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THE VOLUME AND SIZE OF ANTRAL OVARIAN FOLLICLES IN PUERPERAL PERIOD AT THE SLOVAK TSIGAYA EWES.

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The ewes reproduction system after puerperium overcomes essential involutions changes. They are described at the ewe´s Merino. The work was focused on monitoring of biometrical parameters of ewe´s ovary on the Slovak Tsigaya in puerperium, from the view of recruitment and selection of the tertials follicles. We measured the antral follicles from two different axis length (perpendicular to the longitudinal axis to horizontal level) and height (perpendicular to the longitudinal axis at vertical level) which were evaluated on 17, 25 and 35 day after delivery. We did not find any statistically significant differences in the length and height between left and right ovaries, the same-recorded changes are in ovarian volumes. The heights concerned of the right ovaries were in average considerably higher than the left ovaries on 35th day after delivery. From the point of view tertials follicles in the time of selection with bigger diameter than 3 mm. There is tendency of occurrence numerous follicles on 35th day, than after 17 or 25th day in puerperal period.

VASCULAR AND BONE CELLS IN CULTURES ON ARTIFICIAL MATERIALS DEVELOPED FOR TISSUE ENGINEERING.

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Behavior of cells on artificial materials designed for tissue engineering is markedly influenced by physical and chemical properties of the material surface. Irradiation of polyethylene (PE) with O^+ or C^+ ions (energy 23-30 keV, doses from 10^{12} to 10^{15} ions/cm^2) resulted in formation of oxygen groups on the polymer surface and increased its wettability (contact angle decreased from 101^o up to 80^o). The adsorption of collagen IV, an important component of the cell basal lamina, was more homogeneous and higher on the modified surfaces (by 21 to 235 %). Vascular smooth muscle cells (VSMC) in cultures obtained from the rat thoracic aorta adhered on the modified PE at higher numbers (2 to 10 times), by a larger cell-material contact area and contained a higher concentration of integrin-associated focal adhesion proteins talin, vinculin, paxillin and alpha-actinin (by 19 to 56 %). Also the concentration of contractile proteins alpha-actin as well as SM1 and SM2 myosins was increased by 9 to 67 %. All these results were most apparent on PE modified by O^+ in the dose about $3x10^{13}$ ions/cm^2. Similar improvement of adhesion and differentiation (measured by production of osteocalcin) was also obtained in human osteoblast-like cells of the line MG 63 in cultures on carbon-fibre reinforced carbon composites (CFRC) after lowering their surface roughness by grinding with metallographic paper, coating with pyrolytic graphite and polishing with diamond paste (original departures of the roughness profile from the mean line decreased from 6.5±1.8 μm to 0.24±0.09 μm, and the mean spacing of the adjacent local peaks increased from 38±11 μm up to 185±76 μm). In the last set of experiments, the adhesion of VSMC on polylactide growth support was mediated by the oligopeptide GRGDSG (i.e.a ligand for integrin adhesion receptors on cells), tethered from the surface through polyethylene oxide chains. VSMC on this material assembled vinculin-containing focal adhesion plaques, synthesized DNA and divided even in serum-free media. These results are important for the establishment of controlled cell behavior on materials developed for construction of bioartificial tissues and organs.

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ONE YEAR OF THE AEROBIC GYMNASTICS TRAINING WAS ASSOCIATED WITH A DECREASE IN QRS AMPLITUDE IN TEENAGE FEMALE ATHLETES

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In our previous study we observed a decrease in QRS amplitude in the initial stage of an experimental model of exercise-induced left ventricular hypertrophy (LVH) in swimming normotensive rats. The aim of this study was to study the changes in QRS amplitude in junior female athletes during one year training in aerobic gymnastics. Somatometric parameters, heart rate (HR), blood pressure (BP) and 12-lead ECGs were recorded in 12 female athletes, aged 13-17 years (average 14,1 years) at 3-month intervals over a period of one year of competitive aerobic gymnastics training. The Sokolow-Lyon index (SLI) and the maximum QRS spatial vector magnitude (QRSmax), approximated from RV5, RaVF and SV2 voltages, were analyzed. The average values of QRSmax gradually decreased significantly as compared to the initial measurement (2.51 mV, 2.25 mV, 2.04 mV, 2.09 mV, and 1.92 mv, respectively), and similarly decreased the SLI values (2.42 mV, 2,27 mV, 2.04 mV, 1.94 mV, and 1.92, mV respectively). The somatometric parameters under study, as well as HR and systolic BP values did not change significantly. This study showed that one year of competitive aerobic gymnastics training led to a decrease in the QRSmax magnitude. This finding is in contrast with the classical hypothesis on the ECG diagnostics of LVH, and is in agreement with an alternative hypothesis on the relative voltage deficit during the early stage of LVH development.

HIPPOCAMPAL LTP AND SPATIAL LEARNING ABILITY IN TWO STRAINS OF MICE WITH CEREBELLAR DEGENERATION.

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We studied possible interactions between cerebellar and hippocampal activity using Lurcher mutant mice (LMM). 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 (1). Loss of granule cells and inferior olivary neurons is secondary. We compared a hippocampal long-term potentiation (LTP) and spatial learning in Morris water maze between two strains (C3H and C57B1/7) of LMM. Healthy mice (wild-type) were used as a control group. LTP was performed under urethane, 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). For final evaluation differences in the magnitude of population spikes and slope of EPSP were used (2). LMM of both strains revealed higher LTP ability than wild type, but only in C57Bl/7 strain was statistically significant. A comparison of spatial learning ability in both strains showed that C57B1/7 mice reached shorter latencies then C3H strain. The difference is high in wild type animals, lower in LMM. C57B1/7 strain showed better ability of spatial learning. Also the differences between LMM and wild type animals are higher than in the C3H strain. Our results clearly show that two strains of mice with identical type of cerebellar degeneration reveal different spatial learning ability in Morrris water maze. Spatial learning is influenced in these mice strains not only by the presence of the cerebellar degeneration, however its role is indisputable. Evaluation of hippocampal LTP ability shows that a similarity between higher LTP production on the one side and higher spatial navigation ability on the second one does not exist. These findings strongly support ideas that spatial learning tasks involve a

number of nonspatial components (motor control or stress factors) that are not hippocampal dependent and can influence the results (3).
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ACTION POTENTIAL VOLTAGE CLAMP ANALYSIS OF RATE-DEPENDENT CHANGES OF ACTION POTENTIAL IN RAT VENTRICULAR MYOCYTES.

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The rate dependence of cardiac action potential (AP) configuration shows marked tissue and species differences. In rat ventricular myocytes, AP duration is prolonged with increasing frequency of stimulation [1]. The participation of underlying current components has not been definitely established so far. In the present study, we examined the role of individual current components in the rate-dependent changes of AP configuration using the AP voltage clamp method. The experiments were performed at room temperature on myocytes isolated from right ventricles of adult rats. APs were recorded in the current clamp mode during restored stimulation (2.5 Hz) after a period of rest (60 s) until the steady state. In the voltage clamp mode, the stimulating pulses were replaced by the steady-state pattern of AP. After subtraction of the steady state records from all current traces, changes of ionic currents could be directly visualized and correlated with the altered AP waveform. APs recorded during the train of stimuli in the current clamp mode were gradually prolonged. In the AP clamp mode, the fast sodium current I_{Na} remained almost unaltered. Prominent changes, however, occurred in the subsequent current waveform that was apparently composed of more than one component. In the presence of I_{Ca}-blocker Co^{2+} (2 mmol/l), the less pronounced prolongation of AP was observed that corresponded with gradual decline of I_{to}. Under the conjoined effect of Co^{2+} and of I_{to}-blocker 4-aminopyridine (4-AP, 3 mmol/l), the restored stimulation induced contrariwise small gradual shortening of AP that resulted from reverse use-dependent properties of 4-AP induced I_{to}-block. We conclude that the main determinants of rate dependent changes of AP duration in rat ventricular myocytes are variations of I_{Ca-L} (gradual increase caused by modulatory effect of the Ca^{2+} transient) and of I_{to} (decrease due to cumulative inactivation). These contemporary variations of I_{Ca-L} and I_{to} supported each other in the rate-dependent modulation of AP prolongation. Under our experimental conditions, participation of other currents was negligible.

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ERYTHROCYTE DEFORMABILITY IN ONCOLOGIC PATIENTS.

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The aim of the work was the erythrocyte deformability (ED) monitoring in oncologic patients with carcinoma (CA) of the colon before and after operation and in the patients with carcinoma of the mamma before and in the course of radiotherapy. The group of patients with carcinoma of the colon consisted of 15 subjects, aged 62,5±12 y. ED was monitored before and after operation, as well as in the 1st and 3rd day after operation. The second group consisted of 17 patients with carcinoma of the mamma aged 55±12 y. DE was determined before radiotherapy (X rays, 50 Gy) and on the 1st, 3rd and 4th week after radiotherapy. The control group to the both groups of oncologic patients involved 12 healthy subjects aged 31±11 y. The average ED value in the group of patients with CA of the colon was 65.6±3.2 % before operation, in the group of patients with CA of the mamma 65.2±4.1 %. ED values were significantly lower in comparison with the average value of ED in the control group 72.9±5.4 % ($p<0,05$). In the patients with CA of the colon was ED value decreased to 62.0±4.0 % after operation. In the 1.st and the 3.rd day after operation an increase to 66.7±4.1 % and 67.8±5.4 % was recorded. By the influence of radiotherapy in the group with CA of the mamma the decline of ED value from 65.2±4.1 % to 60.8±3.8 % after the 1st week radiotherapy was found. After the 3rd and 4the week another decline of ED to 59.1±4.0 % and 59.6±4.8 % ($p<0,05$) was recorded. The ED values in oncologic patients with CA of the colon and in patients with CA of the mamma were significantly lower in comparison with the control group of healthy subjects. ED in patients with CA of the mamma undergoing a radiotherapy was significantly declined, what may be the impulse of the hemodynamic disorders.

IMMEDIATE CARDIOVASCULAR EFFECTS EVOKED BY COLD FACIAL IMMERSION DURING EXERCISE

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Application of cold stimuli (C) to facial area evokes in a man series of immediate and prolonged respiratory, cardiovascular, haematological and neurovegetative metabolic reflex responses called trigeminal diving reflex (DR). The most prominent effects of DR are bradycardia and hypertensive reaction. Present work was undertaken to examine timing and intensity of cardiovascular changes upon stimulation of most prominent responsive sites of DR under control conditions and physical workout. Respiratory rate, heart rate (HR) and arterial blood pressures (BP) evoked by 20-60 s long cold stimuli (gel-filled plastic applicator; <10 °C) to orbital - frontal (OA+FA) trigeminal areas, respectively, were evaluated in 16 volunteers (17-42 years) during the rest with (n=4; C+A) and without (n=12; C-A) breath-holding (A). Under rest conditions C-A stimulation produced immediate (10-15 s after onset) fall in HR both in OA+FA mode (n=12; Δ 8.2-23.2 %; Δ 7-26 $c.min^{-1}$) and OA (n=9/11, Δ 10.5 %) or FA (n=6/11; Δ13.6 %) which lasted for 36-76 s (OA+FA > FA > OA) until BP returned to control values. In C+A regiment OA+FA stimuli evoked stronger (Δ 11.2-26.2 %) bradycardia compared to FA and OA (Δ 15±4 % and Δ 13±5 %, M±SD). Response persisted for 43-71 s, similar to C-A. Effects of upper facial C stimuli (OA+FA) were examined during sustained tachycardia - hypertensive response modelled by continuous exercise on bicycle ergometer (HR ≥150 $b.min^{-1}$, mean BP >140 mmHg). Stimuli resulted in short–onset (7-16 s) reversal of tachycardia (Δ 9.3 %) which was further strengthened over 60-80 s of exercise (Δ 19.2±9.95 $c.min^{-1}$, Δ 12.2 %). Onset latencies of BP changes paralleled those in HR, but their course and duration was less consistent. Under rest condition, initial (~15-45 s) rise in BP (Δ 5.3-11.5 %) was followed in C-A group mostly (n= 7/12) by normalisation or by small decrease (n=2/12). During continuous exercise, OA+FA stimulation prevented or even reversed ongoing hypertensive response for 42±6 s (25-55 s). In conclusion, DR reversed sustained tachycardia and hypertensive response due to exercise and might be of a value as a simple cure of various paroxysmal tachycardic-hypertensive states.

