years (A: n=15; H: n=23).
Twenty-four hour recordings of systolic and diastolic blood pressure (BP) were taken by the device Space Lab International. The device was programmed to take blood pressure measurements every 15 min (day-time) or every 20 min (night-time). The mean values and standard deviations from each hour were further evaluated. The statistical significant difference between A and H was found during the night hours (20 p.m.–2.a.m.) in the group of 19-21 years old only. The mean values of systolic BP from this period were 104.6±9.3 mmHg in A and 117.8±12.3mmHg in H ($p<0.01$) and diastolic BP were 58.2±7.2 mmHg in A and 67.7±10.4 mmHg in H ($p<0.01$).
It is concluded that several years after anthracycline treatment, the values of blood pressure at night are lower than in healthy subjects. This indicates an impairment of the sympathetic nervous system activity.

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THE EFFECT OF OXYTOCIN ON CARDIOVASCULAR FUNCTION AND ISCHAEMIA/REPERFUSION INJURY IN ISOLATED RAT HEART

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Oxytocin (OT), a nonapeptide hormone is expressed in a variety of organs, such as brain, heart, blood vessels and kidney. Last decade of research has shown that OT, besides its involvement in reproduction, stress (1) and social behavior, participates in regulatory mechanisms in cardiovascular system. Its action on the myocardial level is supported by formation of this peptide in cardiac cells and presence of specific OT receptors on the surface of cardiomyocytes (2,3). However, its effect on cardiovascular function and its role in the pathological situations, such as myocardial ischemia/reperfusion (I/R), is not completely understood. We hypothesized that OT released by stress could play a role as a mediator of protective responses in the myocardium during I/R.
The aim of the study was to characterize the effects of acute administration of OT on haemodynamic parameters in isolated rat heart under baseline conditions and in the hearts exposed to I/R. The role of OT was investigated by subjecting the hearts to 25-min perfusion with OT applied in a dosage of 123 nmol/l (10^{-7} M) using isolated Langendorff-perfused heart and measuring coronary flow (CF), heart rate (HR), left ventricular developed pressure (LVDP), and dP/dt_{max} (index of contraction) in 5-min intervals. Test ischemic challenge was induced by 25 min global ischemia followed by 120 min reperfusion, where we evaluated postischaemic recovery of mechanical function during 40-min reperfusion, whereas the size of myocardial infarction (tetrazolium staining and planimetry) was determined after 120-min reperfusion. Acute administration of OT over 25 min significantly decreased HR by approximately 21 % ($p<0.001$), CF by 30% ($p<0.01$) and contractility of the heart by approximately 24% ($p<0.01$). The above concentration of OT attenuated postischaemic contractile dysfunction and modulated the extent of necrotic changes (infarct size). The results point to an important role of OT in the regulation of heart function under normal conditions and indicate its potential protective role in I/R injury. Supported by grants VEGA SR 2/5110/25, European Social Fund (4-068) and APVT 51-02740.

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SALIVARY TESTOSTERONE IN GIFTED CHILDREN DURING PUBERTY ONSET

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Background. Salivary testosterone has been suggested to play the role in the development of intellectual giftedness (1). Our previous studies have shown that salivary testosterone levels are significantly lower in gifted boys (IQ>130) in comparison to normal boys (70<IQ<130). One of the possible explanations is that the underlying cause of this finding is a later maturation of gifted boys.
Aim. This study should shed light on the dynamics of salivary testosterone levels during puberty onset in gifted and normal children of both genders.
Subjects & Methods. Seventy girls (38 control and 32 gifted girls) and 92 boys (32 control and 60 gifted boys) were sampled every year in autumn during 10 years (from 7-16 years in girls; 8-17 years in boys). Salivary testosterone levels were measured using radioimmunoassay. The time series of gifted and normal children were compared. Additionally, age at menarche was compared in gifted and normal girls.

Results. No difference has been found between gifted and normal girls (neither in age at menarche, nor in salivary testosterone dynamics). Significant differences were found between gifted and normal boys at the age of 8-10 years, gifted boys having lower salivary testosterone levels. This difference disappeared during the puberty onset. The dynamics showed no difference in the onset of endocrine maturation.
Discussion. According to our knowledge this is the first study dealing with the dynamics of salivary testosterone during puberty onset in normal and intellectually gifted children. Our longitudinal data showed no difference in endocrine maturation onset. Our previous finding of lower testosterone in gifted prepubertal boys has been confirmed, but the underlying cause remains unclear. Genetic aspects and specific epigenetic factors should be followed in further studies.

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ECG BODY SURFACE MAPPING (BSM) IN DIABETIC PATIENTS (TYPE 1) WITH AUTONOMIC DIABETIC NEUROPATHY

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Diabetes mellitus is a risk factor of cardiovascular diseases. The diabetic autonomic neuropathy (AN) is known to be risk factor for sudden death. The aim of the present study was to evaluate the association of autonomic diabetic neuropathy (AN) and abnormal findings in ECG BSM in patients with DM 1. We have measured 145 parameters of heart electric field (ECG, VCG, BSPM) in 25 outpatients (11 men, 14 women, mean age 32±10.5) with DM1 suffering from AN, 29 with DM 1 without complications including AN (20 men, 9 women, mean age 33 ± 11.7 years) and in 30 healthy controls (11 men, 19 women, mean age 30 ± 3.2) years. AN was diagnosed when at least 2 Ewing tests were abnormal. The parameters were registered by diagnostic system Cardiag 112.2 (1) and statistically evaluated by Student t-test and test of Mann-Whitney. The faster heart rate (92 beats per min) and shortening of RR interval (668 ms) were observed in DM1 patients with AN when compared with diabetic patients without AN or healthy controls (82 resp. 74 beats per min, RR= 753 resp. 751 ms, $p<0.05$). In patients with DM 1 and AN we have found shortening QRS (75.5 ms) and QT (351 ms) when comparing with healthy controls (QRS 87.6 ms, QT 377 ms), ($p <0.01$). Other significant findings in DM 1 patients with AN like in diabetics without this complications comparing to healthy controls were: higher maximum in depolarization isopotential maps (DIPMmax) in the initial phase of QRS and less positive in the terminal phase, more negative minimum (DIPMmin) during QRS similarly as the minimum in depolarization isointegral maps (DIIMmin) and the minimum in isointegral map of the Q wave (Q- IIMmin), lower maximum in repolarization isopotential maps (RIPMmax) and less negative minimum (RIPMmin), more negative amplitude of Q wave (Q-IPMAM) and more pronounced spread of depolarization (activation time). The patients with DM 1 and AN have similar changes in ECG BPM like diabetic patients without this abnormality. AN cannot explain the abnormal changes seen on ECG BPM in diabetic 1 patients.

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VASCULAR SMOOTH MUSCLE CELLS ON PLASMA-MODIFIED LOW- AND HIGH DENSITY POLYETHYLENE FOR POTENTIAL TISSUE ENGINEERING

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The attractiveness of synthetic polymers for cell colonization can be affected by physical and chemical modification of the polymer surface. In this study, high density polyethylene (HDPE, m.w. 0.952g/cm^3) and low density polyethylene (HDPE m.w. 0.922g/cm^3) were modified by Ar plasma discharge using Balzers SCD 050 device (exposure time 10, 50, 150 and 400 seconds, discharge power 1.7 W). The material was then seeded with rat aortic smooth muscle cells (RASMC; passage 8, 9, 17 000 cells/cm²) and incubated in medium DMEM with 10% of fetal calf serum. On day 1 after seeding, the highest number of initially adhered cells was found on both HDPE and LDPE samples exposed to Ar plasma discharge for 150 seconds. On day 2, the cell number on all modified HDPE foils was significantly higher than that on non-modified HDPE. In contrast, in LDPE, only the values on samples modified by 150 and 400 seconds were significantly higher. On the 5th and 7 th day, there were no significant differences in cell number among all LDPE samples. However, on HDPE, the significant differences persisted on the samples modified for 400 seconds. The cell spreading areas, measured on day 1 after seeding, were significantly larger on all modified LDPE samples and HDPE samples exposed for 150 s. The increased cell colonization was probably due to the formation of oxygen-containing chemical functional groups in the polymer [1]. These results indicate that the cell responsiveness to the changes in physicochemical surface properties was more pronounced in HDPE than in LDPE. On both types of polyethylene, the most appropriate exposure time for the enhancement of cell adhesion and growth was 150 and 400 second.

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PHYSIOLOGICAL CONSEQUENCES OF ION CONCENTRATION CHANGES IN THE TRANSVERSE-AXIAL TUBULAR SYSTEM OF RAT AND GUINEA PIG VENTRICULAR CARDIOMYOCYTES

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To explore the physiological consequences of ion concentration changes in the transverse-axial tubular system (TATS) of rat and guinea-pig cardiac ventricular myocytes, mathematical models of their electrical activity that include a quantitative description of the TATS were developed. The geometrical characteristics of the TATS, the characteristics of ion transporters and their distribution between the surface and tubular membranes were modelled using available experimental data from both species.
In both models, transient depletion of tubular Ca^{2+} during each action potential decreased intracellular Ca^{2+} load and consequently the amplitude of systolic Ca^{2+} transient. However, this effect and particularly its frequency dependence were different in the two species. In the rat model, the maximal depletion of tubular Ca^{2+} during a single action potential, at a stimulation rate of 1 Hz, was 7%. With increasing stimulation frequency, tubular Ca^{2+} depletion increased, reaching 13.1% at 5 Hz. This depletion induced a cumulative beat-to-beat decrease in SR Ca^{2+} content that resulted in 3% and 20% decrease of steady-state Ca^{2+} transient amplitude at 1 Hz and 5 Hz, respectively. In the guinea pig model, the maximal depletion of tubular Ca^{2+} was 13.8% at 1 Hz, which decreased with stimulation frequency to 6.5% at 5 Hz. The

reduction of Ca^{2+} transient amplitude was lower than in rat: 5% and 2% at 1 Hz and 5 Hz, respectively.
These differences arise because: (i) the fraction of I_{Ca} in the TATS, which is responsible for tubular Ca^{2+} depletion, is lower in the guinea-pig (64 %, [1]) than in the rat (87 %, [2]); (ii) the sensitivity of I_{Ca} to the changes of membrane potential induced by tubular Ca^{2+} depletion is lower in guinea pig; (iii) unlike the rat model, the magnitude of I_{Ca} decreases with stimulation frequency in the guinea pig model; (iv) ion diffusion between the tubular lumen and external space is significantly faster in the guinea pig model (τ_{Ca}= 240 ms, [1]) than in the rat model (τ_{Ca}= 500 ms, [3]). These data suggest that changes of ion concentrations in the TATS lumen modulate cell function in species dependent manner.

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THE LEVELS OF CATECHOLAMINES IN HYPOPHYSIS OF EWES AFTER HORMONAL STIMULATION

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The effect of hormonal stimulation on catecholamine levels and activity of its degradation enzyme monoaminooxidase in the hypophysis of ewes in the oestric period was studied by the radioenzymatic method. Monoaminooxidase activity was determined radiochemically. The oestrus of ewes was synchronized with Agelin sponges (Agelin Spofa, Ivanovice on Hana, Czech Republic) containing 20 μg chlorosuperlutin. After completed synchronization we induced superovulation in the experimental group by means of 1500 IU serum gonadotrophin (SG, Ivanovice on Hana, Czech Republic). The extrahypophyseal hormone SG which show LH and FSH activity has a long half – life of biological degradation in the organism and its application is associated with hyperestrogenization (1). High oestrogen levels have a specific impact on hypothalamic adrenergic receptors and influence catecholamine levels and function. The results indicate that hormonal serum gonadotrophin stimulation increase ($P < 0.01$) the pituitary dopamine and epinephrine levels in ewes significantly. In comparison with the control group norepinephrine concentration did not change in this tissue. MAO activity in the hypophysis decreased significantly to almost one half in comparison with control values ($P < 0.001$). According to our results, the serum gonadotrophin in combination with hyperestrogenization (2, 3) influences dopamine and norepinephrine metabolism in the hypophysis of hormonally stimulated ewes and reduces monoaminooxidase activity selectively.