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COMMON SYNCHRONISATION PATTERNS REVEALED BY HIGH FREQUENCY OSCILLATIONS IN POWER SPECTRA OF INSPIRATORY MUSCLES DURING SPASMODIC EFFORTS

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Various respiratory activities are represented in inspiratory muscle electromyograms (EMGs) by typical patterns of inter-discharge periodicity, which are supposed to reflect activation of distinct neuronal assemblies within the central respiratory structures. These rhythms, so called high frequency oscillations (HFOs), can be detected by the power spectral density (PSD) of Fast-Fourier transforms as a prominent autospectral and coherence peaks over the frequency bands H1 (60-110 Hz), H2 (40-60 Hz) eupnoea, and H3 (20-40 Hz) and 110-150 Hz for gasp. Median frequency (MF), defined as the frequency dividing auto-PSD into two parts of the equal power, was shown to be linearly related to an average conduction velocity of the active motor units in the muscle thus providing an estimate of the recruitment order of motor units during particular activity. HFOs were determined in PDS and coherence spectra (CS) calculated in half- overlying 128 ms - 512 ms - Hanning or Turkey windows in digitally filtered (20-250 Hz), AD-sampled (1024 Hz) EMGs of crural diaphragm (DIA) and inspiratory intercostal muscles (IIC) during 40 secondary gasps (G, 216±21 ms, M±SD), gasp-like bursts (362±22 ms) of 50 sighs (S), 40 end-inspiratory oscillations (OS, 812±32 ms) from 32 hypoxic attacks in 10 anaesthetized cats. Also, we analysed 15 nasal sniffs evoked by air-puffs (SF, 163±23 ms), 40 gasp-like epipharyngeal efforts (AR, 193±18 ms) and 32 hiccup-like efforts from hypopharynx (HR, 212±16 ms). PSD in G revealed typically unique PDS-peak in 128 Hz or double peaks in 88 Hz and 132 Hz in both DIA and IIC. In S we found bilaterally correlating HFOs in DIA/IIC in the eupnoeic bands (48 Hz and 88 Hz peaks) and 136 Hz. In PSDs of OS, marked DIA/IIC coherences were detected in 88Hz and 136 Hz peaks as well as prominent HFOs in 168 Hz. PDS of SF showed two regular spectral peaks: 88 Hz a 136 Hz (142 Hz) and occasionally the peak in 36 Hz, too. AR displayed two HFOs markedly resembling G or AB: 88 Hz and 136 Hz. Significant coherences in DIA/IIC were revealed during HR in peaks over 26Hz, 48 Hz, 88 Hz and 142 Hz. MFs were calculated from sequential PSDs of DIA EMG of 12 normal breaths (NB, 1854±62 ms) and 12 AR. The data showed that AR is one of the strongest inspiratory activities with peak integrated EMG and firing rate being 3-4 times and 2-3 times higher than in NB, respectively. In AR the majority of DIA motor units is supposed to be recruited at once with the beginning of activity.

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OSCILLATORY INSPIRATORY DISCHARGES REVEALED IN EUPNOEIC RESPIRATORY PATTERN AND GASP UNDER SEVERE ANOXIC CONDITIONS.

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Characteristics of non-eupnoeic oscillatory inspiratory discharges (OS) were investigated in 16 anaesthetized unparalyzed (Pentobarbital, 30 $mg.kg^{-1}$ i.p.) cats during 24 severe poikilocapnic hypoxic trials (n=24; FIO<1-3 %). by evaluating ventilatory time, flow and volume variables and parallel records of raw multiunit and integrated EMGs from inspiratory (IIC, T4-T6) and expiratory (EIC, T8-T9) intercostals, costal and crural diaphragm (CO, CR) and abdominal muscles. Bifrontal and bioccipital EEGs and MSEPs from somatomotor cortical lead were recorded in 4 cases. Arterial pressure, heart rate and end-tidal/arterial CO_2&O_2 pressures, were monitored concomitantly.

(1) During hyperpnoea OS (n=36) developed within late inspiration, app. 77-126 ms before the "reversible inspiratory off-switch" as short duration (35.4±0.7 ms, M±SE) repetitive inspiratory efforts (8-15 bursts) of 7.5-14.2 Hz frequency (mean 10.3±0.2 Hz) that increased total duration (2.4 x) and peak flow of inspiration.(1.4-3.1 x). In between the periods of oscillatory bursts (68.3±0.5 ms), discharge ceased and might be replaced by expiratory muscle contra-discharges (68 % of cases). Breaths with OS also revealed prolonged post-inspiratory (3-3.5 x) and total expiratory durations (1.5-1.8x). Hyperpnoeic OS appeared in periodic "all-or-nothing" manner (6 %) or developed over 4-5 breaths by accentuating late-inspiratory oscillatory discharge.

(2) In 2 cases of unsuccessful post-apnoeic autoresuscitation and circulation failure compacted gasp bursts dissipated into series of OS discharge (823±34 ms) consisting from 3-14 bursts (7.8±2.4 Hz) of 79±16 ms duration. In 3 cases of prolonged hypoxic apnoea 13-17 min with sustained arterial pressure (75±11 mmHg) and cortical unresponsively in EEG and MSEP traces gasp-like bursts and OS developed spontaneously and lead into transient recovery of phasic breathing.

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DIFFERENT TIMING AND INTENSITIES OF INSPIRATORY AND EXPIRATORY MUSCLE EFFORTS DURING SEVERE ANOXIA WITH VARIABLE CO_2 LEVEL

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The onset latencies, slopes and peak-amplitudes of hyperpnoeic efforts, magnitudes of postapnoeic tonic activity and gasps, respectively, were analysed in simultaneous multiunit EMG activities of costal (CO) and crural (CR) diaphragms (DIA), inspiratory (IIC, T4-T6)) and expiratory (EIC, T7-T9) intercostal muscles and abdominal muscle units (ABD) at 17 anaesthetized (Pentobarbital) unparalyzed cats, which were exposed each to hypocapnic ($PaCO_2$ <20 mmHg), normocapnic ($PaCO_2$~ 35-45 mmHg) and hypercapnic ($PaCO_2$ >65 mmHg) anoxias (n=56; FIO<1-3 %) in 30-45 min intervals. Tracheal airflows, respiratory volumes, arterial pressure, heart rate, end-tidal/arterial CO_2&O_2 pressures and pH were monitored concurrently. 1) During hyperpnoeic stage all inspiratory muscles revealed biphasic pattern. In excitatory portion significantly faster onset, steeper slope of rise, higher magnitude of augmentation and longer duration was always found in IIC compared to CR and CO. Amplitudes of CR were regularly higher than in CO. Intensity of pre-apnoeic depression was stronger on CO compared to CR and IIC. These differences regularly strengthened at larger hyperpnoeic $PETCO_2$. 2) Expiratory muscle activities have been gradually inhibited or abolished during hypoxia. Inhibition was faster and stronger in EIC than in ABD, especially during hypocapnia. In both muscles, the latency of inhibition prolonged, and its strength markedly lowered with higher hypoxic $PETCO_2$. 3) In 65 % of cases inspiratory muscles developed tonic activities during apnoea. Unlike the hyperpnoeic efforts, the muscle tone amplitudes showed nor marked CO_2 -dependence nor the differences between muscles. Contrarily, expiratory muscle tone in ABD showed marked CO_2 - dependent augmentation. 4) The order of inspiratory muscle activation during gasping was similar as in hyperpnoea (IIC > CR > CO), although their relative amplitudes showed little or no apparent CO_2 - dependency. In conclusion, distinct regulation exists between different respiratory muscles during phasic breathing upon extreme chemical drive and dysrhythmic activities as apnoeic tone or gasps.

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HYPOXIA REDUCES eNOS EXPRESSION IN HYPOXIC RAT PLACENTAS

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Hypoxia in fetoplacental vessels is believed to be one of the most important factors in the pathogenesis of fetal and neonatal morbidity, such as intrauterine growth retardation. Chronic hypoxia regulates the expression of endothelial NO synthase in different species and tissues. Typically the expression of endothelial NO synthase (eNOS) rises under chronic conditions. The expression in rat placenta has never been determined in hypoxia. The aim of our study was to analyze the effect of chronic hypoxia on the expression of endothelial NO synthase (eNOS) in rat placenta. Female rats were exposed to hypoxia (10 % O_2) for the last 10 days of pregnancy. Control group of rats remained in normoxia. Experiments were conducted in accordance with the Guide for the Care and Use od Laboratory Animals as adopted and promulgated by U.S. National Institutes of Health (agreement number B 67 900). One day before the calculated day of delivery placentas were removed under thiopental anesthesia (60 mg/kg). Placentas were rinsed in saline solution and immediately homogenized. eNOS expression was determined by western blot with immunodetection using rabbit anti eNOS (St. Cruise). The results were quantify by densitometry. We found significantly lower expression of eNOS in hypoxic placentas compared with normoxic controls. We suppose, that lower expression of eNOS could contribute to the lower blood flow through the hypoxic placenta and deterioration of fetal blood oxygenation and nutrition.

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DOES TACHYCARDIA CORRELATE WITH HYPOTENSION DURING ACUTE BLOOD LOSS?

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Textbook descriptions of hemorrhagic hypovolemia usually list tachycardia as an early sign of blood loss. Decrease in heart rate is described as an element of the irreversible shock, which precedes cardiac arrest. Only a few reports have emphasizes the occurence of bradycardia during reversible schock. Absence of tachycardia in this situation was named as „relative bradycardia“. The aim of this study was the observation and evaluation of bradycardia presence during reversible experimental hemorrhagic shock. The acute experiments were performed in 50 pentobarbital - anesthetised cats (40 mg/kg). Hemorrhagic hypovolemia was induced by removal of 10 %-35 % of the total blood volume. The bleeding was gradual, each 10 % of blood volume with 5-min intervals between the bleeding epizodes, until elicitation of the respiratory depresion. Then the blood volume was immediately restored. The blod pressure, ECG, breathing, hematological markers and acid base balance were analysed. In experimental animals occured three types of „relative bradycardia“ inspite of the hypotension due the bleeding. The first was the rare initial bradycardia at the start of bleeding(at 10 % of animals), than the early bradycardia during loss of 10-20 % of the blood volume (at 70 % of animals) and the late bradycardia during loss of 30 and more % of the blood volume(at 20 % of animals). The blood pressure during the 30 % of blood volume decreased approximately upon to 1/3 of initial value, the first sign of the decompensation of bleeding was the decreasing of the rate and depth of breathing. Quick restoration of the original blood volume by the transfusion or infusion of the physiological solution adjusted the blood pressure and heart rate to the intial levels or slightly above, restored the breathing, each of animals survived. Our findings account for the fact that heart rate is not good predictor of hemorrhagic hypotension and is unreliable sign of blood loss. The „relative bradycardia“ during rapid bleeding is probably common and may lead to confusion or a delay in diagnosis. Relative bradycardia have to be treated with rapid fluid loading. The responsive mechanisms of the relative bradycardia and final bradycardia are different.

CARDIORESPIRATORY RESPONSES TO EXPERIMENTAL HYPERTHERMIA AND ITS PHYSICAL TREATMENT DURING ACUTE NORMOVOLEMIC HEMODILUTION

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Cardiorespiratory responses to hyperthermia and its physical treatment during acute normovolemic hemodilution/anemia were studied in 16 anaesthetized adult rabbits The animals were divided into two groups: hemodiluted group (Hct 18.6±0.4 %) and control group (Hct 41.1±0.9 %). In the hemodiluted group, acute normovolemic hemodilution was achieved by 60 % replacement of total blood volume with dextran. Hyperthermia (the rise in body temperature to 42 °C by a gradual body surface heating) produced significant increase in minute ventilation (V_E) in both groups, however, the hemodiluted rabbits, unlike the controls, were not able to start panting and to keep physiological P_aO_2 in hyperthermia despite the significantly higher values of V_E. The heart rate was higher ($P<0.05$) during hyperthermia in the hemodiluted rabbits compared to the controls. Central venous pressure decreased ($P<0.05$) during hyperthermia only in the controls in the panting phase. The changes in mean arterial pressure were not significantly influenced by hemodilution. Physical treatment of hyperthermia by body surface cooling was accompanied by recovery of the cardiorespiratory variables. There were found no significant differences between the two groups. The results indicate that acute normovolemic hemodilution/ anemia mobilizes mechanisms maintaining the arterial blood pressure and organ perfusion and thus intensifies the cardiovascular effects of hyperthermia. Acute normovolemic hemodilution/anemia did not influence cardiorespiratory recovery during physical treatment of experimental hyperthermia.

CARDIORESPIRATORY RESPONSES TO EXPERIMENTAL HYPERTHERMIA DURING ISOSMOTIC DEHYDRATION.

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Under conditions of heat stress and hyperosmotic dehydration, both animals and humans reduce thermoregulatory evaporation (sweat rate and respiratory evaporative heat loss) and regulate deep body temperature at elevated levels. Hyperosmotic dehydration attenuates the heat stress-induced cutaneous vasodilation. However, little is known about thermoregulatory and cardiorespiratory effects of heat stress under conditions of isosmotic dehydration/hypovolemia. Therefore, cardiorespiratory responses to hyperthermia during isosmotic dehydration/hypovolemia were studied in 17 anaesthetized adult rabbits divided into two groups: normovolemic group (NV; n=10) and hypovolemic group (HV; n=7). In the HV group, hypovolemia (16 % decrease in plasma volume) was induced by furosemide administration (5 mg/kg i. v.) During hyperthermia (the rise in body temperature /BT/ to 42 °C by a gradual body surface heating), the HV rabbits had the breathing frequency lower ($P<0.05$) than the NV animals. The panting was absent in the HV rabbits at the BT of 42 °C, unlike the NV animals. From cardiovascular parameters, the vasoconstrictor response in visceral (mesenteric) region during hyperthermia was attenuated ($P<0.05$), whereas the heat stress-induced cutaneous vasodilation was not significantly influenced by hypovolemia. Ion conclusion, the lower frequency of breathing, thus lower respiratory evaporative heat loss during exogenous hyperthermia in dehydrated animals are present not only during hyperosmotic dehydration, but they occur also under conditions of furosemide-induced isosmotic dehydration/hypovolemia.

ALTERATIONS IN GEOMETRY OF THORACIC AORTA AND CAROTID ARTERY IN THE SHR DURING ONTOGENY.

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The aim of study was to evaluate the geometry of the thoracic aorta (AT) and carotid artery (AC) of Wistar rats and SHR during ontogenic development. Four groups of Wistar rats of the age: 3 weeks (3w), 9 weeks (9w), 17 weeks (17w) and 52 weeks (52w), and four groups of the age matched SHR were taken for the study. Blood pressure (BP) was measured noninvasively on the tail artery by plethysmographic methods. Under anaesthesia the rats were perfused with glutaraldehyde fixative under the pressure 90 mm Hg (3w) and 120 mm Hg (the rest of groups). AT and AC were excised and processed according to standart electron microscopy procedure. Wall thickness (WT) and inner diameter (ID) were measured on semithin sections using light microscopy, and cross sectional area (CSA) and WT/ID ratio (WD) were calculated. BP of 3w old Wistar rats did not differ from the age matched SHR (83±1.9 mm Hg vs. 84±1.4 mm Hg). The difference in this respect was observed in 9w (107±1 mm Hg vs. 154±1.4 mm Hg), in 17w (114±1.4 mm Hg vs. 214±7.3 mm Hg), and in 52w (114.6±3 mm Hg vs.189.7±2.4 mmHg). The value of heart/body weight ratio was higher in all SHR groups in comparison to controls and indicated myocardial hypertrophy. The geometry of both AT and AC revealed that WT of SHR, contrary to controls, progressively increased during the ontogeny. Differences were observed in development between AT and AC. WT and CSA of AT in 3w and 9w old SHR was decreased. WT and CSA of AC in 3w old SHR did not change from the age matched Wistar rats and they were increased from 9w. In 17w and 52w old SHR both WT and CSA were increased in both arteries. WD in AC of SHR was increased in all periods, in AT it was increased only in 17w and 52w old SHR. In conclusion: In comparison to Wistar rats we have already found in SHR remodelled wall of thoracic aorta and carotid artery in prehypertensive period. The alterations in structure continue remarkably with differences in blood pressure level.

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PERIODIC COMPONENTS IN INFRADIAN DYNAMICS OF SALIVARY TESTOSTERONE IN MALE*

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Although the dynamics of testosterone (T) during female menstrual cycle is described (1), no such cyclicity is known in male. Previous studies have indicated a periodic component in the dynamics of T in male (2). The aim of our study was to analyze infradian dynamics of salivary T in human male regarding possible periodic components. Salivary T concentrations were measured in 31 young healthy men during a period of 2.5 months (every second – 1 month; every third day – 1.5 month). The results were analyzed using two different and independent methods. The method Zones of extremes – moving averages was used as a standard, Analysis of Rhythmic Variance (ANORVA) was used as a newly developed method (3). A periodic component with a period length of 30 days was found using both analytic procedures in the time series. Moreover, ANORVA analysis revealed a further rhythm with a period length of 20 days. The differences between extreme values in both periodic components were significant ($p<0,001$). Using two independent statistical approaches we have found two periodic components in salivary T infradian dynamics in male – a circatrigintan (30 days) and a circavigintan (20 days) rhythm. Both cycles seem to coexist and this might be one of the reasons why male infradian rhythms of T have not been described previously. The design of this study enabled us to analyze periodic components with a period length between 4 and 36 days. Further studies should concentrate on longer periods and especially on the possible implications of our results.

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THE BEHAVIOR OF NORMAL AND NEURODEFECTIVE LURCHER MUTANT MICE OF THE C3H AND C57BL/7 STRAINS IN THE MORRIS WATER MAZE

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Lurcher mutant mice (+/Lc) represent a natural model of olivocerebellar degeneration, which is caused by a mutation in the δ2 glutamate receptor gene (1). They suffer from cerebellar ataxia. Wild type (+/+) littermates are completely healthy. In our laboratory we use Lurcher mutant mice of two strains – C3H and C57Bl/7. There are significant strain differences in spatial learning tests. Some of the C3H mice are affected by hereditary retinal degeneration which is one of the reasons of their worse results in the tests. We assess the specific spatial learning disorder in C3H mice and possible differences of maze exploration strategy in Lurcher and wild type mice of both strains. Two experiments were arranged. First, spatial learning was tested in the Morris water maze (2) for 5 days. The platform was hidden under the water surface. For the second procedure new group of mice was used. The platform was removed and the movement trace and swimming speed was registered for 4 days. Then the C3H mice were sacrificed and their retina was examined histologically to recognize presence of retinal degeneration. Only mice with normal retina were involved in statistical analysis, so that the effect of retinal degeneration on the results was excluded. The C57Bl/7 Lurchers showed significantly longer latencies as compared with wild type mice. Within the C3H strain the difference between both types of animals was not found. Learning ability of C57Bl/7 wild type mice was higher than in the C3H wild type animals, whereas Lurchers of both strains did not differ. Both Lurcher mutants and wild type mice of the C57Bl/7 strain spent more time in the central part of the maze than correspondent C3H animals. The results proved spatial learning deficit in C3H wild type mice, which is not caused by the retinal degeneration and resulting problems with orientation. The strains differ in the strategy of new environment exploration. Lurchers of the C57Bl/7 strain showed a learning deficit and their longer latencies cannot be adjudged to the ataxia, because their swimming speed is not decreased.

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DETECTION OF XRCC1(399) POLYMORPHISM IN PATIENTS WITH LUNG CARCINOMA

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Effective repair of DNA lesions is very important factor, affecting an individual sensitivity of cells to initiation of tumorigenesis. Decrease of effective DNA repair may be linked to increased lung cancer risk. Analyses of gene expression help to detect genes included in DNA repair processes. The outcomes of many experiments give on tumorigenesis new view. Damaged molecule of DNA is oncogenic and its lesion depends on somatic or hereditary changes. The XRCC1 gene (X-ray repair cross-complementing group 1), responsible for BER (base excision repair), is involved in repair processes of lesions which can rise during DNA replication. The function of XRCC1 protein is ligation of DNA single strand breaks (SSBs) caused by irradiation or by cytostatics. Total capacity of DNA repair precesses depends on polymorphisms of various repair genes. There was found strong correlation between genetic polymorphisms and lung cancer risk. Different combinations of DNA repair genes polymorphisms interact and may be linked to increased developement of lung cancer. These findings signalize marked influence on susceptibility in relationship with lung carcinoma developement. We assume that disability of DNA repair processes increase level of more agressive forms of lung carcinoma which causes faster metastatize of tumor cells into other organs. This fact negatively affects decreasing of patients survival. Our experiments are focused on XRCC1 repair gene and monitoring of its polymorphism distribution in patients with lung carcinoma. We are focused on exon 10, codon 399 (arg399gln). We have observed susceptibility of three genotypes of XRCC1(arg399gln) polymorphism for lung cancer developement by comparison with results of other authors. Interim we have genotypisized group of patients (n=58) with lung carcinoma, in which we examined following distribution of XRCC1(arg399gln) polymorphism: GG=14 %, AG=58 %, AA=28 %. From actual results is untimely to deduce outcomes which genotype is the most susceptible for lung cancer developement, because of small quantity of patients. We may assume that different genotoxic factors and changes of DNA repair genes function, include polymorphisms, markendly affect process of carcinogenesis and antitumor therapy.

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CHANGES IN SUPEROXIDE DISMUTASE AND CATALASE ACTIVITIES DURING TRANSIENT FOREBRAIN ISCHEMIA/ REPERFUSION IN RAT BRAIN

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Under normal conditions, ROS produced in the course of metabolism are converted to harmless molecules, such as, water and molecular oxygen by the natural antioxidant defence system which consists of numerous antioxidant compounds and enzymes. The primary ROS generated in the body is superoxide, which is converted to hydrogen peroxide by superoxide dismutase (SOD). Hydrogen peroxide is, in turn, converted to water and molecular oxygen by catalase (CAT) or glutathione peroxidase. Three isoforms of SOD are known: cytosolic CuZn-SOD (SOD 1), mitochondrial Mn-SOD (SOD 2), extracellular CuZn-SOD (SOD 3). One form, dependent on presence of copper and zinc at its active site, is found in the cytoplasm of cells and an isoform of this molecule is present in extracellular fluids such as plasma. A third isoform containing manganese at its active site is located in the mitochondria. Trace metals such as copper, zinc and manganese are essential for maintaining the antioxidant activity of SOD. We used 5 min of ischemia (four vessels occlusion) as a preconditioning and followed the reaction both main endogenous antioxidant enzymes SOD and CAT. The activities of the antioxidant enzymes CuZn-SOD, Mn-SOD and catalase were measured in the hippocampus, striatum and cortex after 5 min transient forebrain ischemia and 5 hour, 1 or 2 days reperfusion. The activity of total SOD after 5 minutes of ischemia used as a preconditioning and 5 hours of reperfusion is increased by 3-times. This increase persists during next 24 hours, and drops to nearly control values 2 days after preconditioning. Though activity of CuZn-SOD is significantly increased at 5 hours as well as 1 day of reperfusion in all studied brain regions most expressive increase can be seen in more vulnerable hippocampus and striatum. Very similar results as in the case of total and CuZn-SOD were found in Mn-S0D. The activity of CAT is increased too. Most noticeable difference in comparison to SOD is that CAT reaches peak of its postischemic activity 24 hours after ischemia. As an reaction to preconditioning both main endogenous antioxidant enzymes SOD and CAT are synthesized as soon as 5 hours after ischemia.

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ANALYSIS OF CORRELATION BETWEEN HBA_{1C} AND BLOOD GLUCOSE

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Blood glucose and glycated haemoglobin (HbA_{1c}) are the basic indicators of glycaemic compensation in diabetic patients and the degree of compensation is the key factor in the pathogenesis of diabetic complications. HbA_{1c} concentration depends on blood glucose values of previous weeks and therefore it can be influenced only indirectly, through action on blood glucose values. The mathematical model of haemoglobin glycation makes calculation of HbA_{1c} concentration from blood glucose values possible. We analysed the agreement between calculated and measured values of 373 results from 282 patients with diabetes mellitus. The values were sorted into 3 classes. In the first class there was agreement between calculated and measured results (16.9 % of all results). The real value of HbA_{1c} was lower then the calculated one in almost two-thirds (62.2 %) of results. Higher measured HbA_{1c} than calculated was in 20.9 % of results. The course of regression line calculated from linear regression analysis between HbA_{1c} and blood glucose values was in agreement with the model only in the first class of results. In classes where the calculated and measured HbA_{1c} were different the slope of regression line was considerably flatter than expected. The deviation from expected values of regression analysis was not influenced neither by changing the values of constants applied in the equation nor by recounting the HbA_{1c} values to new IFCC units. According to the analysis the value of Hb_{A1c} is at relatively low values of blood glucose higher than expected from the model. At very high values of blood glucose the opposite is true. This „seesaw“ effect can be explained by the fact that blood glucose values do not reflect exactly the glycaemic compensation. The results confirm the view that assessment of compensation is possible only through complex view on both short and long term parameters of glycaemic control.

EFFECT OF INORGANIC MERCURY ON THE $CA_V3.1$ T-TYPE CALCIUM CHANNEL

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Mercury is quite common environmental contaminant, whose targets include voltage-operated calcium channels. $HgCl_2$ was shown to inhibit various native and recombinant voltage activated calcium channels (1,2). Its effect on recombinant low-voltage activated (LVA) channels was not investigated. Therefore we have analyzed effect of $HgCl_2$ on the $Ca_v3.1$ calcium channel, a member of LVA channels family, heterologously expressed in HEK 293 cells. Whole-cell patch clamp method was employed. 2 mM Ca^{2+} were used as a charge carrier. Inorganic mercury inhibited amplitude of the current through the expressed $Ca_v3.1$ calcium channel in a concentration-dependent manner.