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PARTICIPATION OF S4 SEGMENTS IN ACTIVATION OF THE $CA_V3.1$ CALCIUM CHANNELS

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Opening of voltage dependent channels is mediated by reaction of their voltage sensor to the change of transmembrane potential. Voltage sensor of the low voltage-activated calcium channels is composed of 5-6 positively charged amino acids in S4 segments in each of four domains of α_1 subunit. The aim of our study was to evaluate participation of these segments in voltage dependent activation of $Ca_v3.1$ calcium channel. We have constructed series of mutated channels, in which upper most arginines in individual S4 segments and/or in two adjacent domains were substituted with neutral cysteines. Mutated channels were transiently transfected in HEK293 cells and current carried by 2 mM Ca^{2+}were measured using the whole cell patch clamp method. Voltage dependence of channel activation was evaluated using following protocols: i) the current-voltage dependence activated by a series 100 ms long pulses to membrane voltages between -100 mV and +70 mV with an increment of +10 mV; ii) the instantaneous current-voltage dependence activated by a series 100 ms long pulses to membrane voltages between -100 mV and +70 mV with an increment of +10 mV. Test pulses followed after 10 ms long depolarization of the cell membrane to +60 mV. The ratio of currents amplitudes measured by both protocols represents approximation of relative open probability of the channel. Data were fitted by Boltzman equation. The mutation in domain IIIS4 and double mutations in domains IS4+IIS4 and IIS4+IIIS4 shifted significantly the half-maximal activation voltage ($V_{0.5}$) by -6.9 mV (IIIS4) and -5.2 mV (IIS4+ IIIS4) to hyperpolarized potentials. Mutation in domains IS4+IIS4 caused the significant shift of $V_{0.5}$ to more positive potentials by + 9.9 mV. All these mutations also significantly increased the slope of voltage dependence of activation of mutated channels. Slope factors were decreased 0.66-fold by mutation in IIIS4, 0.78-fold by mutations in IS4+IIS4 and 0.72-fold by mutations in IIS4+IIIS4. In conclusion, most significant and opposite effects on current activation have mutation of putative voltage sensor in domains III and I.

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IMMUNOMODULATION EFFECT OF IMUNOGLUCAN ON OXIDANT-ANTIOXIDATIVE SYSTEM IN EXPERIMENT

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The organism has protective mechanisms by which formation of free radicals (FR) is counteracted, or in case of their formation they are able to reduce negative consequences of their effect by so-called antioxidative systems (1, 2). To find out the effects of beta-1,3/1,6-D-glucan on selected parameters of the oxidative stress and antioxidant protection in rats after their irradiation with the doses of 10 and 20Gy. The experiment was performed after approval of the Ethic commission of the State Veterinary and Food Administration SR on 35 Wistar rats, weighed 350–450 g. The animals were fed with a standard granulated food, and were divided into four groups: In the first control group (CG, n=6), in the second group, where milk with Immunoglucan was in the drinking regimen, 11 rats were included. The third group (n=9) were irradiated by 10Gy, and in the last group (n=9) were irradiated by 20Gy that were administered milk with Immunoglucan in the dose of 2 mg/kg of weight. After 30 days the animals were killed and material collection was carried out. Lipid peroxidase (TBARS), TAS in plasma and activity of antioxidant enzymes (SOD, GPX, catalase in erythrocytes), and of the vit.C were determined. The values were statistically evaluated by the programme Arcus BioQuikstat. A statistically significant difference was found in the activity of catalase and GPX in comparison of CG with the group drinking Immunoglucan ($p=0.01$). In the group of the individuals irradiated with the dose of 10Gy, a significant decrease in the values of all parameters of antioxidant protection (cat. GPX SOD, TAS) was found. The parameter of lipid peroxidation did not change, and vit.C concentration was statistically significantly increased. The individuals irradiated with the dose of 20Gy had significantly increased only the value of TBARS (1.92±0.23 *vs* 1,47±0.20 μmol/l). The correlation analysis revealed the most statistically significant correlations in the CG drinking Immunoglucan. Negative correlation was between TBARS and SOD ($r=-0.79$, $p=0.01$), and between TBARS and catalase ($r=-0.71$, $p=0.05$). Statistically significant relationship was recorded between the concentration of of vit.C and activity of catalase and GPX. Based upon our experiment it can be stated that drinking of Immunoglucan in rats positively influences the organism effort to ensure recovery of the balance between formation of FR and antioxidant protection at which Immunoglucan had a positive effect.

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THE ROLE OF CALCIUM INFLUX AND G_i PROTEINS IN ALPHA1- AND ALPHA2- ADRENERGIC VASOCONSTRICTION ELICITED IN SHR AND WKY RATS

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Increased sympathetic tone and enhanced vascular responsiveness to norepinephrine were reported in spontaneously hypertensive rats (SHR). Our recent studies with acute i.v. nifedipine administration suggested that an important part of hypertensive action of sympathetic nervous system (SNS) was exerted by calcium influx through voltage-dependent calcium channels. The present study is focused on the role of nifedipine-sensitive calcium influx and Gi proteins in alpha1- and alpha2-adrenergic vasoconstriction elicited in normotensive and hypertensive rats. The experiments were carried out in 12-week-old male Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) in which half of animals was injected with pertussis toxin (PTX, 10 ug/kg i.v., 48 h before the experiment) to eliminate G_i proteins. We studied the balance of vasoactive systems as well as blood pressure (BP) responses to acute administration of alpha1- or alpha2-adrenoceptor agonists (phenylephrine, clonidine) before and after nifedipine injection (0.4 mg/kg). The measurements were made in conscious animals subjected to previous blockade of RAS (captopril, 10 mg/kg) and SNS (pentolinium, 5 mg/kg). Pretreatment with PTX decreased basal BP level and reduced BP response to acute ganglionic blockade by pentolinium in both rat strains, although the effect was substantially greater in SHR compared with WKY. In the presence of considerably reduced sympathetic tone, the actual BP level in PTX-treated rats was maintained by enhanced RAS activity. Under the conditions of acute ganglionic blockade, it was evident that clonidine was as potent pressor as phenylephrine. Nifedipine or PTX pretreatment reduced both phenylephrine- and clonidine-induced BP response. Their efficacy was greater on alpha2- than alpha1-adrenergic vasoconstriction. PTX and nifedipine affected alpha2-adrenergic vasoconstriction similarly in both strains, but their influence on alpha1-vasoconstriction was surprisingly smaller in SHR than in WKY. We observed only negligible additivity of PTX and nifedipine effects on pressor response elicited by alpha-adrenoreceptor agonists. In conclusions, our results indicate that G_i proteins and voltage-dependent calcium channels play an important role in the BP response to adrenergic stimulation, but their involvement is greater in alpha2- than alpha1-adrenergic vasoconstriction.

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THE ROLE OF TRPV1 RECEPTORS IN NOCICEPTION AND PAIN MODULATION

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Capsaicin, the active substance from hot peppers, is known to evoke sharp pain after intradermal application. In the last years it was established that this effect is due to activation of transient receptor potential vanilloid receptors (TRPV1), that are present on a subclass of dorsal root ganglion neurons. The TRPV1 receptors are expressed on both the peripheral and central branches of these neurons and can be activated, beside capsaicin, by heat, low pH and possibly by endogenous agonists.
In our experiments we have used single intradermal high concentration capsaicin injection in a model of surgical pain to alleviate postincisional allodynia and hyperalgesia normally present in this model. The best results were obtained when the capsaicin injection was applied 24h before the incision, when it prevented the development of both thermal hyperlagesia and mechanical allodynia. The surgical incision lead also to robust increase of C-fos expression in the dorsal horn, spinothalamic and postsynaptic dorsal column neurons suggesting the presence of central sensitization. This increased c-Fos expression was also prevented by the single high concentration capsaicin treatment.
While the action on the peripheral TRPV1 receptors is relatively well established, the role of the TRPV1 receptors on the central branch of the primary afferents in the spinal cord dorsal horn is much less clear. Our results based on behavioral experiments with intrathecal application of TRPV1 antagonist (SB366791) and *in-vitro* recordings of mEPSC's from the superficial dorsal horn neurons in spinal cord slices suggest, that central TRPV1 receptors may play a crucial role in the modulation of pain transmission at the spinal cord level.

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THE ROLE OF MORPHOGENS IN NEURAL STEM CELL DIFFERENTIATION

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Sonic hedgehog (Shh) and Wnt-7a are secreted morphogens involved in the ongoing neurogenesis in the adult brain. GFP-labeled P0 mouse neural stem cells expressing either Shh (Shh/GFP) or Wnt-7a (Wnt-7a/GFP) were used to study their effect on neural stem cell proliferation and differentiation *in vitro*. Electrophysiological characterisation using the patch-clamp technique and immunohistochemical analysis were carried out 8 days after the induction of differentiation by retinoic acid; wild-type cells (WT/GFP) were used as a control. In WT/GFP cells three distinct cell populations were identified. Large flat cells with a cell-body diameter of 40µm formed an underlying layer (19%), expressed GFAP, and displayed passive, time- and voltage-independent K^+ currents, with an average membrane potential (V_m) of -87 mV and input resistance (IR) of 61MOhms. The second population of cells (16%) with a triangular cell-body (diameter 25 µm) expressed GFAP or NG2 and predominantly displayed passive, time- and voltage-independent K^+ currents together with an inwardly rectifying current activated by hyperpolarisation. Their mean V_m and IR were -90 mV and 72 MOhms, respectively. The third group of cells (65%) with a cell-body diameter of 15µm (termed neuron-like cells) were MAP-2, DCX or β-III tubulin positive with a mean V_m of -83 mV and IR of 357 MOhms and mostly displayed voltage-dependent K_A, K_{DR} and TTX-sensitive Na^+ currents (I_{Na}). Shh expression led to increased numbers of both flat and triangular cells, but their passive membrane properties were not significantly different from those in control cells. The number of neuron-like cells decreased by 25%, and moreover, I_{Na} currents were not detected. In Wnt-7a/GFP cells the number of large flat cells was decreased by 16% and the number of triangular cells increased by 23%, while the number of neuron-like cells was not significantly different when compared to controls. All three cell populations showed increased IR and decreased V_m; Based on electrophysiological data we can conclude that Wnt-7a/GFP cells showed marked differences compared to Shh/GFP and WT/GFP cells, thus this morphogen might play an important role in postnatal neural stem cell differentiation.

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ECC AND LUSITROPY OF VENTRICULAR MYOCARDIUM DURING POSTNATAL ONTOGENY

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The relaxation and inter-beat diastolic properties are termed "lusitropic functions". The role of sarcoplasmic reticulum (SR) dramatically increases during early postnatal period and this development is responsible for diverse regulation of contraction/relaxation cycle in newborn and adult ventricular myocardium. Adult ventricle are most likely dependent on Ca^{2+} release and uptake by SR, whereas immature myocardium is mainly regulated by transsarcolemmal fluxes of calcium. The purpose of the present study was to determine effects of interpolated extrasystole stimulation pattern on the extrasystolic and postextrasystolic isometric lusitropy in immature and adult rabbit right ventricle pappilary muscles. Using the programable stimulator and

mechano-electrical transducer, the following parameters were measured and evaluated: a) maximal isometric force (MG), b) maximal rate of isometric force decline during relaxation (-F), c) time required for the fall relaxation force to half of its peak value (R/2), d) ratio between maximal rate of isometric force decline and MG (-F/MG). The results were plotted as a function of T_e (time of interpolated extrasystole induction). While the adult myocardium exhibited a pronounced postextrasystolic potentiation (maximal MG in the range of short T_e), this feature was absent in newborn ones. The –F of extrasystolic beat in adult myocardium dramatically decreased with the prolongation of T_e. The newborn extrasystolic and postextrasystolic lusitropic parameters were relatively insesitive to the changes in T_e. The accordance between the age-related changes in lusitropic properties and the excitation-contraction coupling developmental pattern supports the hypothesis that the role of SR in both contraction and relaxation increases during the postnatal development.

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DIFFERENT CARDIAC RESPONSE TO ISCHAEMIA/ REPERFUSION INJURY IN NORMOTENSIVE AND HYPERTENSIVE RATS EXPOSED TO CHRONIC SOCIAL STRESS

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Chronic psychosocial stress increases a risk of impaired regulation of vascular function with subsequent development of hypertension and cardiac hypertrophy that might exert a negative impact on the outcome of myocardial ischaemia/ reperfusion (I/R) injury. On the other hand, adaptation to various stressful factors can modulate cardiac ischaemic tolerance. The study was designed: i. to compare the effect of chronic crowding stress (S) on the heart function of normotensive (WKY) and hypertensive (SHR) rats under normal conditions and ii. to characterize their response to I/R. S was induced by caging adult male rats 5 per cage (200 cm^2/rat, 8 weeks), while controls (C) were kept 4 per cage (480 cm^2/rat). S did not affect parameters of body and left ventricular weight in any group; on the other hand, it further increased blood pressure and reduced elevation of myocardial NOS activity in SHR. Neither hypertension nor crowding modified cardiac function evaluated in Langendorff-perfused hearts, however, both conditions substantially reduced coronary perfusion of the myocardium, and this effect was much more pronounced in S-WKY. Ischaemic tolerance was tested by subjecting the hearts to 25-min global ischaemia and 40-min reperfusion, when recovery of left ventricular developed pressure (LVDP) at the end of reperfusion and the incidence of lethal arrhythmias served as end-points of injury. Sustained ventricular fibrillation (SVF; lasting >2 mins) that occurred in none of the hearts from C-WKY group and total duration of ventricular tachycardia (VT; 30 ± 12 s) were significantly increased to 25% and 84 ± 22 s, respectively, in C-SHR group ($P<0.05$) coupled with a lower recovery of LVDP (14.8 ± 6.3% of pre-ischaemic value vs. 60 ± 1.5% in the C-WKY group; $P<0.05$). In the hearts of WKY rats, S exacerbated arrhythmias (SVF 40%, VT 70 ± 20 s) and impaired LVDP recovery (33 ± 6.3%; $P<0.05$ vs. C-WKY). In contrast, SVF was completely suppressed in S-SHR hearts, in conjunction with shorter duration of VT (22 ± 5 s; $P<0.05$ vs. C-SHR) and better recovery of contractile function (LVDP 36.5 ± 11%; $P<0.05$ vs. C-SHR). Conclusions: under normal conditions, neither hypertension nor stress themselves affect heart function substantially. However, response to ischaemia is altered in unstressed hypertensive rats. On the other hand, ischaemic challenge superimposed on the chronic psychosocial stress represents a higher risk of lethal arrhythmias and contractile failure in normotensive rats indicating a potential development of cross effect of adaptation in hypertensive individuals.

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CHANGES IN THE NITRERGIC NEURONAL POPULATION OF THE RAT´S HIPPOCAMPUS FOLLOWING NICOTINE AND KAINIC ACID ADMINISTRATION

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Using histochemical NADPH-diaphorase analysis, we studied the influence of intraperitoneal administration of nicotine (NIC), kainic acid (KA) and combination of both these substances on hippocampal neurons and their changes. In experiments, 18-day-old male rats of the Wistar strain were used. 30 minutes prior to the kainic acid application (10 mg/kg), animals were pre-treated with 1mg/kg of nicotine. After 2 days, 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 the CA1 and CA3 areas of the hippocampus, in the dorsal and ventral blades of the dentate gyrus and in the hilus of the dentate gyrus. In animals exposed only to nicotine the number of NADPH-diaphorase positive neurons in the CA1 and CA3 area of the hippocampus and in the hilus of the dentate gyrus was higher than in controls. In contrast, KA administration lowered the number of NADPH-diaphorase positive cells in all observed hippocampal areas except both blades of the dentate gyrus. In the CA3 area of the hippocampus the number of NADPH-positive neurons in rats exposed to both of substances were higher, when compared to control animals. These findings support the hypothesis, that nicotine as well as kainic acid act on neuronal nitrergic system.

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Na^+ ABSORPTION IN RAT COLON DURING EXPERIMENTAL COLITIS

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The inflammatory bowel disease (ulcerative colitis, Crohn's disease) is a disease characterized by intestinal inflammation and profound alterations of ionic transport, which result in diarrhea. Although the inflammatory mediators are predominantly prosecretory some data indicate that the response of the inflamed intestine to secretagogues is often depressed. However, diarrhoea may relate not only to an increase in electrolyte secretion but also to a decrease of electrolyte absorption and in healthy animals some of the inflammatory mediators cause not only increase of chloride secretion but also impairment of sodium absorption. The crucial mechanism of sodium absorption in rat colon is the electroneutral sodium absorption via Na^+/ H^+ exchanger, the isoform NHE3, localized in the apical membrane of colonocytes. The aim of this study was therefore to assess the changes of NHE3 mRNA expression in rat colon during acute colitis. Colitis was induced by intracolonic administration of trinitrobenzenesulphonic acid (TNBS) and NHE3 mRNA and the levels of pro-inflammatory cytokines interleukin-1beta mRNA and tumor-necrosis factor alpha mRNA by real-time quantitative RT-PCR seven days after TNBS administration. Using quantitative RT/PCR we have shown that NHE3 mRNA was markedly reduced in colonic mucosa of TNBS-treated rats in comparison with control animals. These data indicate that colonic electrolyte absorption has to be reduced significantly during experimental colitis.

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THE EFECT OF CORTICOSTERONE AND CORTICO TROPINE-RELEASING HORMONE (CRH) ON BEHAVIOUR OF RATS IN ACTIVE ALLOTHETIC PLACE AVOIDANCE (AAPA) TASK

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Corticoids are now widely used in clinical medicine. Higher levels of corticoids were reported in some diseases, as Cushing disease, depression, posttraumatic stress disorder and dementia of alzheimeric type. It has been shown long-time increased plasma levels of corticoids cause neurodegeneration in CNS, namely hippocampus. For this reason it is important to study how corticoids affect cognitive functions. In the present time, many studies of corticoids and CRH are published, but most of them use pharmacologic, endocrinologic and molecular views. Only a few papers paid attention to the effects of corticoids and CRH on behaviour and memory. We had four groups of Long-Evans rats. The A group was implanted with subcutaneous pellets continuously releasing high level of corticosterone. The C group was adrenalectomized and together with the B group was implanted with osmotic pumps releasing CRH into cerebral lateral ventricle. These groups modeled various dysregulations of hypothalamic-pituitary-adrenal (HPA) axis. The K group consisted of unoperated controls. All four groups were tested in a spatial task, called AAPA, which requires rats to remember location of a room frame-fixed sector on a circular arena; to neglect intramaze cues which provide irrelevant information. This newly developed behavioural task, requiring cognitive coordination, is sensitive to hippocampal impairment. We found that the administration of corticosterone alone as well as the CRH alone did not affect the rats' performance in the AAPA task. On the contrary, the behaviour of B group indicated a cognitive deficit. Together with corticosteroids, for long time elevated levels of CRH can participate on the changes of behaviour. We suggest that by means of behavioural methods, it is possible to study the role of CRH in HPA dysregulation and relationship between behavioural and structural changes in the brain.

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BLOOD PRESSURE VARIABILITY IN PATIENTS WITH CARDIAC TRANSPLANTATION

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Background: Cardiac component of the baroreflex is responsible for attenuation of blood pressure fluctuations in the range of frequencies 0.04 – 0.15 Hz (low frequency, LF). Deinervation of the heart in patients after orthotopic cardiac transplantation (OTC) eliminates this mechanism.
Aim: The aim of the study was to compare Low Frequency – blood pressure variability in patients after OTC with healthy controls.
Methods: We examined 7 patients (age 55.7±9.7 years) after 2-8 years after cardiac transplantation. ECG, blood pressure (BP) and thoracic impedance were recorded beat-by-beat during 20 minutes (Task Force Monitor, CNSystem, Austria) in supine position during spontaneous breathing and breathing controlled by metronome (5 min, 0.33 Hz).The results were compared with the examination of the group of 7 healthy subjects (C) of similar age (50.0±2.8 years).
Results: Both groups did not differ (OCT versus C, mean ± SD) in heart rate (80.4±10.8 versus 72.2± 6.9 beats/min), in systolic (119.4±11.8 vs. 124.9±11.0 mmHg) and diastolic BP (80.6±10.3 vs. 85.7±8.3 mmHg), in stroke volume index (32.6±8.9 vs. 39.9±6.6 ml/m^2), in cardiac index ((2.54±0.55 vs. 2.90±0.58 l/(min.m^2)) and in total peripheral resistance index (2863±552 vs. 2754±845 dyn.s.m^2/cm^5). On the other hand heart rate variability spectra (ms^2) was decreased in OTC (LF heart rate variability: 8.43±12.09 vs.164.29±171, $p<0.01$). No difference was seen in diastolic BP variability spectra (mmHg^2): LF dBP (5.00±8.82 vs.3.10±1.94, n.s.).
Conclusion: It is concluded that LF dBP variability is unchanged in patients after OTC despite of the deinervation of the heart.

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THE ROLE OF MITOGEN-ACTIVATED PROTEIN KINASES IN PROCESSES ASSOCIATED WITH DEVELOPMENT OF DOXORUBICIN CARDIOMYOPATHY

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Doxorubicin (Dox) is a type of anti-cancer drug, that induces apoptosis of cardiomyocytes, causes chronic cardiomyopathy and contributes to the development of myocardial dysfunction and heart failure. Although the mechanisms of doxorubicin-induced cardiomyopathy is multifactorial, increased oxidative stress due to overproduction of free radicals and antioxidant-deficit play an important role. In addition, cardiac cells apoptosis has been suggested to be associated with Dox cardiomyopathy. Mitogen-activated protein kinase (MAPK) signaling pathways are the primary intermediator of induction of apoptosis by oxidative stress. The study has been designed to characterize the protein systems involved in the mechanisms of Dox-induced cardiomyopathy. Our further goal was to look for the changes in protein system of MAPKs and to define the involvement of some stress inducible proteins (hsp) in Dox-induced cardiomyopathy. Doxorubicin was administered to rats by intraperitoneal injections over a period of 6 weeks (cumulative dose of 15 mg/kg). The tissue samples were obtained from saline-treated and Dox-treated animals at the end of the application period. The protein pattern was determined after electrophoretic separation followed by silver staining. The levels and phosphorylation state of specific proteins were determined by ECL-Western blot analysis. The levels of proteins connected with glutathion detoxification system were determined electrophoretically after separation using glutathion-Sepharose chromatography. Protein studies revealed significant differences in the pattern and amount of some proteins between control and Dox-treated hearts. Further analysis showed significant changes also for the proteins bound to the glutathion-Sepharose. The chronic exposure of rats to Dox was connected with increased activation of ERK1/2 and up-regulation of the levels of Hsp 60. On the other hand, Dox induced a down-regulation of protein levels of hsp70. The results show that the development of Dox cardiomyopathy in rats is associated with modulation of protein expression. The data point to the important role of ERK signaling pathway and heat stress proteins in mechanisms underlying the development of doxorubicin-induced cardiomyopathy.

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DOPAMINERGIC REGULATORY SYSTEM IN THE RAT HEART ATRIA

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Dopamine (DA), via different receptor subtypes, regulates cardiovascular functions by actions on the central and peripheral nervous systems, vascular smooth muscle, the heart and the kidney (1). It is generally accepted, that in the heart, DA is synthesised in the postganglionic sympathetic neurones, where it serves mainly as a precursor of norepinephrine (NE).
In our study, we investigated the putative existence of NE-independent dopaminergic regulatory system in the heart atria. Newborn rats were randomly divided into 3 groups: GUAN and 6HD (rats sympathectomized by administration of guanethidine, 50 mg/kg/day on postnatal days 1-21 or 6-hydroxydopamine, 100 mg/kg/day on postnatal days 1-7, 14, 21 and 28, respectively), and controls (CONT). At the age of 20 and 40 days, DA and NE concentrations were measured in the

heart atria by radioimmunoassay, atrial whole-mount preparations were subjected to immunohistochemistry for tyrosine hydroxylase (TH), dopamine β-hydroxylase (DβH), NE and DA transporters (NET and DAT, respectively), and chronotropic effect of DA was evaluated on the isolated spontaneously beating right atria. In addition, the expression of TH mRNA by reverse transcription-polymerase chain reaction (RT-PCR) and concentration of DA were determined in HL1 cells derived from mouse atrial myocytes. After either type of sympathectomy, NE levels in the atria decreased to 2-5% of control values, whereas DA concentrations were ~40% and ~65% of CONT in 6HD and GUAN atria, respectively. Immunohistochemistry of GUAN and 6HD atria showed intact TH-positive small intensely fluorescent (SIF) cells displaying also DAT immunoreactivity. In the CONT right atria of 20-day-old rats, DA had no effect the spontaneous beating rate (180±11 min^{-1}), whereas in the atria of 6HD rats, DA significantly decreased the beating rate in a concentration-dependent manner (10^{-8}-10^{-5} mol/l). HL1 cells expressed TH; DA concentration in these cells reached ~2ng/g. In conclusion, in the heart atria, at least two NE-independent pools of DA seem to exist: dopaminergic SIF cells and atrial cardiomyocytes.