Threshold for the inhibition was above 10 nmol/l and 10 μmol/l $HgCl_2$ blocked the current completely. Half-maximal inhibitory concentration for amplitude inhibition was between 0.1 and 1 μmol/l. At all tested concentrations, inhibition was virtually irreversible upon washout. Additionally, $HgCl_2$ shifted current-voltage (IV) relation to more negative membrane potentials. Concentrations 100 nmol/l and bigger activated a background current in HEK 293 cells. This phenomenon was previously reported for hippocampal neurons, too (2). Our results contrast with those reported by Szücs (2). We did not find transient potentiation of the current by low mercury concentration and we did find shift in IV. Reason for the difference may be different experimental model. In hippocampal cells used by Szücs and coauthors effects of mercury on T-type calcium channel may be obscured by its effect on other ion channels, present in their experimental object.

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EFFECT OF DIVALENT IONS ON SELECTED PARAMETERS OF NITROGEN METABOLISM

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The objective of the present study was to evaluate effect of some pollutants on alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and glutamate dehydrogenase (GGT) enzyme activities of rumen fluid in vivo and in vitro conditions. Rumen contents were collected from sheep using a stomach tube two hours after feeding. Ruminal contents were then strained through eight layers of cheesecloth. The concentrations of copper and zinc were sigificantly higher in rumen fluid of sheep from industrially exposed area (in vivo study). In vitro study, Cd, Cu and Mg were separately added to 10 ml of strained rumen fluid so their final concentrations were 5 mmol/l. After the addition of the metal ion, each mixture was shaken and incubated for 30 minutes at 37 ^{0}C prior to assaying enzymatic activity (1). *In vivo* study, the activity of individual enzymes of the rumen fluid of sheep from industrially exposed area were significantly higher than in sheep from non-industrially exposed area. In vitro experiment, magnesium stimulated the activity of all enzymes tested whereas the cadmium and copper effect was enzyme specific. Results of this study indicate that pollutants can affect ruminal enzyme activities of sheep and there are differences between effect of pollutants added individually to rumen fluid and effect of pollutants which are animals exposed to chronically in industrially exposed area. In vivo conditions, resulting enzyme activity reflects mutual action of individual pollutants on enzymatic system in the rumen environment.

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NON-ENZYMATIC ANTIOXIDANTS IN CHILDREN WITH CYSTIC FIBROSIS IN DEPENDENCE ON CLINICAL STATUS

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Patients with cystic fibrosis (CF) have a high free radical load due to chronic infection and inflammation. Recent studies demonstrate protective effects of antioxidants vitamins and co-factors. Optimal vitamin E status has been shown to reduce lipid peroxidation and optimal alfa-1-antitrypsin levels are positively related to lung function. The aim of this study was to correlate some of the non-enzymatic antioxidant levels in pediatric patients with cystic fibrosis. This was a prospective cohort study of pediatric patients from our cystic fibrosis center in Košice aged 2-18. All patients are with mild to severe pulmonary disease and pancreatic insufficiency. They were treated with standard doses of vitamin E as part of multivitamin prescription according patient´s body weight. Serum level of vitamin E, Alfa-1-antitrypsin were collected. Body mass index (BMI) of the most our patients were significantly decreased, as well as vitamin E and alfa-1-antitrypsin. Deficiency of antioxidant in our cystic fibrosis patients was common. New strategies in supplying vitamin might help to better balance the vitamin and other non-enzymatic antioxidants status of CF patients.

BIOMECHANICAL PROPERTIES OF THE ARTIFICIAL FIBRIN CARTILAGE

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Pathological changes in cartilages during ageing, acute or chronic diseases, such as osteoarthritis and/or rheumatoid arthritis often lead to pain, decreased mobility and invalidism. Contemporary approaches in cartilage repairing employ chondrocytes embeded in biodegradable polymers, such as collagen, hyaluronic acid, polyesters, fibrin, chitosan, etc. Three-dimensional scaffolds are reported to improve chondrocyte re-differentiation capacity, supply them with nutrition, and provide them with an appropriate mechanical support (1,2). The aim of this study was to determine the static loading diagram, the Young`s modulus, the Poisson`s ratio, and also the failure strength of the composite collagen/hyaluronate/fibrin scaffold and their changes after drying up. The static loading as well as dynamic forced vibrations have been applied. We have found a good correlarion between static and dynamic values. Several hours lasting drying resulted in a substatial increase of stiffness and flexibility decrease which was strongly dependent on the thickness of dryed layer. While a considerable increase of the elasticity modulus has been reached in 3 to 4 hours for a 1-mm-thick layer, a similar elasticity modulus for the 2-mm-thick layer has been observed after 6 to 24 hours.

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VASCULAR SMOOTH MUSCLE CELLS ON SYNTHETIC POLYMERS PATTERNED WITH ADHESIVE MICRODOMAINS

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We prepared synthetic polymers patterned with cell-adhesive and non-adhesive microdomains by plasma polymerization. The following monomers were used for plasma polymerization: acrylic acid (AA) as hydrophilic layer suitable for cell adhesion and proliferation and 1,7-octadiene (OD) as a hydrophobic material to which the cells attach poorly (1). The second monomer was polymerized through a mask with slots that were 75 or 150 μm wide. Vascular smooth muscle cells (VSMC) derived from the thoracic aorta of Wistar rats (passage 4) were seeded on the surface at the initial density of 50 000 cells per ml and well. The number and distribution of cell on the samples were evaluated microscopically from 6 hours to one week after seeding in one-day intervals. The VSMC adhered and proliferated preferentially on the strip-like AA domains i.e., 84.5 % and 63.3 % of cells were found on these regions on day 1 and 7 after seeding, respectively. The population density of VSMC on AA strips was significantly higher than that on regions formed by 1,7-octadiene, and even exceeded the values on pure AA without microdomains on day 1, 3 and 7 after seeding (by 25, 37 and 6 %, respectively). These results suggest that plasma polymerization is a suitable method for producing surfaces useful for

regionally selective adhesion and directed growth of cells on biomaterials for tissue engineering.

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CARDIORESPIRATORY RELATIONS IN GENERAL ANAESTHESIA

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Each anaesthesia, all anaesthetic drugs and methods of anaesthesia have either stimulatory or inhibitory impact on the cardiovascular system. That's why the tonus of the vegetative nerve system, its changes and influence of drugs applied during anaesthesia are important. The direct inhibitory impact of some anaesthetic drugs on the myocardium (eg. thiopental) is clinically drab or does not show on ECG, because at the same time the catecholamines are released (1,2). Some anaesthetic drugs have influence on the heart, others on the blood vessels. Cardiac rhythm disturbances during anaesthesia are frequent, but usually remain clinically undetected. Besides effect of the anaesthetic drugs the reason for arrhythmias can be reflectoric influences from the operation field and the respiratory pathways, but also hypercapnia or hypocapnia (1,2,3). The hemodynamic influence of the ventilation is discussed in the second part of the poster. The mutual influence of circulation and ventilation is during spontaneous and artificial ventilation. Changes of the lung volume during respiration have influence on the right afterload, on direct mechanical interactions heart-lungs and on the change of the vegetative tonus (4). Spontaneous inspirium has a vagolytic influence. Liberal hyperventilation or directed hyperinflation has on the other side vagostimmulatory influence. Changes of intrathoracic pressure have an impact on the systemic venous return and left preload. Preload of the left ventricle decreases by increase of the respiratory volume over 15 ml/kg, i.e. above the functional residual capacity volume, which leads to compression of capillaries in the alveolar septa. These changes have a great role eg. at hypovolaemia (5).

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STRESS INDUCED CHANGES OF FORMAZAN PRODUCTION IN RAT BONE MARROW CELLS

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Rats (Sprague-Dawley) were exposed to the subsequent short termed stressors: whole body irradiation (6 Gy), immobilization 1x45 min, resp. 7x45 min (for 7 days), injection, high temperature (hot plate 55 °C, max. 2 min) and frequent handling. Naloxone - antagonist of endogenous opiates was i.p. injected in a single dose (8 mg/kg b.w.) 30 min after the last immobilization. Bone marrow cells were isolated from rat femur and colorimetric MTT microassay was performed immediately and after 24 h of cells cultivation. Hematocrit values were also examined. Our results showed that: the lowest formazan values and the decrease in hematocrit was found in irradiated rats. Succinate-tetrazolium reductase activity dropped by 27 % in 7x immobilized rats compared to controls. Naloxone injection eliminated stress induced decrease of formazan production in bone marrow cells indicating that the rats with administrated naloxone withstood stress better than those with saline solution. Protective effect of naloxone might be important in resistance to reactive oxygen species in bone marrow cells.

NON ALCOHOLIC STEATOSIS OF LIVER IN CHILDREN (NASH) -PATOPHYSIOLOGICAL ASPECTS

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First information about child NAFLD appeared in English literature in 80-ties. Spectrum of histological abnormalities defined as Non-Alcoholic Fatty Liver Diseases (NAFLD) includes condition ranged from simple steatosis to the extreme form called Non-Alcoholic Steato-hepatitis (NASH). Basic characteristics of NAFLD are 2 conditions: 1. presence of steatosis of liver, that is state, when the amount of lipids in the tissue of liver rises from 10 to 15 %. The second condition is that the steatosis of liver is not caused by excessive intake of alcohol. Etiology: Etiological agents which participate in the beginning of non alcoholic steatosis of liver according to Mehta can be divided into primary and secondary. The primary are associated with insulin resistance: Diabetes mellitus 2. type, B. Obesity, C. Hypertriglyceridaemia. Secondary agents: A.NAFLD caused by drugs- methotrexat, corticoids, B. Surgical Interventions, C. Metabolic influences: acute starving, MAS. There are two important conditions in the pathogenesis of NASH: accumulation of triglycerides in hepatocyte and contemporary metabolic or inflammatory stress in the liver. This theory is called „two hits theory". Steatosis is defined as accumulation of fat in hepatocytes, mainly triglycerides. We accuse oxidative stress which leads to damage of hepatocytes, necrosis and fibrosis and consider it as a basis of second hit in the steatotic liver. Excess of free fatty acids in the cycle of mitochondrial beta-oxidation casues production of reactive oxygen species ($O2^-$ and OH^+). Both of them cause peroxidation of lipids. The products of peroxidation of lipids- aldehydes are highly reactive and they react with the molecules of respiratory chain of mitochondrias and disable the transfer of electrons. The respiratory chain is reduced. It transmits the electrons to O_2, thus increases the productions of ROS and cells are damaged. These aldehydes can activate stellate cells as well, and participate on fibrosis. Chronic inflamatory stimulation in the liver leads to proliferation of stellate cells. Activated cells produce extra cellular matrix. When the synthesis surpasses the ability of degradation, accumulation of matrix occurs. We can find marks of fibrosis in liver biopsy.

INFLUENCE OF HEPARIN ON THE APOPTOSIS OF JURKAT CELLS

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The use of anticoagulants is an inevitable part of the oncological disease treatment. The anticancer therapy (i.e. chemotherapy and surgical therapy) may increase the occurrence of the thromboembolic complications. In this context, the use of heparin and its derivatives is of a preventive character. Heparin eliminates the thromboplastic activity of the circulating tumour cells, and influences the microvasular permeability, thus blocking the progression of the metastatic process in fact. The antiproliferative effects of heparin also seem to be very interesting. Lately, there have also increased the proofs of antioncogenic and apoptosis inducing effects of heparin (1,2,3). The mechanism of these effects is not clear. In this context, it is inevitable to focus on the influence of a possible proliferation of healthy tissues. The potential weakening of lymphocytes population in oncological patients can have a negative effect. The aim of this study is evaluating the abovementioned apoptosis induction effects of heparin on lymphoblastic Jurkat T cells model population. Jurkat T cells were cultivated in- 10 x 10^3 i.u., 5 x 10^3 i.u., 2,5 x 10^3 i.u. unfractionated

heparin/ 5ml of cultivation medium. Viable cells were evaluated by MTT test after 72 hours cultivation. Apoptic and necrotic exact ratio in each concentration was evaluated by flow cytometry. The outcomes have not established a significant effect of heparin on T cells proliferation and apoptosis induction. Therefore, it is not possible to generalize the proapoptic effects of heparin, which were presented in the literature.

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THE CYTOGENIC AND HORMONAL ACTIVITY OF THE EWES OVARY IN THE COURSE OF OESTRUS

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The histological changes in follicular populations and endocrine activity in the ewes ovary of 10 intact (K), 19 synchronized (S) as well as 10 synchronized + PMSG stimulated oestrus (SS) groups were studied. The oestrous were synchronized by 20 mg of chlorsuperlutine vaginal sponges 12 days. The ovary were stimulate by i.m application of 500 I.U of PMSG on last day of synchronization. The pituitary gonadotropins (Gn), progesterone (P4) and 17-beta oestradiol (E2) were monitored in the course of 2 days before and 3 subsequent days after oestrus by RIA. The ovary were processed by standard histological method and stained by haematoxilin-eosin. The size-category type of the ovarian follicles were processed according (1). The follicular atresia and karyometric investigation of granulose and lutein cells were analysed ia application of Lucia system softwer and atresia criteria (2). We found the significant increase of average number of ovulations after combine hormonal synchronization and Gn stimulation in seasonally anoestrous ewes (0.8 versus 1.7). The number of gonadotropin depending antral follicles (category 6) and degree of atresia is seems to be significantly different after hormonal synchronisation and ovary stimulation (SS=11n/33 %) in comparison to simple synchronized (S= 5n/9 %) or intact ewes (K=1./5 %) . The FSH:LH ratio in the day of oestrus decreases from 1:4.4 in intact ewes till 1:1.7 in synchronized and Gn stimulated ewes, where as in the course of first days after oestrous the ration FSH: LH levels were significantly close and became to be nearly similar in intact and stimulated synchronized ewes 1:1.5, 1:1.1, 1:1 and 1:1.4. The P4 levels on the third day after oestrous were in the similar manner as FSH and LH in all groups but E2 levels does not copy the FSH and LH levels with exception only in stimulated synchronized ewes where these remain relative in high level in as well as after oestrous. These hormonal and cytological consequences in time of the fertilization are depended on previous hormonal status in course of pro- and oestrous phase.