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MERCURY INDUCED ALTERATIONS IN RAT KIDNEYS

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Mercury is a naturally occurring element found in air, water and soil. It exists in several forms – elemental or metallic, inorganic and organic mercury compounds. All forms of mercury may cause kidney damage if significantly large amounts enter the body. Kidney is sensitive to the effects of mercury, because mercury accumulates in the kidney and higher exposure to the tissues causes more damage. The aim of the present study was to detect effects of mercury on the kidney structure of adult rats. Rats received mercury (as $HgCl_2$) in single intraperitoneal doses of 20 mg $HgCl_2$ (group A), 10 mg $HgCl_2$ (group B) and 5 mg $HgCl_2$ (group C) per kilogram of body weight respectively and were killed 48 hours after mercury administration. After the preparation of histological samples the results were compared with the control group (K). Results suggest that kidneys of experimental rats have a typical bean shape with soft surface. Qualitative microscopic analysis of kidney revealed structural alterations characterized mainly by reduced diameter of glomeruli and renal corpuscles, damaged tubules with affected quality of tubular cells and diapedesis (infiltration of interstitium by erythrocytes). Quantitative analysis showed decreased relative volume of interstitium in all the experimental groups, significantly in group C. Relative volume of tubules was increased in all the experimental groups. The diameter of glomeruli and diameter of renal corpuscles decreased significantly in all experimental groups in comparison to the control group. However, the number of glomeruli per constant area significantly increased in group A and B. The number of nuclei per constant area also significantly increased in all the groups of animals in comparison to the control group. The height of tubular epithelium showed significantly decreased tendency in the animals of group B and C. This study demonstrates negative effects of inorganic mercury on the structural and functional parameters of kidney, which is the most important place of filtration of blood and production of urine.

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ANALYSIS OF COUPLING OF M_2 MUSCARINIC ACETYLCHOLINE RECEPTORS TO $G_{I/O}$, G_S AND G_Q HETEROTRIMERIC GTP-BINDING PROTEINS

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We have shown recently in our laboratory that activation of individual subtypes of muscarinic acetylcholine receptors leads to changes in several second messenger pathways via coupling to different G-protein subtypes (1,2). To study interaction between muscarinic M_2 receptor and different G-proteins in detail we adopted a scintillation proximity assay (3). We show that under identical conditions different agonists activate different sets of G-protein subtypes. The extent of activation of G_s and G_q G-proteins does not correlate with the magnitude of stimulation of the preferentially coupled G-protein, $G_{i/o}$. On the other hand, it correlates with the magnitude of allosteric interaction between agonist and GDP on the receptor-G protein complex. We conclude that conformations of the M_2 receptor induced by interaction with agonists are agonist specific and differ in interaction with G-proteins.

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THE LEVELS OF AMINO ACIDS IN PORK MEAT OF ALTERED QUALITY

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Qualitative alterations in pork – PSE (pale, soft, exudative) - are an accompanying feature of intensive breeding. Changes in internal biological conditions lead to an increased sensitivity of high yield pigs to stress. PSE meat is characterised by rapid glycolysis. Interaction of increased temperature in qualitatively changed PSE muscular tissue and its increased acidity results in denaturation of muscular proteins (1). Our investigations focused on changes in nutritional characteristics (total proteins and amino acids) of normal quality pork and pork with abnormal course of ageing and on subsequent qualitative changes - PSE. The following methods were used to identify PSE meat (n=6): - pH determination ($pH_{1hod.}$ and $pH_{24\ hod.}$ p.m. - *post mortem*); - colour radiance determination; - loss of meat juice determination. Quantitative determination of total amino acids was carried out after acid hydrolysis at 110ºC during 24 hours (2). According to our findings regarding total proteins and amino acids, the nutritional value of PSE pork meat is not lower than that of qualitatively unchanged pork. The results showed generally higher values of essential isoleucine, threonine, valine, leucine and phenylalanine, non-essential tyrosine, glutamic acid, cystine, aspartic acid and alanine in the fresh musculature of pigs with PSE meat. The finding was confirmed by the sum of essential amino acids in the PSE meat and correlated with significantly higher values of total proteins in the PSE meat. An increased secretion of somatotrophic hormone (STH), which is caused by dopaminergic stimulation (3, 4), is a part of stress reaction. STH stimulates synthesis of nucleic acids and thus supports significantly the proteosynthesis which may serve as evidence of positive nitrogen balance in the organism of stressed animals.

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RELATIONSHIP BETWEEN TISSUE LIPID PEROXIDATION AND TISSUE CONCENTRATION OF COPPER, CADMIUM, MERCURY AND LEAD IN SHEEP

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Heavy metals have been reported to affect health status both in human and animals. Long-term occupational exposure to mercury is one of the risk factors for increased lipid peroxidation in miners (1). Peroral administration of mercuric chloride increases lipid peroxidation in the liver and kidney tissue of Japanese quails (2). Chronic exposure to heavy metals alters the ruminal enzyme activity of sheep (3, 4). The aim of the study was to determine the effect of heavy metal intake by diet on the malondialdehyde (MDA) level in the tissue of gastrointestinal tract, kidney and liver of sheep. The experiment was carried out on twelve female sheep of mixed breed, weighing from 30 to 35 kg. Six sheep in the first group were from a laboratory farm and were fed with hay *ad libitum* and 300g of barley for one day. The second group was grazed on natural pasture in a territory near a copper production works. Mercury, copper, cadmium and lead concentrations were determined by atomic absorption spectrophotometry. The second group of sheep had significantly higher concentration of copper and cadmium in the tissue of rumen wall, duodenum, colon wall, liver and kidney. Lead concentration was significantly higher in the rumen wall, colon, liver and kidney, but not differences were found in duodenum. Mercury concentration was significantly higher only in kidney tissue. Malondialdehyde concentration was significantly higher in reticulum epithelium, omasum, duodenum, colon, colon wall and kidney medulla. The highest concentration of MDA was found in liver. Our results show that the lipid peroxidation is one of the molecular mechanisms of cell injury in chronic heavy metal poisoning.

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ARE THERE ANY DIFFERENCES IN THE EXPRESSION OF MYOSIN HEAVY CHAIN ISOFORMS BETWEEN SLOW AND FAST RAT SKELETAL MUSCLES?

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Skeletal muscles of small rodents contain four main fiber types, namely type 1, 2A, 2X/D and 2B fibers containing myosin heavy chain (MyHC) 1, 2a, 2x/d and 2b isoforms. Each of these MyHC isoforms is the product of a distinct gene and their expression is believed to be primarily transcriptionally controlled. In most rat muscles, messenger *RNA (mRNA)* transcripts for MyHC1, 2a, 2x/d and 2b and their corresponding protein products were found with the exception of the slow antigravity soleus (SOL) muscle, where typically only MyHC1 and 2a transcripts and protein isoforms were demonstrated under normal conditions. We have shown that both fast extensor digitorum longus (EDL) muscle and slow SOL muscle express all four MyHC1, 2a, 2x/d and 2b *mRNA* transcripts under normal conditions in euthyroid, as well as in experimental hypothyroid and hyperthyroid (i.e. after chronic treatment with methimazole and T_3, respectively) 2- to 7-month-old female inbred Lewis rats (1-4). In the SOL muscle, this is not matched, however, by the expression of corresponding four isoforms, as we have found that 2x/d and 2b protein isoforms are not normally present at levels detectable by SDS-PAGE. We have also shown that the chronic hypothyroid and hyperthyroid status affects the expression of MyHC isoforms both at the *mRNA* and protein levels. From our results we can conclude that alteration of the thyroid status leads to typical changes in the expression of MyHC mRNA transcripts and MyHC protein isoforms in the EDL and SOL muscles, due to the different fiber type composition based on different physiological demands of either muscle. These changes correspond to those described after shorter periods of altered thyroid status. The characteristic phenotype differences between EDL and SOL muscles remain thus preserved even after 7 months of thyroid hormone status alteration.

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QUALITATIVE AND QUANTITATIVE CHANGES IN THE OVIDUCTS OF EWES AFTER HORMONAL STIMULATION

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Qualitative and quantitative histological changes in the ovarian oviducts of slovak merino ewes were studied in the anoestrous period after treatment by Oestrophan and hormonal stimulation. Observations were studied in 40 ewes, 2 – 4 years, the mean weight 40 – 50 kg. The synchronization of ovaries was achieved by administering of Oestrophan ($PGF_2\alpha$ at a dose of 250 ng/head on days 1 and 11). Hormonal stimulation for superovulation was provided by treatment with 750, 1000 and 1500 IU PMSG. The animals were killed approximately 120 h after the application of the hormone. Samples from their oviducts were processed by the common histological methods for examination under a light microscope and for examination under a scanning electron microscope. We observed that the influence of PMSG in the anoestrous period increased the number of newly formed corpus luteum, i.e. the highest number was observed in ewes stimulated with 1500 IU PMSG. The rinsing of the gonadal apparatus of the sheep of the latter group provided the highest number of ova per ewe sheep. The hormonal stimulation resulted in a significant increase in the weight of uterine cervices and uteri, the number of glands, the height of their epithelia and the height of the cervical surface epithelia also increased significantly (3, 4). The administration of serum gonadotrophins used to induce superovulation in ewes increased the contact surface, caused multiplication of cilia in part of oviduct isthmus. There are also tertiary protuberances not only secondary ones as in the control. The ampular epithelium is more frilled and the cilia were higher. The infundibular surface was also in addition covered by a fold with cilia well-groups in tufts and there are a lot of spherical protrusions. From these results it follows that the hormonal preparation used in the anoestrous period to stimulate seperovulation became ovident also in the changes of the oviducts as in the natural oestrous period (1, 2).

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THE INFLUENCE OF CADMIUM IONS ON CADMIUM AND LEAD ACCUMULATION IN THE ORGANISM OF PHRYNOHYAS RESINIFICTRIX

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The studies aimed to detect cadmium accumulation in larval *Phrynohyas resinifictrix* and its relationship with lead accumulation. The studied material consisted of 105 live tadpoles of *Phrynohyas resinifictrix*. The larvae were divided between 3 experimental groups. Group I consisted of control animals grown in clean water. Group II contained larvae exposed to Cd^{2+} concentration of 1 mg/l. Tadpoles from group III were exposed to Cd^{2+} in concentration of 2 mg/l. The lead level was constant in water of all experimental groups (0.3 mg/l). After various time of exposure i.e. 2, 4, 6, 8 and 16 hours and 1 and 2

weeks 5 tadpoles from each group were taken and submitted to Cd and Pb accumulation analysis. The Cd and Pb were measured with AAS technique. Results were expressed in micrograms per gram of dry weight. Statistical analysis was performed using Friedman ANOVA, Kendall coefficient and Mann-Whitney U test. The studies indicated that the differences concerning tested elements between consecutive groups were statistically significant. Cd ions can influence on metabolism speed it up or lower it or even curb it completely. Significant increase of Cd content inside the cell decrease speed of metabolism but low dose of Cd can act as an enzyme activator. The results of our investigation indicate that cadmium ions affect the lead accumulation and processes are dependent on many different factors – in particular from duration of cadmium ions influence on organism. The lead content changed significantly after one week of living in the experimental conditions. The increase of their content is probably connected with cadmium elimination.

LOCALIZATION AND QUANTIFICATION OF MT2 RECEPTORS IN GASTROINTESTINAL TRACT OF RAT

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Melatonin receptors MT1 and MT2 are members of the superfamily of G-protein coupled receptors. The melatonin receptors are activated by hormone melatonin, which is secreted primarily by the pineal gland at the night. In addition to synchronization of biological rhythms and protective action, melatonin can modulate some functions in the gastrointestinal tract (GIT) (1, 2). Melatonin is present in the gut but there are limited data about its receptors in this system.
The aim of our study was to investigate MT2 receptors in tissues of stomach, duodenum and colon of Wistar rats in the middle of the day and night. The MT2 receptors were determined by immunohistochemistry and quantified by Western blotting analysis.
The florescence signal was high in the mucose and submucose layers and present also in muscularis layers. No signal was detected in the epithelial layer of the tissues. The highest level of MT2 receptors was found in the cerebellum as a positive control and in the distal part of GIT (colon). Stomach exhibited lower levels and the lowest levels of MT2 were found in duodenal tissues. There were no significant differences between day and night in MT2 in all analyzed tissues. Our study demonstrates presence of MT2 receptors in GIT. This finding suggests a physiological role of melatonin in gut, especially in colon.

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ROLE OF PARIETAL CORTEX AND HIPPOCAMPUS DURING AVOIDANCE OF A MOVING OBJECT IN RATS

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There is now substantial evidence from rodent studies showing dorsal hippocampus (DH) involvement in spatial processing, particularly during navigation based on the use of distal landmarks, eg. in water maze (1). The role of parietal cortex (PC), however, is far from fully understood. Spatial deficits observed after PC lesions are only mild in contrast to lesions of DH. Recent studies suggest that PC may play its major role in tasks in which navigation is based on the use of proximal landmarks (2). This might support findings from monkies and humans which assigned to PC function in coding spatial properties of the landmarks within a frame related to the body of the subject (egocentric frame). Moreover, PC may use other reference frames, including object-centered reference frame. Since most of tasks examining spatial behavior in rodents takes place in stable environments, our aim was to assess role of DH and PC in environment containing a dynamic element (i.e. moving proximal landmark). For this purpose a new behavioral paradigm has been recently introduced (3). In Robot Avoidance Task (RAT) rats were trained on a dry circular arena (d=85cm) to keep a minimal safety distance (25cm) from a moving, programmable robot. The robot was programmed to move straigth forward (15cm/s) until it hit the wall, then it waited about 15s, turned 180 ± 0 to 90 degrees, and ran again. Rats with PC lesion (n=8) showed no impairment in acquiring this task when compared to controls, in fact the acquisition was enhanced during first two sessions. On the contrary, well-trained animals (n=9) with cannuli aimed to DH (AP -4.5, ML ±3, DV -3) displayed a markedly worse performance ($P<0.001$) during temporarily blocking DH function by tetrodotoxin. However, this temporal inactivation had no effect on distance estimation. These results suggest that PC is not involved in avoidance of a dynamic object, while DH plays an important role during execution of the escape reaction but not in estimation of the distance from the moving object.

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LOW CONCENTRATION OF ISOFLURANE CAUSES NEUROGENIC PULMONARY EDEMA IN SPINAL CORD INJURED RATS

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The role of isoflurane in the development of neurogenic pulmonary edema was examined in 131 male Wistar rats using 1.5%, 2%, 2.5%, 3%, 4% or 5% isoflurane anesthesia in air. Epidural balloon compression of the spinal cord at the Th8 level was performed. The development of neurogenic pulmonary edema was examined using X-ray imaging, an examination of subpleural bleeding, the pulmonary index and histological evaluation of the lung tissue. In animals anesthetized with 1.5% or 3% isoflurane, neurological outcome was monitored using the BBB and plantar tests for 7 weeks postinjury, while morphometric analysis of the volume of spared white and grey matter was performed and the spinal cord examined using MRI. X-rays, the pulmonary index and the histological picture revealed a massive pulmonary edema in all animals from the 1.5% and 2% groups. Almost 42% of the 1.5% isoflurane group animals died of severe pulmonary hemorrhage and suffocation. Only 29% of animals from the 2.5 % and 3% groups developed low-grade pulmonary edema; no animal died. All animals from the 4% and 5% groups died of anesthesia overdose. Animals anesthetized with 3% isoflurane recovered behaviorally significantly more rapidly than did the animals anesthetized with 1.5% isoflurane, but at 4 weeks the recovery rate was comparable. Morphometric measurements after 7 weeks survival and MRI showed no differences in the lesions. We conclude that 1.5 - 2% isoflurane anesthesia causes neurogenic pulmonary edema in rats with an experimentally compressed spinal cord. The optimal concentration of anesthesia for performing a balloon-induced spinal cord compression lesion is between 2.5 – 3% isoflurane in air. Neurogenic pulmonary edema significantly complicates the recovery of rats with spinal cord injury.

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EXCITATION INDUCED SPATIAL AND TEMPORAL CHANGES OF [Ca^{2+}] IN CARDIAC T-TUBULES

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The transverse-axial tubular system (TATS) represents a complex restricted diffusion space. Majority of L-type calcium channels are located in TATS membrane, where they are inhomogenously distributed in clusters within dyads. This may have important consequences for distribution of intra-luminal Ca^{2+} concentration within the TATS lumen ($[Ca^{2+}]_t$) and indirectly for cellular Ca^{2+} homeostasis. To calculate distribution in space and time of $[Ca^{2+}]_t$, we proposed a model described by partial differential equations. The equation of Ca^{2+} diffusion was accompanied by equation of calcium binding in the membrane surface and/or in the lumen of cylindrical (254 nm diameter) T-tubules. The aim of numeric computations (software MATLAB, version 6) was to evaluate the effect of tubular Ca^{2+} buffering and of inhomogeneity in Ca channel distribution on $[Ca^{2+}]_t$ distribution. The model responded to sudden increase in bulk extracellular $[Ca^{2+}]$ from 0 to 1 mmol/l in a way that depicted well the reported wave-like gradient of $[Ca^{2+}]$ propagated along the T-tubules (1). The velocity of propagation decreased strongly with the increase of T-tubular length and varied between 5 and 60 μm/s in the middle of 5 to 30 μm long T-tubules. If only two separated clusters of Ca^{2+}-channels were considered along the T-tubule, non-homogeneous Ca^{2+}-depletion was prominent during the first 60 ms which, due to the effect of diffusion and Ca^{2+}-buffering, became negligible after 150 ms following I_{Ca}-activation. If, however, the clusters of Ca^{2+}-channels were distributed in agreement with realistic distance between dyads (420 nm) the irregularities of Ca depletion practically disappeared even in the absence of Ca buffers. For comparison, the simulation was repeated assuming uniformly distributed I_{Ca} transferring the same electrical charge across the t-tubular membrane. The distribution of Ca^{2+} depletion with depth along the t-tubule was practically the same as in the case of clustering of channels within dyads. Greater local depletions in the regions adjacent to dyads were compensated by lesser depletion in the neighbouring segments and the irregularities were rapidly equalized by diffusion. We conclude that the observation of slower velocity of Ca^{2+} diffusion along TATS lumen (related to expected velocity considering diffusion coefficient of bulk extracellular medium) can be explained at least partly by Ca binding within TATS. The inhomogeneities in Ca^{2+}-channel distribution in TATS due to clustering within dyads has little effect on spatial and temporal changes in $[Ca^{2+}]_t$ induced by excitations.

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RATE CONSTANTS OF OPEN CHANNEL BLOCK OF CARDIAC TRANSIENT OUTWARD CURRENT

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The interaction of ionic channels with biologically active substances (particularly channel blockers) depends generally on membrane voltage and channel state. This study was focused on quantitative description and modelling of the block of transient outward current (I_{to}) in open channel state. The main objective was to elaborate an improved approach to the assessment of association and dissociation rate constants (k_{on}, k_{off}) of drug-channel interaction from analysis of concentration-dependent drug-modified currents. The effect of open channel block is characterized by modification of apparent inactivation. Single exponential I_{to} inactivation often observed in drug free conditions (time constant τ_i) is converted into double-exponential time course (time constants τ_f and τ_s). The rate constants k_{on}, k_{off} are usually calculated from approximate formula (1) based on linear relationship between the drug concentration c and reciprocal of τ_f [1]. Analysis of a kinetic diagram of I_{to}-channel gating supplemented by drug-channel interaction in the open channel state led us to derivation of an improved formula (2) enabling more precise calculations of association and dissociation rate constants. The new

$$1/\tau_f = k_{off} + ck_{on} \quad (1) \qquad 1/\tau_f + 1/\tau_s - 1/\tau_i = k_{off} + ck_{on} \quad (2)$$

formula was verified by simulations on a quantitative 9-state model of drug-channel interaction described by a set of differential equations. Numeric values of k_{on} and k_{off} were used in agreement with published data for different drugs. Simulations of voltage clamp experiments were performed using the numeric computation software MATLAB, version 6 (The Math Works, Inc.). Computed I_{to} in the phase of apparent inactivation were fitted to exponentials and the resulting time constants were used to plot left sides of equations (1) and (2) against drug concentration c. The points plotted according to (1) revealed considerable differences from a straight line in the low concentration range and the best fit to linear function led to 90 % and 5 % overestimation of k_{off} and k_{on} at average, respectively. The points plotted according to (2) presented linear function perfectly and the error in estimation of both rate constants did not exceed 3 % under the following two conditions: activation of I_{to} must be much faster than the fast phase of apparent inactivation and full inactivation must be achieved during the imposed test depolarizing pulses.

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DIFFUSION IN THE RAT CEREBRAL CORTEX DURING STATUS EPILEPTICUS AND HYPOXIA-ISCHEMIA

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The objective of the present work was to compare the diffusion parameters in the brain cortex of adult rats during pilocarpine-induced status epilepticus and during and after hypoxia-ischemia. Status epilepticus was induced in urethane anesthetized and artificially ventilated adult male Wistar rats by the administration of pilocarpine (300 mg/kg, i.p.). To potentiate the effects of pilocarpine, lithium chloride (127 mg/kg, i.p.) was given to the animals 14-18 h before the experiment. Hypoxia-ischemia was induced by ventilation with 10% O_2 in N_2 plus unilateral carotid artery occlusion for 30 minutes. The real-time iontophoretic method using tetramethylammonium-selective microelectrodes was used to measure the ECS volume fraction (α) and tortuosity (λ). The mean values of α and λ before the application of pilocarpine were α=0.19 ± 0.004 and λ =1.58 ± 0.01, (n=7, mean ± SEM). Following pilocarpine administration, there were no significant changes in λ. The volume fraction started to decrease several minutes after the administration of pilocarpine, reaching a minimum (0.13 ± 0.01) 80 - 100 minutes later. At 120 minutes, α started to increase and reached 0.18 ± 0.01 four hours after the administration of pilocarpine. During hypoxia/ischemia, α decreased from 0.19±0.01 (n=12, mean±SEM) to 0.07±0.03 and λ increased from 1.55±0.01 to 1.88±0.03. During 90 minutes of reperfusion α increased to 0.23±0.03, while λ decreased to original values (1.53±0.04). We conclude that pathologically increased neuronal activity during pilocarpine-induced seizures causes cell swelling followed by a long lasting reduction in the ECS volume fraction. Compared with status epilepticus, changes in the diffusion parameters during hypoxia-ischemia are more pronounced, with a greater decrease in volume fraction accompanied by a concomitant increase in tortousity.

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ANXIOGENIC EFFECT OF METHAMPHETAMINE IN THE TEST OF SOCIAL INTERACTION IN ADULT MALE RATS

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Amphetamines are known to affect human behavior in serious manner; specifically they induce aggressive behaviors and impair social interaction. Results of experimental studies are, however, not so clear. There are studies showing both increasing and decreasing effects of amphetamines on social interaction in rodents. The aim of the present study was to assess the effect of low doses of methamphetamine (MA) on social interaction in adult male rats. Rats were tested in different stress conditions: low stress (dimly lit, known environment), medium stress (dimly lit, unknown environment) and high stress (intensely lit, unknown environment). In each stress condition different set of animals was used. Rats were always divided into five groups. Control (without injection), Saline (with 1ml/kg saline injection) and three MA groups (doses: 0.5; 1 and 1.5 mg/kg). Injections were applied 30 minutes prior to testing and animals were kept for 30 minutes in separate cages in a dimly lit room. Always two strange rats of the same treatment group were tested. Their behavior was recorded for 5 minutes in an open field by using camcorder. Data were analyzed by ODLog software. Times spent in social interaction, locomotion and comforting behavior were analyzed using a One-way ANOVA (drug treatment) for each condition separately. Our data demonstrate that MA dose dependently decreases social interaction, while does not change comforting behavior. Locomotion was increased only after application of the highest dose of MA (1.5 mg/kg). There was no stereotypic behavior after using these small doses of MA. While intensity of social interaction is often associated with anxiety in rats, our results also suggest that MA may have anxiogenic effect.

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THE EFFECT OF CHRONIC NOS INHIBITION ON ACTIVITY OF REGULATORY PROTEINS IN RAT HEARTS

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Intracellular signaling mechanisms mediated by NO involve regulation of expression and activities of proteins involved in remodelation of extracellular matrix, matrix metalloproteinases (MMP). Our aim was to characterize the effects of chronic NO synthase inhibition by L-NAME treatment on the MAPK cascades and alterations of MMPs and its tissue inhibitor (TIMP). NO deficiency (NOD) was induced by L-NAME (40/mg daily, 4 weeks). Activities of MMPs from serum and tissue samples were analyzed by zymography in gels containing gelatine as a substrate and protein levels of MAPKs, MMPs and TIMP-2 proteins were determined by Western blot analysis using specific antibodies. We found that the development of NOD was connected with inhibition of ERK pathway characterized by decreased phosphorylation (activation) of ERKs, down-regulation of aFGF and H-Ras protein levels. In hearts from rats with NOD we found also decreased activities of tissue MMP-2. Analysis of serum metalloproteinases showed that gelatinolytic activity of approximately 20 kDa proteinase was increased in serum of L-NAME-treated rats. Results of specific immunoprecipitation assay and Western blot analysis indicate that changes in serum proteinase activities are not connected with decrease in activities of tissue MMP-2. Moreover, the protein contents of MMP-2 in the ventricular tissue and serum were not different in the control and L-NAME-treated hearts. We found that L-NAME treatment was associated with decrease in levels of tissue MMP inhibitor TIMP-2. The results point to the role of ERK signaling pathway and proteins involved in remodeling of extracellular matrix (MMP-2) in cardiac responses connected with development of chronic NOD.