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DIFFERENTIAL EFFECT OF STREPTOZOTOCIN-INDUCED DIABETES ON CENTRAL AND PERIPHERAL STRUCTURES OF THE CIRCADIAN SYSTEM OF WISTAR RAT

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Most living organisms from prokaryotes to humans express a variety of physiological and behavioural rhythms, endogenous in their nature, that are driven by self-sustained oscillators. In mammals, the master circadian pacemaker localised in the suprachiasmatic nuclei of the hypothalamus has dominant role in regulation of circadian rhythms. Lately peripheral oscillators in the heart, liver and kidney were discovered. Pathways that are used to synchronize components of the circadian system have been shown to be susceptible to several pathophysiological conditions. Alterations in input pathway or peripheral oscillators and consequently inappropriate internal synchronisation can negatively influence function of desynchronised structures. In our study we investigated effects of streptozotocin (STZ)-induced diabetes mellitus on the circadian system at the level of melatonin synthesis and expression of per2 and dbp in the heart and liver in 8 weeks old Wistar rats. This model allows investigation of relationships inside the circadian system under conditions of precisely defined metabolic disturbances and unchanged neural pathways. Expression of clock gene per2 and transcriptional factor dbp were measured by real time PCR. STZ-induced diabetes mellitus had more pronounced effect on per2 expression in the liver than in the heart. We observed a pronounced phase advance in the rhythm of dbp expression in both, the liver and the heart. The melatonin rhythm was not influenced by streptozotocin administration under LD conditions. Changes in gene expression in the heart and liver can be caused by altered input pathway or changed function of the peripheral clock.

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CHANGES OF ANTIOXIDANT VITAMINS IN RATS AFTER LONG-TERM ADMINISTRATION OF CADMIUM

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Cadmium (Cd), as a toxic metal, still attracts an attention because it is often detected in the air, water and food products extending the maximum allowable limits. The aim of the study was to evaluate the effects of cadmium on antioxidant vitamins in rats after a subchronic peroral administration. Male and female Wistar albino rats aged 74 days, of average weight 235.0±43.26 g were housed in conventional condition on a normal laboratory diet and supplied with drinking water. Cadmium was applicated to the experimental group (n=12) as a cadmium chloride compound ($CdCl_2.2H_2O$, Sigma) in dose LD_{50} of Cd in drinking water divided into 90 daily doses. The LD_{50} value of Cd as $CdCl_2$ per os for rats is 225 mg/kg. The rats in the control group (n=12) received drinking water without Cd. After 90 days of treatment, rats were anaesthetized with Natrium pentobarbitale (50 mg/kg, i.p.) and blood samples were taken from the heart using heparin as an anticoagulant. The concentrations of vitamin E was determined by HPLC, and vitamin C by colorimetric method according to Roe and Kuether. The measurement of plasma total antioxidant status (TAS) with Randox kit (Randox, lab., UK) was carried out on an automatic spectrophotometric analyser Cobas Mira S (Roche, Switzerland). Content of Cd in the liver was analyzed using an atomic absorption spectrophotometer (Unicam Solar, 939). During experiment the content of Cd in the liver of rats in the experimental group was increased significantly ($p<0.001$). The plasma total antioxidant status as an integrated marker of all plasma antioxidants was decreased significantly ($p<0.001$) from 1.19 to 0.88 mmol/l. The decrease of TAS could be an answer of plasma antioxidants to an elevated production of reactive oxygen species. The concentration of vitamin E was reduced significantly ($p<0.05$). As a result of rats possibilities to synthetize vitamin C and reduce the negative effects of cadmium intoxication we found a non significant increase the concentration of vitamin C. These results showed that cadmium intoxication has an unfavourable effect on antioxidant status caused consumption of extracellular antioxidant.

ADHESION AND GROWTH OF VASCULAR ENDOTHELIAL AND SMOOTH MUSCLE CELLS ON POLYTETRAFLUORO-ETHYLENE VASCULAR PROSTHESES

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Clinically used vascular prostheses are constructed as bioinert, i.e. not allowing for adhesion of thrombocytes, vascular smooth muscle cells (VSMC) and immunocompetent cells in order to prevent restenosis of these grafts. We focused on the adhesion and growth of VSMC and vascular endothelial cells (EC) in cultures on blood vessel grafts made of expanded polytetrafluoroethylene (ePTFE, Goretex®). The prostheses were cut into samples 1 x 1 cm in size, sterilized in autoclave, put into polystyrene test plates TPP (Switzerland; 24 wells, diameter 1.5 cm), and seeded on the luminal surface with VSMC derived from the rat thoracic aorta (seeding density 15700 cells/cm^2) or bovine pulmonary artery EC (line CPAE, 8100 cells/cm^2). VSMC were cultured for 7 days in 1 ml of the medium DMEM supplemented with 10 % of fetal bovine serum (FBS), and EC in the medium MEM with 2 mM L-glutamine, Earle`s BSS with 1.5 g/l sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate and 20 % of FBS. The test plates TPP or glass coverslips (Menzel-Glaser, Germany) served as control surfaces. Two days after seeding, both cell types adhered to the prosthesis by a markedly smaller adhesion area and in lower numbers, which reached in VSMC only 42 % and 57 % of those on the glass and polystyrene, respectively. EC on ePTFE reacted even more sensitively (37 % and 31 % of the values on the glass and polystyrene, respectively). Their number did not change significantly during the whole experiment, whereas VSMC exhibited a certain growth activity. Their population doubling time between the 2nd and 7th day after seeding was 114, 48 and 43 hours on the prosthesis, glass and polystyrene, resp. Although VSMC on the prosthesis did not reach the final population density found on the control standard cultivation materials, the results suggest that the PTFE grafts are not absolutely bioinert, and significant restenosis due to VSMC proliferation could occur after some time of exposure to these cells. Therefore, it seems to be more appropriate to design the vascular grafts as bioartificial, i.e. with confluent anti-thrombogenic and anti-immunogenic endothelial cell layer which helps to maintain the VSMC in a non-proliferative contractile phenotype.

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GLUCOCORTICOID EFFECT ON SELECTED CELL LINES AND ITS MODULATION BY LIGANDS OF PERIPHERAL BENZODIAZEPINE RECEPTORS

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Glucocorticoids play important roles in many biological processes including metabolism regulation, cellular proliferation and differentiation (1). They exert antiproliferative effect on a number of cell types, such as those of lymphoid, fibroblastic, epithelial and bone origin, which is the main reason for their use in immunosuppressive, anti-inflammatory and anti-cancer therapy. One of the most important problems associated with glucocorticoid therapy is development of resistance. This study was designed to investigate if a synthetic glucocorticoid, dexamethason, is able to induce apoptosis in selected human cell lines (Jurkat- T-lymphoblastic leukaemia, HeLa-cervical carcinoma, U-87MG and U-373MG-human malignant gliomas). At the same time, we attempted to modulate its effect by diazepam a peripheral benzodiazepine receptors ligand. In connection with selected cell lines and their response to glucocorticoid action we were interested in basal expression of antiapoptic protein bcl-2 and expression of tumor suppressor p53. We estimated cell survival by standard MTT test. Using flow cytometric analysis, we have determined changes in cell cycle and basal expression of MDR1 protein, which is considered to be responsible for a multidrug resistance in many types of cancer. Basal expression of bcl-2 and p53 protein was detected by western blot. Seventy-two hour incubation with both tested substances resulted in significant decrease in cell survival in Jurkat, HeLa, and U-87MG cell lines. We did not observe any changes in cell survival of U-373MG cell line. Subsequent cell cycle analysis revealed apoptosis induction in HeLa and U-87MG cell lines after 24-hour incubation with dexamethason. This effect was potentiated by diazepam in U-87MG. No changes in cell cycle were observed in Jurkat and U-373MG. Finally, we investigated if such a response related to basal expression of MDR1 in examined cell lines. However, no such a relation was observed. To conclude, we suppose, that resistance to glucocorticoid action, might have direct connection with expression of mutant p53 tumor supressor in U-373MG and over expression of bcl-2 in Jurkat cells. However, to prove this hypothesis, further investigation will be required.

Miller et al.: Endocrinol Metabol 555-711, 1995

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HEART RATE INCREASE AT THE ONSET OF EXERCISE: RELATIONS TO HEART RATE VARIABILITY

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During physical exercise heart rate (HR) increases by parasympathetic withdrawal and increase of sympathetic activity to the heart. The analysis of HR variability (HRV) in time and frequency domains provides information about autonomic control of cardiovascular system. Nonlinear analysis using the Poincaré plot method is able to provide supplementary information about cardiac autonomic control. The aim of this study was to determine the association between HRV parameters and the rate of initial increase of HR at the onset of exercise (on-response). HR was continuously monitored in 17 healthy male subjects (mean age: 20.3±0.2 (SEM) years) at rest (25 min supine; 5 min standing), during exercise (8 min of step test at 70 % of maximal power output) and in recovery phase (30 min supine). HRV analysis in time and frequency domains and evaluation of the Poincaré plot measures (length, widths) were performed on selected segments of heart rate time series. HR on-responses were quantified using exponential curve fitting technique. The time constants T_{on} respresenting the rate of on-responses to exercise were computed for each recording. The lower time constant is an indicator of faster HR increase at the onset of exercise. Post-exercise HRV indices and time constant of on-response – Ton – to exercise were negatively correlated. From the pre-exercise HRV indices only several Poincaré plot parameters were correlated with Ton. In conclusion, the pre-exercise HRV parameters are not closely correlated with the rate of cardioacceleration at the onset of exercise. On the other hand, post-exercise HRV parameters are related to the rate of initial adjustment of HR to exercise referring to the importance of rapid HR on-response for a faster recovery after exercise.

BAROREFLEX SENSITIVITY AS AN INDIVIDUAL CHARAC-TERISTIC FEATURE

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It is known that a decrease in 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 therefore useful to know, whether BRS/BRSf characterizes also a healthy individual. The aim of this study was to test reproducibility of baroreflex sensitivity with respect to coherence between the variability in systolic blood pressure (SBP) and pulse

intervals (PI). SBP and PI were recorded beat-to-beat for 5 min (Finapres, controlled breathing at 0.33 Hz) in 116 subjects (aged 19-24 years) sitting at rest three times in periods of one week. The power spectra of SBP and PI, cross-spectra and coherence were calculated. BRS was determined in a frequency range of 0.067-0.133 Hz. Eight indices were tested: $BRS_{0.1Hz}/BRSf_{0.1Hz}$ - the value at a frequency of 0.1 Hz; $BRS_{COHmax}/BRSf_{COHmax}$ - the value at a maximum coherence; $BRS_{Wcoh}/BRSf_{Wcoh}$ - weighted value with respect to coherence values in the whole frequency range; BRS_{Wpcoh}/BRS_{WPcoh} - weighted value with respect to coherence for frequencies with coherence above 0.5. Significance of the difference between intraindividual and interindividual variability of baroreflex sensitivity with respect to the method of BRS/BRSf determination was evaluated by MANNOVA test. Correlation between mean values of BRS/BRSf and distribution of values measured in each subject was evaluated by Pearson correlation coefficient. All the indices revealed lower intraindividual than interindividual variability (MANNOVA test; $p<0.001$). Significant correlation was found between average BRS/BRSf and standard deviation of individual BRS/BRSf values for all indices (Pearson´s correlation coefficient ranged between 0.43 and 0.98; $p<0.001$). In spite of resting variation of BRS/BRSf, baroreflex sensitivity is an individual characteristic feature with the highest reproducibility at its low values. Increased dispersion of resting BRS/BRSf values at their higher mean values could reflect reactivity of a high central gain of the baroreflex.