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EFFECTS OF HEMOFILTRATION AND OF HEMOFILTRATE ON ELECTRICAL PROPERTIES OF HEARTS IN PIGS WITH HYPERDYNAMIC SEPTIC SHOCK

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Severe sepsis and septic shock represent a major cause of morbidity and mortality in intensive care units. Continuous hemofiltration has been suggested as possible therapeutic option that may remove inflammatory mediators. On the other hand, hemodialysis and hemofiltration were reported to influence cardiac electrophysiological parameters and to increase the arrhythmogenic risk. Therefore, in this study we have investigated the effects of hemofiltration on electrophysiological properties of the septic pig heart.

30 pigs of both sexes, in which the sepsis was induced by fecal peritonitis, were divided into 3 groups: 1) standard septic group with 24 hours-maintained sepsis; 2) group with conventional hemofiltration (CH, 35 ml/kg/h), CH applied for the second 12 hours of the sepsis; 3) group with high volume hemofiltration (HVH, 100 ml/kg/h), HVH applied for the second 12 hours of the sepsis. ECG was measured just before and after 24 hours period of sepsis. Action potentials were recorded in isolated ventricular preparations obtained from the hearts at the end of experiment.

Sepsis significantly shortened RR and QT intervals in all 3 groups. Action potential duration (APD) was at slow pacing rates significantly longer in standard septic group than in CH and HVH groups. Hemofiltrate from both CH and HVH prolonged significantly and reversibly APD at all pacing rates. Substitution solution alone had no effect on APD. Hemofiltrate obtained from control non-septic group did not influence the action potential of isolated ventricular preparation at all.

We conclude that the hemofiltration and the septic hemofiltrate influence significantly the electrophysiological properties of the septic heart, probably due to removal/content of various inflammatory mediators in the septic hemofiltrate.

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DO HIPPOCAMPAL CONCENTRATIONS OF EXCITATORY AMINO ACIDS IN ANIMAL MODEL OF PSYCHOSIS PREDICT THEIR LEVELS IN A SCHIZOPHRENIC BRAIN?

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Kim et al. (1) first reported decreased glutamate (GLU) levels in cerebrospinal fluid (CSF) from patients with schizophrenia (SCZ) but subsequent studies have been inconsistent as for the GLU concentrations in serum, CSF or in brain tissue (2, 3). To solve the inconsistencies we used an animal model of SCZ based on neonatal lesioning of the hippocampus by quinolinic acid (QUIN). Infusion of QUIN (250 nmol into each lateral cerebral ventricle) to 12-day-old rats led to a 15% decrease in tissue concentrations of GLU (but not that of aspartate; ASP) on day 50. In parallel, hippocampal microdialysis revealed a decrease in basal GLU release which was more pronounced in perfusates collected from ventral parts of the right hippocampus (comparing to controls). Although concentrations of ASP in the hippocampal perfusates exhibited a certain degree of laterality in controls, in the QUIN-treated animals the differences in dialysates slowly decreased to below the sensitivity of ASP detection. As little is known on whether antipsychotics can improve the low levels of GLU/ASP in a SCZ brain (4), we injected a dose of typical (haloperidol; 0.1 mg/kg b.w., s.c.) and/or atypical antipsychotic (clozapine; 5 mg/kg b.w., s.c.) to increase the low GLU levels in dialysates from the ventral hippocampi of 50-day-old rat males treated with QUIN. The acute administration of haloperidol and/or clozapine appeared to increase a basal GLU release in ventral hippocampi of rats with modelled psychosis. In the case of ASP the acute administration of these

antipsychotics had no significant influence on its concentrations in the hippocampal dialysates, but clozapine elevated the ASP levels to detectable rates in almost all dialysates during 6-h microdialyses. In the used model of psychosis, cognitive functions were impaired in parallel to a decline in GLU/ASP in the hippocampal interstitial fluid while antipsychotics improved the extracellular levels of excitatory amino acids as well as psychotic symptoms.

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EXTRACTION METHOD OF HIGH-QUALITY RNA FROM ENDOSCOPIC BIOPSIES

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Biopsy sampling and subsequent molecular analyses besides histological examination have become a fundamental part of clinical research in the field of inflammatory and neoplastic gastrointestinal lesions. For performing quantitative gene expression studies, the quality of extracted RNA is of paramount importance. Endoscopic mucosal biopsies are limited not only with respect to yield but especially to integrity of RNA molecules. To determine the expression of pro-inflammatory and neoplastic markers we developed a method for identification and quantification of transcript levels in human biopsies. During colonoscopy of ten patients (four with ulcerative colitis, two with Crohn disease and four controls), thirty mucosal biopsy specimens were collected in 1ml RNA-later (Ambion) and stored at -80°C until use. Transferred tissue was disrupted by one run in the MagNA Lyser Instrument (Roche) and total RNA was extracted using a GenElute Mammalian Total RNA kit (Sigma-Aldrich) according to the manufacturer's instructions. To assess the integrity and concentration of isolated total RNA by microcapillary electrophoresis, the Agilent 2100 bioanalyser was used in conjunction with the RNA 6000 Nano LabChip kit. Synthesis of cDNA was performed in 20µl reaction using random hexamer primers and MMLV reverse transcriptase (Invitrogen). Real-time quantitative RT-PCRs for cyclooxygenase-2 (*COX-2*) and TATA-binding protein (*TBP*) were carried out by standard fluorogenic 5' nuclease assay using the ABI PRISM 7000 sequence detector (Applied Biosystems). The results showed RNA Integrity Number (RIN) in the range between 7,5 and 9,8 (average value 9,06), indicating excellent RNA integrity. The level of transcripts for COX-2 mRNA, a marker of inflammation, was the highest in the biopsies from the areas of histologically proven high inflammatory activity. This simple and rapid method for isolation of high quality RNA from endoscopic biopsies along with prerequisite RNA integrity control ensures quality input for downstream transcriptomic applications which are often expensive and time-consuming.

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PARASYMPATHETIC INNERVATION OF THE HEART IN A RAT MODEL OF CHRONIC RENAL FAILURE

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Chronic renal failure (CRF) is associated with a high risk of sudden cardiac death due to atherosclerosis, hypertension and dysfunction of the autonomic nervous system, which includes the alteration of both sympathetic and parasympathetic nervous systems (1). The aim of our work was to study the impact of CRF on parasympathetic innervation of the rat heart. Male rats were randomly allocated to undergo sham operation or 5/6 surgical nephrectomy (SNX) in two steps. Blood pressure and the resting heart rate were measured 3, 6 and 9 weeks after initiation of the CRF or sham operation. Successive bilateral vagal stimulation before and after decentralization, recording of the heart rate of the isolated right heart atrium and contraction experiments on the right and left papillary muscles were performed 10 weeks after operations. To verify the effectiveness of nephrectomy, we monitored the plasma concentrations of creatinine and urea by photometric methods.
In SNX rats, urea concentration in plasma was four-times higher and creatinine concentration three-times higher than in the control ones. The resting heart rate, systolic and diastolic pressures of SNX rats were higher than in control rats. The parasympathetic tone, measured as the positive chronotropic effect of muscarinic blocker atropine after administration of beta-blocker metipranolol was significantly lower in SNX rats. On the other hand, there was no difference in the heart rate of the isolated atria, contraction force and duration of both contraction and relaxation after administration of parasympathomimetic drug carbachol between control and SNX rats. Likewise, the effect of left and right vagal stimulations on the heart rate in SNX rats did not differ from their respective controls. Although the uremic rats in vivo were less sensitive to the parasympathetic blocker than the control rats, their efferent part of the cardiac parasympathetic innervation was not affected by CRF. Our results suggest that CRF has probably the deleterious effect only on the central part of parasympathetic nervous system.

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CHRONOPHYSIOLOGICAL VIEW ON THE VENTRICULAR ARRHYTHMIA THRESHOLD CHANGES DURING APNOE AND REOXYGENATION IN WISTAR RATS

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At the present time it is known that practically all cardiac functions show a marked circadian rhythmicity, which can play a crucial role in the development of hypoxia/reoxygenation induced ventricular arrhythmias. Because the onset and development of ventricular arrhythmias depends on many factors to which some disorders of pulmonary ventilation also belong, the circadian link between disorders of pulmonary ventilation and incidence of ventricular arrhythmias can be important. The aim of our study was to determine the dependence of the changes in the electrical stability of the heart on the light-dark cycle (LD cycle) during the apnoe – induced hypoxia and subsequent reoxygenation in *in vivo* rat experiments. Experiments were performed in female Wistar rats in ketamine/xylazine anesthesia (100 mg/kg + 15 mg/kg, i.m.) after adaptation on the LD cycle 12 : 12h, with the dark part from 6.00 to 18.00h. The animals were artificial ventilated by respirator with ventilatory parameters for normal ventilation and reoxygenation: V_T = 1ml/100g, respiratory rate 40–50 breaths/min. The apnoic episode was simulated by swiching off ventilator for 2 minutes. The electrical stability of the heart was measured by the ventricular arrhythmia threshold (VAT). This parameter of the electrical stability of the heart was introduced because the ventricular arrhythmias were the mixed type including the spontaneous mutual transitions between ventricular fibrillation, ventricular tachycardia and flutter. The VAT was defined as minimal amount of the electrical current (in mA) needed for elicitation of the ventricular arrhythmias. The control ventricular arrhythmia thresholds (VAT) were given by electrical stimulation of the right ventricle base after surgical interventions (tracheotomy and thoracotomy) and 5 minutes of the normal ventilation. The VATs were measured after apnoic episode and after 5., 10., 15. and 20. minute of reoxygenation. The average VAT was significantly decreased by apnoic episode in the both light parts of the day (1,32±0,44mA apnoe vs. 1,88±0,71mA control - light part $p<0,001$; 1,52±0,85mA apnoe vs. 2,39±0,89mA control - dark part $p<0,001$). In the course of reoxygenation, although the VAT was decreased gradually in the light part of the day (2,03±0,89mA; 1,88±0,91mA; 1,86±0,74mA; 1,78±0,54 mA), the increasing tendency was recorded in the dark one (2,22±0,96mA; 2,35±0,96mA; 2,62±0,89mA; 2,68±0,63mA). It is concluded that the electrical stability of the rat heart, measured by the ventricular arrhythmia threshold, in *in vivo* conditions is significantly

decreased by apnoic episode independently on the LD cycle but with the preservation of the LD differences. Reoxygenation, after the apnoic episode, acts antiarrhythmically in the dark (active) part of the day in contrast of the light (nonactive) one, where proarrhythmogenic effect was found.

ACTION OF A GABA-A RECEPTOR ANTAGONIST BICUCULLINE ON CORTICAL EPILEPTIC AFTERDISCHARGES IN IMMATURE RATS

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Inhibition mediated by GABA-A and GABA-B receptors may play a different role in suppression of cortical epileptic afterdischarges (ADs) in developing rats (1). Therefore we started to study effects of GABA-A receptor antagonist bicuculline (1 or 2 mg/kg i.p. 15 min before stimulation) in rats 12, 18 and 25 days old. Intensity was increased in a stepwise manner from 0.2 to 15 mA; eight responses were always averaged. An interval between individual stimulations was at least 10 min. Threshold current intensities were estimated for movements induced by stimulation, ADs of the spike-and-wave type, clonic seizures accompanying this type of ADs and transition into the limbic type of ADs. Duration of ADs was also measured. There were no consistent changes in any parameter tested. Threshold for movements induced by stimulation was decreased by the 2-mg/kg dose in 18-day-old rats only. Lower dose of bicuculline decreased threshold for clonic convulsions in 12- and for spike-and-wave type of ADs in 18-day-old rats. Duration of ADs was not significantly changed by either dose of bicuculline in any age group. Our results demonstrated that inhibition mediated by GABA-A receptors does not participate in restriction of cortical epileptic afterdischarges. The results are in marked contrast to those with GABA-B antagonist exhibiting proconvulsant changes of all measured parameters (1).

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DHP ANALOG CEREBROCRAST INHIBITS $Ca_V1.2$ AND $Ca_V3.1$ CALCIUM CHANNELS

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Cerebrocrast is a novel analog of a 1,4-dihydropyridine (DHP) synthesized in the Latvian Institute of Organic Synthesis. It does not antagonize calcium influx in neuronal tissue. Cerebrocrast inhibited KCl – induced arterial contraction. It was hypothesized that cerebrocrast is a vascular – specific calcium antagonist. Direct effect of cerebrocrast on calcium channels in native or expression system was not established yet. In our experiments we have used HEK 293 cells as an expression system either permanently transfected with cDNA encoding for main subunit for $Ca_V3.1$ calcium channel or transiently transfected with cDNAs encoding for smooh muscle isoform of the α_1 subunit of $Ca_V1.2$ calcium channel together with α_{2a} and $\alpha_2\delta$ subunits. 2 mM of Ca^{2+} were used as a charge carrier in experiments with $Ca_V3.1$ calcium channel. In experiments with $Ca_V1.2$ calcium channel Ca^{2+} concentration was raised to 10 mM. Cerebrocrast inhibited current through $Ca_V1.2$ calcium channel in submicromolar concentrations. 1 μM of the drug inhibited approximately 50 % of the current amplitude measured at holding potential of -80 mV. In contrast to known DHPs such as nifedipine or isradipine, cerebrocrast was also able to inhibit neuronal $Ca_V3.1$ calcium channel. Nevertheless, almost two order higher concentrations were needed to reach 50 % inhibition of the current amplitude. Effect of cerebrocrast on $Ca_V3.1$ was potentiated when holding membrane potential was shifted from -100 mV to -70 mV.

In conclusion, cerebrocrast is less effective L-type calcium channel blocker but more effective T-type calcium channel blocker than formely used DHPs.

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PERMEABILITY PROPERTIES OF RAT CARDIAC RYANODINE RECEPTOR

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Ryanodine receptor (RYR) is the major intracellular Ca^{2+} release channel required for excitation-contraction coupling in cardiac and skeletal muscle. The channel controls Ca^{2+} flux from the storage site in the sarcoplasmic reticulum to active site on the contractile apparatus. Crystallographic structure of the RYR channel is currently unavailable, therefore the architecture of the conductive pore involved in ion handling can be probed only indirectly by examining permeation properties of the channel using various ions as a charge carrier. Till now, all available evidence is compatible with the proposal that the conduction pathway of the RYR channel can be occupied by only one ion at a time. The purpose of our study was to re-examine this conclusion under asymmetrical ionic conditions that have not yet been tested. RYR channels were isolated from the rat heart and reconstituted into planar lipid membrane. We revealed that the zero-current potential determined under bi-ionic conditions showed clear concentration dependence, when $[Li^+]/[Ca^{2+}]$ ratio was held constant at 12:1. Furthermore, the zero-current potential did not show minima or maxima with varying Ba^{2+}/Ca^{2+} mole fraction. In the light of barrier model of ion permeation through the channel, the concentration dependence of the zero-current potential is one of the characteristics predicted for multi-ion channels. Thus, our results weaken for the first time the hypothesis about the single-ion nature of the RYR channel conduction pathway.

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Q-EUROCARDIO-QUANTITATIVE SEISMOCARDIOGRAPHIC METHOD (Q-SCG) STUDYING BOTH HEART INOTROPY AND CHRONOTROPY

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The movement of the heart and blood is a primary source of the force acting on the body. The classic ballistocardiographic method measuring the cardiac output is obsolete in cardiology due to the impossibility of objective calibration. In 1956 we developed the quantitative ballistocardiograph (G-BCG) (1). In Q-BCG method the amplitude of the force is registered in sitting person, calibrated and expressed in Newtons. The „systolic“ (F) and minute (MF) cardiac force of the heart are evaluated. These parameters were studied in different age, different pressure of oxygen, and in physical training. The linear relation was found between the force of skeletal muscles and F. The Q-BCG method was completed by the measurement of the heart rate variability (Quantitative seismocardiography- Q-SCG) (2). The Q-SCG method is capable to study both the heart chronotropy and inotropy.

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HISTOLOGICAL AND USG MONITORING IN PUERPERAL EWES AND THEIR INDUCTION TO OESTRUS DURING GALACTOPOIESIS

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Folliculogenesis in postpartum period occurs in certain waves similarly to cows (1). Laparotomy with following ovariectomy was realized after lamb weaning (spring) on day 17, 24 and 32 after parturition (Experiment 1, E1). The ovaries were USG analyzed with 5-MHz linear transducer. Follicular data were analyzed quantitatively and qualitatively (2, 3). The ovaries were picked up at the end of laparotomy and fixed in 10 % formalin. Sections of ovary tissue were stained with haematoxylin and eosin. Ovarian slides were microscopically studied and analyzed by LUCIA-G version 4.6. The second experiment (E2) was undertaken to verify biotechnological methods in breeding oriented to lamb and milk production. Anaestrous Improved Vallachian ewes milked two times a day were induced to oestrus 40 – 50 days after parturition with FGA sponges for 12 – 13 days. After sponge withdrawal the ovulation rate was stimulated with 1000 IU eCG. Control group (not milked) was treated with FGA sponges for 12 days and 500 IU eCG. E1: Differences in ovary size were not statistically significant. Number of follicles < 3 mm monitored by USG on day 32 was increased against day 17 after parturition. Image analyses of ovary sections determined significant increasing in total number of follicles ($P<0.05$) and follicles > 3 mm ($P<0.001$). The rate of atresia increased from 82 % to 89 % on day 32 postpartum. E2: We observed nearly 85 % fertility similar to control group. The fertility was not significantly lower but fecundity increased to 130 % in difference to control group ($P<0.001$). Prolificacy is significantly higher than in control group (150 % – 160 % vs 116 %; $P<0.001$) with twinning rate 0.5 – 0.8. Higher dose of eCG and galactopoiesis evidently influence the ovulation and prolificacy of Improved Vallachian ewes.

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ANGIOGENESIS AND ENDOTHELIN OF CRITICAL LIMB ISCHAEMIA

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Endothelial dysfunction (ED) is also a part of pathogenesis of hypoxic-reperfusion syndrome. The endothelial cells (EC) are able to replace damaged endothelium in a vessel, but also to form new blood vessels by the process of angiogenesis (A). Angiogenesis is a creation of so-called "biological bypasses" with growth of small vessels that grow around the obliterated artery, and so they improve the arterial flow into ischaemic tissues. At therapeutic angiogenesis (TA) either budding of new vessels (A), or differentiation in situ of EC from precursor stem cells (vasculogenesis) is induced. The main using of TA is supposed at critical limb ischaemia, in patients for who conventional re-vascular processes are not suitable. N=82 randomly selected patients in control group (CG, n=42) operated for non-ischaemic disease of lower limbs (LL) and 40 with ischaemic disease of LL (ICHLL) of average age of 50 years. The ration of men to women is 3.5:1. In the patients with ICHLL 2. pilot groups were formed: 1. N=27 without critical limb ischaemia (CLL), all underwent revascul. operation with saving their limb. 2. N=13 with CLI: 12x attempts for revascul. with limb saving in n=5 (7 patients–amputation sec. Callender), 1 patient without attempt for revascul. with primary amputation. Examination of peripheral limb congestion: angiography, Doppler USG, malleolar -brachial indices. In a link with these examin. the values of antioxidant protection were found, i.e. determination of total antioxidative capacity in plasma (TAS, TBRS); of the vitamin A,B,C, ceruloplasmin (CPL), transferrin (Tf); of the lipid parameters–apoB, of IL-6, TNF-α and endotelin. It was found that concentr. of vitamins A,B,C were decreased at the initial sampling in the patients with ICHLL vs CG. The values of IL-6, TNF-α and endotelin were increased at the first sampling in ICHLL vs CG. Increased values of endotelin were a sign of the loss of the functional characteristics of endothelium, and so also of the development of ED. Increased values of inflammatory markers (CRP, fibrinogen, proteins of acute phase of inflammation) unambiguously reflect the inflammatory process in the arterial wall. Increased concentration of CRP is a consequence of smoking and is regulated not only by the two given IL-6, IL-1, but also by TNF-α. Of this set, n=27 underwent the primary operation, n=12 patients were re-operated. N=7 patients despite revascul. had to undergo amputation, and in 7 ones complications – phlebothrombosis was recorded. Our aim remains–to search for the most suitable and specific markers that could help at precise dg. and moving away the negative effect of the disease–amputation.

DOWNREGULATION OF ADRENERGIC RECEPTORS IN COLD ADAPTED HUMANS

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To specify changes in adrenergic functions after adaptation of humans to cold, effect of administration of increasing concentrations of beta1 and beta2 adrenomimetics (Dobutamine, Bricanyl) on resting metabolic rate, heart rate, blood pressure and finger skin temperatures of young men (22,7 years, body mass 79 kg) and of cold adapted winter swimmers (37,3 years, body mass 83 kg) was studied. It was found that the increase in metabolic rate, mediated by beta1 and beta2 adrenomimetics was attenuated after cold adaptation, indicating downregulation of beta1 and beta2 adrenoceptors. Since cold adapted humans have greater capacity of nonshivering thermogenesis (1), than that mediated by both beta1 and beta2 adrenoceptors, the role of other types of adrenoceptors can be anticipated. Heart rate increased after administration of the beta2 agonist, but was not influenced by the beta1 agonist. The role of beta2 adrenoceptors in mediating heart rate was attenuated after cold adaptation. Skin temperature of the finger was lowered after administration of the beta2 agonist and this effect became more prominent after cold adaptation, indicating greater vasoconstriction tone. No changes in blood pressure after administration of beta1 and beta2 adrenomimetics were observed. Data indicate crucial role of adrenoceptors in all physiological functions responsible for adaptation of humans to cold.

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TEMPERATURE DEPENDENCY OF NMDA RECEPTOR EPSCs

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Patch clamp technique was used to characterize temperature dependency of the time course and amplitude of NMDA receptor mediated component of EPSCs (NMDA receptor EPSC) induced in lamina 2/3 pyramidal neurons by focal electric stimulation of adjacent (~50 μm) tissue. Neurons were visualized in brain slices prepared from juvenile rat using IR/DIC microscopy.
The amplitude of evoked NMDA receptor EPSCs and spontaneously occurring miniature NMDA receptor EPSCs exhibited only little temperature dependence between 25 - 40°C when normalized with respect to the single channel conductance. The time course of NMDA EPSCs was accelerated with the temperature increase. Rise time (10 - 90%) was 6.1 ± 0.6 (*n = 13*) at 35°C and the Q_{10} was 1.4. The deactivation of evoked NMDA receptor EPSCs was double exponential being τ_{fast} = 49 ± 7 ms (A_{fast} 54 ± 8%); τ_{slow} = 171 ± 13 ms at 35°C. The Q_{10} for τ_{fast}, τ_{slow}, A_{fast} was 1.7, 1.8 and 1.0, respectively. Ifenprodil (10 μM), a selective inhibitor of NMDA receptors containing NR2B subunit, reduced the amplitude of NMDA receptor EPSC (by 65 ± 11; *n = 6*), however, had no effect on the deactivation kinetics of both the fast and slow component of NMDA receptor EPSCs. Temperature dependency of the deactivation kinetics of NMDA receptor EPSC was significantly different from that determined for recombinant NR1/NR2B

receptors when activated by 1 mM glutamate for 2 ms. These were characterized by Q_{10} for τ_{fast}, τ_{slow}, A_{fast} 3.7, 2.7 and 1.0, respectively.
The results of our experiments indicate that: (i.) the value of Q_{10} for deactivation kinetics of NMDA receptor EPSCs is low, (ii.) the kinetics of synaptically activated and recombinant NMDA receptors is different and (iii.) in contrast to the previous hypothesis (1) it is unlikely that desensitization of NMDA receptors play a prominent role in shaping NMDA receptor EPSCs.