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THE LINK BETWEEN THE LEVELS OF CIRCULATING INFLAMMATORY CYTOKINES AND PULMONARY HYPERTENSION IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Pulmonary hypertension is a frequent complication in the course of the chronic obstructive pulmonary disease (COPD) and has a negative impact on the prognosis in such patients. In COPD patients, elevated levels of various circulating inflammatory cytokines have been described, namely tumour necrosis factor- α (TNF-α), C-reactive protein (CRP), and interleukines (IL-8 and IL-6). Transgenic mice overexpressing TNF- α, developed emphysema and pulmonary hypertension, associated with changes in the pulmonary vessel wall. The objective of our study was to determine whether the presence of pulmonary hypertension in COPD patients is linked with the elevated levels of TNF-α and CRP. In 43 consecutive patients with COPD, lung function was assessed using bodyplethysmography, pulmonary artery pressures (PAP) were determined using echocardiography. In all patients, peripheral venous blood samples were collected, while no clinical and/or laboratory signs of acute infection nor other ilness known to raise the levels of cytokines, were present. Plasma TNF-α level was measured by using enzyme-linked imunoassay (ELISA) and plasma CRP level was measured by using spectrophotometry. Patients were divided into two subgroups depending on pulmonary artery pressures (for the diagnosis of pulmonary hypertension, the cut-off value of the systolic PAP was ≥ 30 mm Hg). 23 patients showed the presence of the pulmonary hypertension, whilst in 20 patients the systolic PAP below the cut-off level was found. Both mean TNF-α and CRP levels were significantly higher in the pulmonary hypertension subgroup compared to the subgroup without elevated PAP (23.32±8.31 pg/ml versus 5.82±3.11 pg/ml, $p<0,005$; 6.13±1.19 mg/l versus 3.06±0.83 mg/l, $p<0.05$, respectively). In COPD patients with the evidence of pulmonary hypertension, the chronic systemic inflammatory process is more pronounced. Further studies are needed to clarify the effects of circulating cytokines on the pulmonary vascular changes leading to pulmonary hypertension.

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POSSIBILITY OF H_2 BREATH TEST USING IN DIAGNOSTICS OF GASTROINTESTINAL TRACT BREAKDOWNS

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Hydrogen breath testing is a precisious, non-invasive and powerful procedure for measuring expired hydrogen concentrations (in parts per million-ppm), in order to diagnose the cause of gastrointestinal symptoms. The tests are based on the change in breath-hydrogen concentration from basal (fasting) levels after using a test dose of an appropriate sugar. The production of H_2 by bacterial fermentation of carbohydrate substrate in the colon is the basis for the tests of malabsorption, bacterial overgrowth and small intestinal transit time.

The next diagnoses can be auxiliary by H_2 breath test:

- Carbohydrate malabsorption
- Lactose intolerance
- Carbohydrate breakdown deficiencies
- Bacterial overgrowth
- Intestinal transit time
- Sucrose malabsorption
- Fructose malabsorption
- Sorbitol malabsorption

Specificity and sensitivity of H_2 breath test to gastrointestinal disorders will be evaluated in comparison to ordinary gastrointestinal diagnostic methods. The results of H_2 breath test depending on diagnoses of patients will be presented after two months of H_2 measurements in patients with gastrointestinal problems hospitalized in the II. Department of Children and Adolescents Medicine in Košice.

THE ONSET OF ESTRUS CYCLE AND HORMONAL CHANGES IN FARMED FALLOW DEER (Dama dama) FOLLOWING SEASONAL ANOESTRUS

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In our experiment we monitored concentration of progesterone, estradiol 17β, LH and onset and repetition of estrus cycles with its detection after anoestrus period in fallow deer. There were used five fallow deer does (n=9), three to five years of age and 30-35 kg of body weight. Concentration of hormones was monitored in animals reared in north hemisphere in mild climatic region. Fallow deer does are profound seasonaly polyestric animals. Signs of estrus were monitored in our latitude at the end of October and during November. The lenght the estrus cycle increased and became more variable as the season proceeded but was not affected by age of the doe or liveweight. The onset of the first signs of estrus following the anoestrus period is in 13-14 days which is characterized by occurence of one or more silent ovulations what confirms concentrations of progesterone manitored at this period, associated with short-lived corpora lutea. Mean duration of the estrous cycle in farmed fallow deer during the major rutting season is 21 days with a range of 19-22 days. Our results pinpoint these hormonal changes: preovulatory level of LH release reached 20 ng/ml approximately 4h following the onset of estrus, the range of progesterone concetrations was 0,1-0,3 ng/ml and estradiol 17β level reached 23-25 pg/ml. These hormonal changes found in farmed fallow deer are similar to those of other deer species so far investigated.

PRIMARY CHANGES IN VENTRICULAR REPOLARIZATION RELATED TO BLOOD PRESSURE VALUES AND R-R INTERVAL DURATION IN DIFFERENT SYMPATHERGIC SITUATIONS

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The pattern of ventricular repolarization is considered to be physiologically con-trolled by the variation of the adrenergic discharge to the ventricular myocardium. The aim of this study was to assess the effect of R-R interval duration and of the blood pressure (BP) on the ventricular repolarization variability at rest and in different situations with increased sympathetic drive of the cardiovascular system (handgrip, MA, head-up tilting), as well as in relation to the new JNC and EHS BP classification. Altogether in 211 male subjects (aged 24.5±13 y., HR 74±14/min, BP 126±13/81±9 mm Hg, BMI 24.9±4.4) - ECG, Frank lead system VCG and 80 electrodes body surface maps were recorded at rest and in above mentioned test situations, using a PC based system CARDIAG. BP was measured oscillometrically by an OMRON device. Selected parameters were evaluated by ANOVA, regression analysis and the Student's t-test. Compared to the subgroup with BP < 120/80 mmHg, already in the range of resting BP 120-135/80-85 mmHg, a decreased magnitude of the maximal spatial repolarization vector (sTmax) by 14 %, of the tip-to-through amplitude of the QRST body surface map (AMPL) by 19 %, an increased spatial angle between integral QRS and STT vectors (ANGLE) by19 % and shortened R-R by 9 % ($P<0.02 – 0.01$) were found. Analogical changes were observed also during the sympathergic test situations, namely to the head-up tilting, with significant R-R shortening by 21 %, diminution of sTmax by 27 % and AMPL by 25 % and an increase of ANGLE by 57 %. At rest there was a strong correlation between sTmax and R-R intervals+BP, accounting for 53 % of sTmax variance. It was weaker in reactive situations, e.g. during MA test it participated only on 25 % of the sTmax variability or less A complete dissociation between the reactive repolarisation and HR changes was observed in 22 subjects. They responded to different stimuli by the same tachycardic reaction, but by significantly different changes of repolarization parameters. Over the dividing value of casual BP ≤120/80 mmHg, already in the class defined as prehypertensive (JNC-VII) or normal (EHS 03), the repolarization parameters point to some increase in the sympathetic outflow to the ventricles. These changes are similar to those, observed under mental or physical strain. BP and HR changes contributing to the repolarization parameters variability, may reflect an increased sympathetic outflow to the cardiovascular system in general, but not necessarily the site-specific sympathetic drive of the ventricular myocardium.

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SINGLE NUCLEOTIDE POLYMORPHISMS AND BREAST CANCER IN EAST SLOVAKIA REGION

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Breast cancer is the most common malignant neoplasm in women worlwide and the first leading cause of cancer deaths in women today. Geographic variations in breast cancer incidence an survival have been reported in the World. These differences are unexplained, but it is suggested that genetic alterations may play role in breast cancer development. A limited number of genes have been identified that explain heritable risks of breast cancer. The association between breast cancer risk and genetic polymorphisms of p53 at codon 72 (Arg72Pro), XPD exon 23 and XRCC1 exon 10 has been investigated by several studies, but the result are not consistent. In the present work we studied association between XRCC1, XPD and p53 polymorphism and breast cancer risk in the East Slovak population involving 60 cancer patients and 49 controls. Individuals having the genotype of the Arg/Arg polymorphism at codon 72 in the p53 gene showed a significant increased risk of breast carcinoma compared with control group. On the other hand, the genotypes of the Lys/Gln and Lys/Lys at the codon 751 in the XPD gene and the genotypes of the Arg/Gln and Arg/Arg at the codon 399 in the XRCC1 gene showed only nonsignificant increased risk of breast carcinoma.

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BEHAVIOR OF RATS IN THE MORRIS WATER MAZE - EFFECT OF AMPHETAMINE TREATMENT

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Studies in humans have shown that there exists an individual predisposition to addiction. The exact mechanisms for differential vulnerability to reinforcing effects of drugs of abuse among individuals are not known. Psychostimulant drug amphetamine (AMPH) belongs among the most frequently misused addictive drugs and therefore it deserves thorough behavioral studies of their own actions and its interactions with stressors. In our previous studies we demonstrated that AMPH influenced the performance of laboratory rodents in several models of learning and memory depending among others on the dose and time of the administration. In this study we investigated the spatial learning and memory of Wistar rats in the Morris Water Maze (1). Treatment of animals was in accordance with the Declaration of Helsinki Guiding Principles on Care and Use of Animals. After three days lasting training we explored the effects of two acute doses of AMPH. The rat performance in space memory was expressed as mean latency and distance to reach the hidden platform. AMPH was administered i.p. in a single dose of 1 or 8 mg/kg. In a dose of 1 mg/kg AMPH slightly decreased the mean latency and distance to reach the platform; this effect disappeared after next 3 hours. AMPH in a dose of 8 mg/kg produced very strongly impaired performance in spatial memory. Most rats did not find the platform in the time limit of 60 s and these rats remained usually in one segment and moved only in the outer ring of the water maze. Also this effect disappeared in the next 3 hours in spite of the fact that the behavior of rats was still strongly modified in their home cages. In a test lasting for 12 days, chronic application of AMPH (4 mg/kg) produced only minor effects that were slightly different in three used rat strains. The effect of AMPH on spatial memory seems to be different from the effects demonstrated in other behavioral tests; examples of these differences are given for the effects of AMPH in the passive avoidance test.

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ISCHEMIC HEART DISEASE IN PATIENTS WITH COPD TREATED BY LONG-TERM OXYGEN THERAPY

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Recent studies indicate that chronic obstructive pulmonary disease (COPD) may be associated with increased risk for the development of cardiovascular diseases. The objective of our study was to determine the prevalence of coronary artery disease (CAD) in patients with COPD treated by long-term oxygen therapy (LTOT). Our study group included 143 patients with severe COPD and chronic respiratory failure, in whom LTOT was initiated. The diagnosis of COPD was based by assessing pulmonary function test using bodypletysmography (Jaeger); the variables evaluated were the forced vital capacity (FVC), the forced expiratory volume in 1 second (FEV1), the FEV1/FVC ratio, the residual volume (RV), the total lung capacity (TLC), and the RV/TLC

ratio. Arterial blood gases were assessed from an arterial sample obtained by the puncture of radial artery. In the cohort of 143 patients with COPD, 116 (81.1 %) were, whereas 27 (18.9 %) were not diagnosed with CAD. Depending on the age, the cohort was divided into three subgroups (group A: < 60, group B: 61-70, group C: > 70 years). The prevalence of CAD increased progressively with increased age (ANOVA, $p<0.001$). In contrast, pulmonary function tests (FEV1 and FVC) were the lowest in group A compared to group B and C. The 1-year survival was not different between patients with CAD (65.5 %) compared to patients without CAD (66.7 %). Our study demonstrates that the prevalence of CAD is very high in patients with COPD. However, 1-year survival does not differ between COPD patients with and without CAD, apparently as a consequence of significantly worse pulmonary funtion in the younger age group of COPD patients.

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STEROID DEHYDROGENASES AND THEIR ROLE IN TRANSFORMATION OF GLUCOCORTICOIDS IN CHICKEN

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Transformation of glucocorticoids into inactive derivatives represents an important mechanism controlling cellular levels of active hormones. These reactions are catalyzed by several enzymes especially by 11β-hydroxysterid dehydrogenase and 20β-hydroxysteroid dehydrogenase. This question has been studied in mammals in detail but much less is known about it in birds. Similarly, the mRNA sequences of mentioned enzymes are known in mammals but not in birds. The aim of our study was: 1) to demonstrate the presence of steroid dehydrogenase activities in different tissues, 2) to determine their kinetic parameters and 3) to assemble possible cDNA sequences of chicken steroid dehydrogenases using unidentified cDNA clones of EST databaze in GenBank. Enzymatic activity was measured on tissue slices of several different tissues in nutrient rich medium in the presence of various cofactors and ^{3}H corticosterone in nM concentration. Kinetic studies were done on microsomal and cytosolar fractions of tissue homogenates in the presence of appropriate cofactors, and increasing substrate concentrations. Steroids were extracted from incubation medium, separated and identified by HPLC using liquid-scintillation detector. We found high activity of 11β-hydroxysteroid dehydrogenase in kidney and oviduct and slightly lower in small intestine. High activity of 20β-hydroxysteroid dehydrogenase was found in kidney, medium in small intestine and oviduct and relatively low in liver. K_M for 11β-hydroxysteroid dehydrogenase was found in nM range whereas K_M for 20β-hydroxysteroid was in μM range. We also designed cDNA sequences for 11β-hydroxysteroid dehydrogenase isoform 1 and isoform 2 and for 20β-hydroxysteroid dehydrogenase. The experiments demonstrating their functional activity in S.cerevisiae and E.coli expression systems are in progress now. Our results suggest that reduction on C20 is a more frequent mechanism of inactivating glucocorticoids in avian tissues than oxidation on C11.

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THE COMPARISON OF NOS ACTIVITY IN THE SPINAL CORD AFTER ISCHEMIA IN VIVO AND IN VITRO

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The activation of the Ca^{2+}-dependent nitric oxide synthase (NOS) during ischemia may lead to overproduction of nitric oxide (NO) in neuronal and endothelial cells and contribute to pathogenesis of ischemia/ reperfusion injury. The aim of the present study was to investigate the catalytic NOS activity in the spinal cord after ischemia/reperfusion and to find the correlation between ischemia in vivo and in vitro. In the experiment we have used the lumbosacral segments of the rabbit´s spinal cord. Spinal cord ischemia was induced by infrarenal balloon occlusion of the abdominal aorta for 15 min (1), ischemia in vitro was performed in conditions of oxygen/glucose deprivation by the incubation of spinal cord slices for 45 min (2). Incubation of ischemic slices in the presence of oxygen and glucose (for 30 min) was used to simulate the sudden restoration of blood supply to ischemic tissue. The catalytic NOS activity was determined by the conversion of L-[^{14}C]arginine to L-[^{14}C]citrulline (3). The control value of the catalytic enzyme activity in the spinal cord achieved 45.422 pmol/mg protein. In comparison to control, the decrease of the enzyme activity was reached after 45 min ischemia (40.55 pmol/mg protein) in vitro and 15 min ischemia (38.394 pmol/mg protein) in vivo. The restoration of oxygen and glucose during 30 min reperfusion caused a marked increase of NOS activity after ischemia in vitro (48.717 pmol/mg protein) and nonsignificant increase during spinal ischema in vivo (46.34 pmol/mg protein). Our results show the enhancement of NOS activity due to reperfusion period after both experimentaly induced types of spinal cord ischemia and suggest that 45 min incubation of spinal cord slices in conditions of oxygen/glucose deprivation is comparable to 15 min ischemia performed in vivo. The model of oxygen/glucose deprivation is a reliable model that could be used for the pharmacological treatment of deleterious effect of free radicals during ischemia and reperfusion.

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ATP-CHLORIDE CHANNEL INTERACTION IN INNER MITOCHONDRIAL MEMBRANE OF RAT HEART

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Ion channels selective for chloride ions have been found in the outer and inner mitochondrial membranes using the electrophysiological methods: patch clamp and bilayer lipid membrane (BLM) method. The observed differences in their single channel conductance, anion selectivity and mechanism of regulation suggest on the presence of several different chloride channels in the mitochondrial membranes. In our study we measured single channel properties and modulation of the chloride channels using BLM method. The submitochondrial particles ("inside-out" vesicles of inner mitochondrial membrane), isolated from rat heart muscle, were incorporated into artificial lipid membrane and the single chloride channel currents were measured. The observed mitochondrial chloride channels at asymmetrical 250/50 mM KCl (cis/trans) gradient had the high variety of conductances, about 60-160 pS and the single chloride channel amplitudes ranged between 1.3-5.0 pA at 0 mV. ATP (0.5-2 mM) differently modulated activity of the chloride channels. They were irreversible or reversible blocked by ATP, or ATP modulated their kinetic. Disulfonic stilbene (DIDS) irreversibly blocked activity of the chloride channels at 100 μM from the matrix side. The observed results suggest that the inner mitochondrial membranes contain different groups of chloride channels, which are distinctly influenced by ATP. Thus the channel-ATP interaction may regulate different physiological and pathological mitochondrial processes, as e.g. mitochondrial volume regulation, the pH gradient and electrical potential across mitochondrial membrane.

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EFFECT OF LOSARTAN ON GEOMETRY OF CONDUIT ARTERIES DURING DEVELOPEMENT OF HYPERTENSION IN SHR

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We evaluated the effect of AT_1 receptor blocade of losartan on blood pressure (BP), cardiac hypertrophy and structural changes. Losartan was administred to spontaneously hypertensive rats (SHR+Los) and control Wistar rats (W+Los) from ages 4 to 9 weeks (20 mg kg^{-1} day^{-1} b. w. by gavage). Untreated Wistar rats and SHR were also studied. BP was measured by the tail plethysmographic method. Rats were killed, perfused (120 mm Hg) by glutaraldehyde fixative and processed for standard electron microscopy. Wall thickness (WT) and inner diameter (ID) of thoracic aorta (AT) and carotid artery (AC) were messured in light microscopy. Cross sectional area (CSA) and wall thickness/inner diameter (WD) was calculated. BP in Wistar+Los group (101±2.3 mm Hg, $p<0.05$) was decreased in comparison to control Wistar rats (109± 1.7 mm Hg). BP in SHR was in comparison to Wistar rats increased (149±2.0 mm Hg, $p<0.01$). Administration of losartan prevented increase of BP in SHR (136±1.0 mm Hg, $p<0.01$). Heart weight/body weight ratio (HW/BW) in Wistar+Los group (3.0±0.1, $p<0.01$) was decreased in comparison to control Wistar rats (4.6±0.3). HW/BW in SHR was in comparison to Wistar rats increased (5.8±0.2, $p<0.01$). Losartan decreased cardiac hypertrophy in SHR (4.2±0.1, $p<0.01$).

	Wistar	Wistar+Los	SHR	SHR+Los
AC				
WT(μm)	27.0±1.2	21.3±0.6 ^{xx}	33.8±0.7 **	31.9±0.7
ID (μm)	743±23.7	825±23.1 ^{x}	714±21.2	792±18.1^{++}
CSAx10^3μm^2	64.8±2.3	56.4±1.8 ^{xx}	79.1±1.5 **	82.2±2.2
WDx10^{-2}	3.7±0.3	2.6±0.1 ^{xx}	4.8±0.2 **	4.1±0.2 $^{+}$
AT				
WT (μm)	64.7±1,0	52.2±1.5 ^{xx}	58.8±1.5 **	56.9±0.8
ID (μm)	1492±24	1460±21	1487±34	1628±33^{++}
CSAx10^3μm^2	316.3±6.6	247.9±8.6 ^{xx}	285.0±9.4 *	300.2±5.0
WD x 10^{-2}	4.4±0.1	3.6±0.1 ^{xx}	4.0±0.2	3.5±0.1 $^{+}$

* $p<0.05$, ** $p<0.01$ in comparison to Wistar, $^{+}$ $p<0.05$, $^{++}$ $p<0.01$ in comparison to SHR, ^{x} $p<0.05$, ^{xx} $p<0.01$ in comparison to Wistar rats.
In conclusion, losartan reduced BP in SHR and had a positive effect on both reduction of cardiac hypertrophy and remodelling of vessel wall of SHR.

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CONCENTRATION OF Lp(a) AND APOLIPOPROTEINS IN OBESE CHILDREN AND ADULTS

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Obesity is an important risk factor, which associates with premature development of atherosclerosis. Lipoprotein (a) – Lp(a) is an independent risk factor of premature atherosclerosis and myocardial infarction (1). The goal of this study was to determine the concentration of Lp(a), apo B_{100} and lipids (total cholesterol – TCH, triacylglycerols – TG, LDL-cholesterol – LDL-CH, non HDL-cholesterol – non HDL-CH and HDL- cholesterol – HDL-CH) in blood serum in 28 boys with obesity (OB) – age: 12±2 years, BMI: 28±2 kg/m^2 and in 32 obese men (OM) – age: 49±9 years, BMI: 30±2 kg/m^2. In the control group were investigated 21 healthy boys without obesity (KB) – age: 11±3 years, BMI: up to 23 kg/m^2 and 21 healthy men without obesity (KM) – age: 45±8 years, BMI: up to 23 kg/m^2. Lp(a) was determined using the immunoturbidimetric method and apo B by electroimmunoassay. TCH and TG were determined using the biochemical tests of Lachema company. We found significantly elevated serum concentration of apo B_{100} ($p<0.01$), TCH ($p<0.01$), LDL-CH ($p<0.001$) and non HDL-CH ($p<0.01$) in both groups OCH and OB. Concentration of Lp(a) was increased in groups OCH and OB. The combination of elevated serum concentration of Lp(a) and apo B_{100} is considered a severe atherogenic risk (2). Children and adults with obesity are threatened also by high serum concentration of lipids (TCH, LDL-CH and non HDL-CH). For early diagnostics of hyperlipidemy in obese is inevitable a regular control of lipid status already in childhood.

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SINGLE, DOUBLE AND MULTIPLE COUGH SOUND DIFFERENTIATION

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The coughing is presented by a sudden expulsion of air which is accompanied by a typical sound. Cough sound of healthy subjects consists of two or one sound burst regardless of spontaneous or voluntary coughing. More sounds burst indicate cough in pathological conditions. This means that cough sound examination can provide important information about the state of health. The occurence of cough burst we have before evalueted only visually with inspection of cough sound pressure curve, what was not precise. Therefore the aim of this study was to objectivify the evaluation of cough sound burst. We analysed the cough sound of 105 subjects (45 healthy volunteers, 25 F, 20M, mean age 21 yrs; 35 asthmatics, 21 F, 14 M, mean age 47.7 yrs; 25 bronchitics, 6 F, 19 M, mean age 69.1 yrs). The diagnosis of COPD or asthma was on the basis of American and British criteria. Patients in each group were selected with approximately the same severity of illness and requirement for therapy. The analysis of the digitized cough sound records was performed using Fast Fourier Transform - a self-developed algorithm was used for further calculation. The sound intensity as a curve of total spectral power was expressed. The local maxima of cough sound intensity were stated on the basis of steepness of total spectral power curve. Single cough sound record contains only one local maximum, double two and multiple sound several maxima (see on Fig. 1).

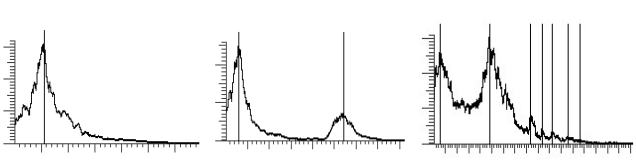

Fig. 1. Recording the total power of the cough sounds. 1. and 2. healthy volunteers, 3. bronchitics. The vertical lines mark the local maxima of cough sound intensity. The number of local maxima of cough sound intensity in patients suffering from chronic bronchitis or asthma was greater than in healthy volunteers.

BRAIN FUNCTIONS IN NORMAL AND NEURODEFECTIVE MICE EXPOSED TO HIGH-FREQUENCY ELECTRO-MAGNETIC FIELD DURING THE FOURTH POSTNATAL MONTH

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We studied the effect of exposure to long-term high-frequency electromagnetic field (HF EMF) on healthy wild type (+/+) and Lurcher mutant (+/Lc) mice of the C3H strain. Lurcher mutants served as a model of olivocerebellar degeneration. They suffer from complete postnatal loss of Purkinje cells, which is caused by a mutation of δ2

glutamate receptor gene (1), and secondary decrease in numbers of cerebellar granule cells and inferior olivary neurons. Mice were chronically exposed to HF EMF (880 MHz) or control conditions for 3 hours a day during the fourth month of postnatal life (day 91-120). After the exposure we examined spatial learning in the Morris water maze (2) and motor skills, CNS excitability by means of the audiogenic epilepsy method. Spontaneous cortical activity was registered using the agar electrodes (3). Spatial learning ability was poor in both wild type and Lurcher mutant mice. Spatial learning, motor functions and CNS excitability were not changed by HF EMF exposure. Frequency spectra analysis showed clear shift to lower frequencies during HF EMF exposure with higher differences in wild type. Our previous experiments showed that HF EMF exposure during the first postnatal month also had no effect on spatial learning, while exposure in young adult mice (the second postnatal month) ameliorated spatial learning ability (4, 5). It means the HF EMF effects depend on the age of the experimental animals. The highest sensitivity was found in young adult mice.

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INFLUENCE OF RUMINANT AMNIOTIC FLUID FRACTIONS ON CELL PROLIFERATION

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Cell proliferation of developing foetus is influenced by insulin-like growth factors (IGF-I and –II) and their binding proteins (IGFBP-1) present in amniotic fluid (1,2). This fluid plays important role in the protection of fetuses from bacterial infections (3) and on the contrary supresses mitogen-stimulated lymphocyte proliferation (4, 5). IGF-I and –II can modulate certain functions of the immune system (6). The aim of this study was to determine the mitogenic effect of some peptide components of bovine amniotic fluid on bovine peripheral blood lymphocytes. The next aim of our work was the determination of mitogenic activity of ovine amniotic fluid fractions to mouse fibroblasts (BP-A31 cells) and in conclusion, the comparison of the mitogenic activities of amniotic fluid fractions of cow and sheep species. The proliferation of lymphocytes was not significantly changed after the addition of natural bovine amniotic fluid likewise when the delipidated ovine amniotic fluid was added to BP-A31 cells, there was no effect. On the other hand both inhibiting and activating effects on tested cells were found after separation of amniotic fluid. The inhibitng effect ($p<0.05$) was found in the case of separated bovine amniotic fluid (Peak I) and ovine amniotic fluid (fraction B). On the other hand we have observed activation of lymphocytes by Peak II of bovine amniotic fluid ($p<0.05$) and also of BP-A31 cells by fraction A in case of ovine amniotic fluid ($p<0.01$). Our results confirm that the same growth factor can act differently on the different cell types.