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PACLITAXEL EFFECT ON BODY CONTENT OF MINERALS AND THE ULTRASTRUCTURE OF LIVER AND KIDNEY CELLS IN RELATION TO Mg^{2+} INTAKE

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Oral supplementation of magnesium does not prevent increased urinary excretion of calcium and sodium in patients with ovarian cancer treated with chemothera- peutic drugs. The aim of this study was to evaluate, in an animal experiment, the effect of paclitaxel (TX) on mineral levels in liver and kidney tissues and on the ultrastructure of the respective cells, as well as to find out a possibility to influence these negative changes by magnesium supplementation.
Material and methods. A total of 70 female laboratory rats were used. Half of them had ad libitum access to drinking water with increased magnesium content (Magnesia, MG; Mg2+, 200 mg/l), the rest of the animal drank water with low magnesium content (Dobra voda, DV; Mg2+, 10mg/l). Tissue samples were collected before paclitaxel administration and then at 1, 8, and 15 days after intraperitoneal injection of the drug (TX, 3 mg/ kg). The control animals were given the same volume of saline intraperitoneally. In each tissue sample, the concentration minerals was assessed by flame atomic absorption spectrophotometry.
Results. In the liver tissue, the loss of minerals differed significantly between the animals with and those without magnesium supplementation; the Mg+TX group showed a drop in Na and K levels only on day 1, while the DV+TX group had a significant decrease in Mg, Na and K levels as late as day 15. In the kidney tissue, on the other hand, the concentrations of all minerals studied increased, and the increase was greater in the DV+TX than the MG+TX group. The ultrastructure of liver cells was not significantly affected by either TX administration or Mg2+ supplementation. In the kidney cells, the proximal tubules showed an increased proportion of dying cells in both groups particularly on day 8. The morphometric evaluation of intact cells in the proximal tubules revealed that pinocytic vesicles and vacuoles increased in volume in the MG+TX group on days 8 and 15, and lysosomes increased in size in the DK+TX group on day 15 (they indicated slight deterioration of cell conditions).
Conclusion. Magnesium supplementation reduces changes occurring in mineral content in liver and kidney tissues, as compared with the control group. These changes are also reflected in some changes detected by electron microscopy in cell morphology.

HARMONOGRAPHIC SOUND VISUALIZATION

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We experimented with sound visualization using generalized Lissajous curves as an alternative to more common visualization based on Fourier analysis in order to estimate how far a pleasurable sound does correspond to an aesthetically challenging harmonographic representation.
Harmonograph invented by French mathematician Jules Antoine Lissajous is nowadays a less common tool for signal analysis. While Lissajous was especially interested in sinusoidal signals, we used his tool after a slight modification to analyze signals of any shape. To visualize sound signals we used a dual-trace storage oscilloscope and spectrum analyzer with harmonographic XY mode.
We analyzed several sound signals of gradually increasing complexity. For each signal studied, we show time-domain signal, amplitude spectrum, and harmonographic curves (traces in XY mode, Lissajous curves). The most aesthetically pleasurable pictures are perhaps obtained at the transition from quasi-periodic to random noise signal (at the edge of chaos on would say), where common amplitude spectrum does not yet reveal anything remarkable.
We notice that the technical tools commonly used for signal analysis share substantial similarities with those used by living creatures. Neural system has tools to calculate e.g. autocorrelation, cross-correlation, deconvolution, harmonic analysis and frequency filtration. If we wish to speculate a little bit we can ask if the brain does also calculate Lissajous curves. Although we do not present any firm evidence, we will find some support for this. First, neuronal analysis of sensory input and other cognitive functions of the CNS is complex and not completely understood. Second, biological hardware is without any doubt able to calculate Lissajous curves as it has available all functional modules needed to perform the task. Third, neural system does perform harmonic signal analysis only partially: it calculates amplitude spectrum, while it is not known if it also calculates and uses phase spectrum. Some information therefore might get lost or is perhaps processed by other means. We conclude that the option that the neural system performs (multidimensional) harmonographic signal analysis should be taken seriously.
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PATIENTS WITH DIABETES MELLITUS TYPE 2 - MALES VERSUS FEMALES – CHARACTERISTICS OF SOME PARAMETERS IN SERUM AND SUBCUTANEOUS ADIPOSE TISSUE

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Adipocytes produce a range of polypeptides and cytokines (e.g . leptins, TNFα, adiponectin, resistin), which modulate tissue insulin sensitivity enabling them to participate in the ethiopathogenesis of diabetes mellitus 2. type (2TDM). The aims of this study were: 1) to determine lipid spectrum values (cholesterol, HDL-cholesterol, LDL-cholesterol, and TAG), insulin resistance markers (C-peptide, insulin, S-glucose, BMI, HOMA and QUICKI indexes). 2) to determine the concentrations of various cytokines in serum and subcutaneous abdominal adipose tissue of male and female patients with 2TDM. 3) to evaluate the reciprocal relations and interactions between these and factors associated with insulin resistance (leptin, TNFα, adiponectin, resistin, A-FABP, E-FABP and PPAR gamma) in the two sexes. This study included two groups: 18 men and 11 women, which had diabetes duration about 20 years and were treated mostly with insulin and antihypertensive drugs and/or diuretics etc. The average ages of the two groups were slightly different (men 60, women 61). 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 most instances the concentration of markers was statistically higher in female diabetics. We found also these relationships: 1) in adipose tissue in females, positive correlation between A-FABP, BMI and TNFα and between resistin, leptin and PPARγ, but in males TNFα positively correlates with leptin and PPAR(and adiponectin positively correlates with A-FABP and negatively with E-FABP. In serum of diabetic men we found positive correlations of TAG both leptin and E-FABP and positive correlation between adiponectin and insulin 2) in serum of diabetic women all significant correlations were between the parameters related to the metabolism of saccharides and lipids but of diabetic men the relationships between tested parameters got rich with the positive

correlations of TAG both leptin and E-FABP and positive correlation between adiponectin and insulin.

EXPRESSION OF CLOCK GENE per2 IN HUMAN COLORECTAL CARCINOMA

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Period genes are key circadian regulators in vertebrates and recent studies indicate that they are involved in control of cell proliferation. Mice with deleted *per1* and *per2* genes exhibit a loss of circadian rhythmicity in locomotor activity and express a significant increase in neoplastic and hyperplasic phenotypes (1). Recent data suggest a close relationship between circadian regulation and neoplasia and it is hypothesized that deregulation of PER proteins can be common in tumour cells. Therefore in our present study we determined expression of *per2* in colorectal carcinomas and adjacent gut tissues resected during surgery.
Our study included 26 patients of both sexes (10 females and 16 males) with progressing colonic cancer. All patients were exposed to a standard hospital practice and exposed to light from 6:00 until 21:00 daily. All surgeries were performed during morning hours. Tissue samples were collected at the time of surgery directly from the tumour as well as from the proximal (at least 10 cm) and the distal part (at least 2 cm) of resected tissue. Samples were put into liquid N_2 and kept at 80ºC until RNA extraction. Expression of *per2* was measured by real time PCR. Expression of *per2* was detected in both the tumour and adjacent tissues without signs of malformation. Higher expression was detected when the tumour was localized in *colon ascendens* as compared to *c. descendens*. Recent published data suggest a common pathway shared by regulators of circadian rhythmicity and cell proliferation (2). More studies are needed to reveal functional relationships between both these processes.

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CYCLIC NUCLEOTIDES AND PACEMAKING ACTIVITY IN PITUITARY LACTOTROPHS

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Lactotrophs isolated from anterior pituitary gland fire extracellular Ca^{2+}-dependent action potentials spontaneously and express numerous plasma membrane channels, but the mechanism underlying their pacemaking activity and its role in prolactin (PRL) secretion is still not known. We studied the relevance of cyclic nucleotide signaling pathways in control of electrical activity and PRL release. In mixed anterior pituitary cells, both Ca^{2+}-sensitive and -insensitive adenylyl cyclase subtypes contributed to the basal cAMP production and soluble guanylyl cyclase was exclusively responsible for basal cGMP production. Inhibition of basal adenylyl cyclase activity but not soluble guanylyl cyclase activity reduced PRL release. In contrast, forskolin stimulated cAMP and cGMP production as well as electrical activity and PRL secretion. Elevation in cAMP and cGMP levels by inhibition of phosphodiesterase activity was also accompanied with increased PRL release. The adenylyl cyclase inhibitors attenuated forskolin-stimulated cyclic nucleotide production, and PRL release but did not abolish pacemaking activity. The cell permeable 8-Br-cAMP stimulated firing of action potentials and PRL release. The stimulatory action of cAMP was dependent on extracellular Ca^{2+}. Protein kinase A inhibitors did not stop spontaneous and forskolin-stimulated pacemaking and PRL release. These results indicate that cAMP facilitates pacemaking and PRL release in lactotrophs predominantly in a protein kinase A-independent manner.

THE CHANGES OF REACTIVITY OF ISOLATED PULMONARY VESSELS AFTER EXPOSURE TO CHRONIC HYPOXIA

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Exposure to chronic hypoxia results in hypoxic pulmonary hypertension (HPH). The aim of our study was to determine the changes of the pulmonary vascular reactivity during exposure to the chronic hypoxia and the potentially protective role of the concomitant hypercapnia in the various phases of chronic hypoxia. We compared 3 groups of pulmonary vessels isolated from adult male rats exposed for 3 weeks to isobaric hypoxia (F_{iO2} = 0.1) (group H). Two groups were exposed to hypoxia and hypercapnia (F_{iCO2} = 0.03) either during the first (group H+early hypercapnia) or the third week of exposure to hypoxia (group H+late hypercapnia). 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 80 mM KCl or 5, 10 and 20 mM $PGF_{2\alpha}$. We observed the significant blunting of the vasoconstriction induced by the KCl in groups H and H+early hypercapnia in comparision to the group H+late hypercapnia. When we gassed the bath by 21%O_2, the responses to the $PGF_{2\alpha}$ were significantly augmented in the both groups exposed to the concomitant hypercapnia in comparision to the group H. After 45 min of the exposition to 95% O_2 we observed the further significant increase of the contractile response to the $PGF_{2\alpha}$ in group H+late hypercapnia. These results indicate that chronic hypoxia induces a oxidative stress of the walls of pulmonary arteries and concomitant hypercapnia during the late phase of hypoxic exposure mitigates the effect of oxidative stress to the vascular reactivity.

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INTERACTION OF CREATINE KINASE IN M-LINE IS STRONGLY pH DEPENDENT

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Creatine kinase is an important ATP regenerator in the cells with highly fluctuating energy demands. Its compartmentalization in muscle cells has been well described (1). The cytosolic isoform (MM-CK) interacts with the M-line of the sarcomere and it is functionally coupled to myosin ATPase during muscle contraction. In order to better understand an interaction of the MM-CK with M-line of myofibrils within the range of physiological pH changes occurring during muscle contraction, we employed an in situ exchange assay. We studied the exchange of naturally bound MM-CK by external MM-CK labeled with iodoacetamidfluorescein (IAF) at the purified myofibrils from rabbit psoas muscle (2). Fluorescence Lost in Photobleaching (FLIP) method and subsequent semi-quantitative evaluation by a fluorometry under different pH conditions (6.80 – 7.15) was used. The results obtained by confocal microscopy showed that the value of pH has a strong influence on the exchange of MM-CK of the M-line. We did not observe any fluorescence signal in the M-line in “acid” pH (pH 6.8 to 6.9). In neutral pH, the exchange reaction occurred and reached its maximum at pH 7.10-7.15. Therefore all following FLIP experiments were performed at this narrow pH range. Semi-quantitative fluorometry measurements confirmed the maximum of creatine kinase exchange at pH 7.15. At acid conditions, the amount of exchanged enzyme dropped to 10%. These results suggest a strong bond of creatine kinase in M-line in acidic pH which occurs during muscle contraction and dynamic association and dissociation of enzyme in resting physiological pH.

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DESIGN OF NEW PRIMERS FOR REAL TIME PCR OF RAT MYOSIN HEAVY CHAIN ISOFORMS

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Myosin heavy chain (MyHC) cDNA sequences are highly conserved among mammals including human, mouse and rat. Although the full sequence of human and mouse type 1, 2a, 2x/d and 2b MyHC isoforms have been well described, the information for rat is still incomplete and often controversial. When we compared the primers used in RT-PCR studies of rat muscles (1-4) with the available database information, we have found that primers for 2b isoform corresponded either to an 2b-like isoform or to a protein catenin. In order to obtain more reliable data for primer construction, we have separated 2b MyHC isoform from the rat EDL muscle by SDS-PAGE and analyzed it by two subsequent mass spectrometry techniques (MS). After tryptic digestion, the obtained peptides were identified by Matrix-Assisted Laser Desorption/Ionisation reflectron Time of Flight (MALDI-TOF) and sequenced by LC/MS Ion Trap technique. The comparison of approximately 50 % of the 2b MyHC isoform sequence revealed that the primary structure is likely identical with a NCBI database record gi|34870888|ref|XP_340819.1| marked "similar to 2b". Interestingly, the XP_340819.1 record was recently replaced by XP_340819.2, which does not match all peptides obtained by MALDI. In order to receive highly specific PCR products for rat MyHC isoforms, all available relevant cDNA sequences were compared. We redesigned the primers according to our findings with respect to their equal melting temperature (Tm) and lengths of PCR products. The new pairs of primers were designed in a way to bind to separate exons. Synthesized primers were used in pilot PCR experiments, which proved an amplification of distinct products for each pair of primers tested. Use of new primers will make the organization of further RT-PCR experiments simpler.

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