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DEVELOPMENT OF THE MOLECULAR CLOCKWORK IN THE RAT SUPRACHIASMATIC NUCLEUS DURING LATE EMBRYONAL AND EARLY POSTNATAL STAGE

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Molecular clockwork underlying circadian rhythmicity in mammals resides in suprachiasmatic nuclei of hypothalamus (SCN) and consists of several clock genes, namely Per1, Per2, Per3, Cry1, Cry2, Bmal1, Clock and kasein kinase 1 epsilon. The clock genes and their products form transcriptional/translational feedback loops. Rhythmic metabolic and electrical activity of SCN starts already during prenatal development. Our previous results did not, however, reveal any rhythmicity in expression of clock genes Per1, Per2, Cry1, Bmal1 and Clock within the SCN of 19-day-old rat embryos (E19) and the rhythmicity was detected only at postnatal day (P) 3 (1). The aim of this study was to find out when exactly the rhythmic expression of clock gene starts. Pregnant dams or mothers with their pups were kept under 12 hours light and 12 hours dark regime. On the day of experiment, animals were released into darkness and sacrificed in 2 hours intervals throughout the 24 h cycle. Expression of Per1, Per2, Cry1, Bmal1 and Clock mRNA was examined by in situ hybridization at E20, P1 and P2. At E20 no significant rhythmicity of the clock gene was detected. At P1 the difference between day/night levels in Per1 and Bmal1 mRNA became significant and only at P2 a clear rhythmicity in expression of all examined genes except Clock was demonstrated. These results suggest that the molecular core clock mechanism within the rat SCN starts to operate just after birth.

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QRS ISOINTEGRAL MAP EXTREMA OF HYPERTENSIVES WITH AND WITHOUT LEFT VENTRICULAR HYPERTROPHY

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When evaluating left ventricular (LV) hypertrophy (LVH) by electrocardio-graphic (ECG) means, increased voltage and prolonged duration of QRS waves is assumed. The voltage-duration products of QRS complex or more precise time integrals in chest and limb leads may be also used as criteria. Spatial analogy of single leads time integral is the isointegral map (IIM) when using ECG body surface mapping (BSM). We wanted to find out whether the hypothesis of increased values of time integrals holds in hypertensive (HT) patients (pts) with and without LVH when using detailed analysis of IIM QRS extrema.Mean IIM of QRS complex and its thirds were constructed using the electrode system after Barr [1] in 33 HT pts (48.5 ± 13.4 years old, 20 men) divided into 3 groups: HT pts without LVH (HT: 9 pts), with concentric LVH (LVH_C: 19 pts), and with eccentric LVH (LVH_E: 5 pts). LVH was based on echocardiographic examination using the LV mass index due both to body surface area and to height raised to power 2.7 ($h^{2.7}$). Concentric LVH was defined as LVH with relative wall thickness $RWT > 0.45$. Else there was eccentric LVH. None of included patients had LV dilation (internal diameter $LVIDd \leq 5.9$ cm for men and $LVIDd \leq 5.4$ cm for women). Values of mean map extrema were analysed. Patients' data were compared with 14 controls (39.2 ± 12 years, 8 men) without cardiovascular diseeases. Extreme values of time integrals in IIM QRS tended to be lower in LVH_C pts than were the values of control group, group HT and/or LVH_E pts. No significant differences ($p < 0.05$) concerning medians of extrema were found. The highest extrema were observed in LVH_E group, while the lowest ones in LVH_C group. Probably a dominant role in this fact plays the geometry of the heart as well as the heart-chest geometry. Higher extrema in HT group compared to controls are not clear yet.

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THYROID HORMONE MODULATES $[Ca^{2+}]i$ AND ARRHYTHMIA SUSCEPTIBILITY BY ACUTE, NONGENOMIC EFFECTS

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Thyroid hormones are known to modulate several systems involved in the control of Ca^{2+} homeostasis by both, nuclear and extra-nuclear (acute) actions. Since intracellular Ca^{2+} ($[Ca^{2+}]i$) disturbances play a key role in the development of cardiac arrhythmias, including ventricular fibrillation (VF), this study was aimed to examine the acute effect of T_3 on $[Ca^{2+}]i$, susceptibility of the heart to VF and its ability to restore sinus rhythm. Two series of experiments were performed: 1) Isolated hearts taken from rats or guinea pigs were perfused with oxygenated Hepes-buffered Tyrode solution at constant flow and loaded with Fura-2. Fura-2 fluorescence was excited at 340 and 380 nm by UV light administered via the optic fibre probe focused on the surface of left ventricle. The ratio of 500 nm fluorescence intensity excited at 340/380 nm was recorded as a quantitative index of the $[Ca^{2+}]i$. T_3 was administered to the perfusate in range 10^{-8}-10^{-4} mol/l during Ca^{2+}overload that was induced by elevation of $[Ca^{2+}]o$. 2) Isolated hearts taken from guinea pigs were perfused with oxygenated Krebs-Henseleit solution at a constant flow. LVP and ECG were continuously monitored. VF inducibility was examined by using 2s burst of 100 rectangular pps, 0.2 ms duration, 1.5 time threshold voltage in train rate of 10s. The time to spontaneous sinus rhythm restoration was examined as well. T_3 was applied in range 10^{-9}-10^{-6} mol/l. Results showed: 1) Administration of T_3 in lower concentration (10^{-8}-10^{-7} mol/l) had no apparent effects, while in higher (10^{-6}-10^{-5} mol/l) attenuated Ca^{2+}overload. The latter was fully abolished by 10^{-4} mol/l of T_3, however, it was followed by transient dramatic increase of $[Ca^{2+}]i$ that triggered arrhythmias. 2) T_3 in range 10^{-9}-10^{-7} mol/l significantly decreased, while in 10^{-6} mol/l increased susceptibility of isolated heart to electrically induced VF. Besides, T_3 in lower (10^{-9}-10^{-7}), while not in higher (10^{-6} mol/l) concentrations facilitated spontaneous sinus rhythm restoration. In conclusion, these results indicate that there is a relatively "narrow cardioprotective window" due to lower dosage of T_3, while potentially harmful effects due to over-dosage.

RESPONSE OF BLOOD PRESSURE AND REMODELLED CONDUIT ARTERY TO ACH AND BK IN SHR AND SHR TREATED WITH NO DONORS

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In NO deficient hypertension (NODH) discrepancy was found in enhancement of hypotension in vivo and attenuation of relaxation of conduit arteries in vitro to acetylcholine (ACh) and bradykinin (BK) (1). The question is whether a similar phenomenon is present in SHR, with different pathogenesis and vascular wall structure (2). Wistar rats, SHR, SHR given either molsidomine (Mols 50 mg/kg b.w. twice daily) or pentaerythrityl tetranitrate (PETN 100 mg/b.w. twice daily) were studied. After 6 weeks in one half of animals carotid artery under anaesthesia was cannulated for BP registration, and jugular vein for administration of drugs. In the other half iliac artery (IA) was used for in vitro studies. To determine the geometry of IA the animals were perfused with a glutaraldehyde fixative (120 mmHg). IA was processed for electron microscopy. BP was: 132.5±2.7 mmHg in controls, 177.1±5.4 mmHg in SHR, 175.2±2.8 mmHg in Mols, and 176.6±5.7 mmHg PETN group, respectively. In SHR enhanced hypotensive response was found to both ACh (1 μg and 10 μg 87.9±6.9 mmHg and 108.1±5.1 mmHg vs. 35.9±4.7 mmHg and 64.0±3.3 mmHg, $p<0.01$, in controls), and BK (100 μg 106.7±8.3 mmHg vs. 53.3±5.2 mmHg, $p<0.01$, in controls). Similar results were found in SHR administered NO donors. In contrast, maximum relaxation to ACh, measured in vitro, expressed as percentage of phenylephrine precontracted IA was attenuated (12.1±3.6 % in SHR vs. 74.2±8.6 %, $p<0.01$, in controls). Geometry of IA: inner diameter in SHR declined (680±46 μm vs. 828±28 μm, $p<0.01$, in controls). Wall thickness (54.1±2.0 μm vs. 29.0±2.1 μm, $p<0.01$, in controls), wall cross-section area ($123.0\pm6.3\times10^3$ μm^2 vs. $77.3\pm4.6\times10^3$ μm^2, $p<0.01$, in controls), and wall thickness/inner diameter ratio ($8.7\pm0.9\times10^{-2}$ vs. $3.6\pm0.4\times10^{-2}$, $p<0.01$, in controls) increased. No differences in these respects were found among the experimental groups. The findings demonstrate enhanced hypotension and attenuated relaxation of conduit artery to NOS activators in SHR and SHR treated with NO donors. The response pattern is similar to that found in NODH.

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LATE VENTRICULAR POTENTIALS IN CHRONIC RESPIRATORY DISEASES OF EASTERN SLOVAKIA PATIENTS

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Late ventricular potentials are markers of prognostically severe arrhythmias or sudden death. The aim of this study was to obtain whether and how frequently the late ventricular potentials are present in Eastern Slovakia patients with chronic respiratory diseases (chronic bronchitis, chronic obstructive pulmonary disease and chronic respiratory insufficiency partial and/or global). Normal and short-time signal-averaged electrocardiograms were recorded (device Schiller) and electrocardiographic parametres (heart rate, RR and PR intervals, high frequency QRS and QTc durations, amplitude of RMS40 ms, RMS50 ms, high frequency QRS 25-250Hz and 40-250Hz, LAHF 25-250Hz and 40-250Hz durations, axes of maximum P, QRS and T vectors) were compared in 19 young healthy persons (age 19.37±1.12) and 65 patients (40 men, 25 women, age 61.57±13.73) with chronic respiratory diseases (nonparametric z-test). Pathologic late ventricular potentials were evaluated by method of Simson, positive results were in presence of at least 2 pathologic criteria occurrence. Significant differences ($p<0,05$) compared to young healthy persons were obtained in QTc interval duration (442.32±33.43 to 420.58±20.33 ms), RMS40 25-250 Hz (61.42±52.54 to 42.16±18.40 ms), angle of P_{max} (26.52±40.5 to 41.89±17.36 grades) or QRS_{max} vectors (20.0±39.91 to 48.21±27.14 grades) only. Our first results indicate that late ventricular potentials occurr in 26.3 % of Slovak young healthy persons and in 35.4 % of patients with chronic respiratory diseases (insignificant difference, chí-square test). A higher occurrence of late ventricular potentials in chronic respiratory diseases can support the hypothesis on their connection with a higher occurrence of cardiac arrhythmias in those patients and can be connected with very different values of markers of health state in some minorities of Slovak inhabitants which is similar to a situation in other countries.

LATE VENTRICULAR POTENTIALS IN CHRONIC RESPIRATORY DISEASES OF EASTERN SLOVAKIA PATIENTS AND GENDER

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Women are considered to be an arrhythmogenic gender and late ventricular potentials are a marker of severe arrhythmias. The aim of this study was to obtain the gender differences in the frequency of late ventricular potentials in patients of Eastern Slovakia with chronic respiratory diseases (chronic bronchitis, chronic obstructive pulmonary disease and chronic respiratory insufficiency). The ECG and late ventricular potential parametres (heart rate, RR, PR, QRS and QTc interval durations, amplitude of RMS-40 ms, RMS-50 ms, high

frequency QRS 25-250 Hz and QRS 40-250 Hz, LAHF 25-250 Hz, LAHF 40-250Hz durations, axes of maximum P, QRS and T vectors were measured (device Schiller) according to the Simson`s method and compared in both genders during short-term signal averaged ECG in 40 men and 25 women with chronic respiratory diseases (partial and/or global). Significant differences between men and women (at least $p<0,05$, nonparametric z-test) were obtained in high frequency QRS 25-250 Hz only, borderline differences in RMS40 25-250 Hz, RMS50 25-250 Hz, and angle of QRS_{max}. Late ventricular potentials occurred in 16 of 40 men (40 %) and 7 of 25 (28 %) women (insignificant difference, chi-square test), borderline values in 5 of 40 men (12.5 %) and 1 of 25 (4 %) women (insignificant difference). Our first results indicate that a higher occurrence of late ventricular potentials in chronic respiratory diseases of Eastern Slovakia inhabitants can support the hypothesis on their connection with a higher occurrence of cardiac arrhythmias in them, the gender differences of electrocardiographic parametres are mostly insignificant. Maybe larger groups of patients can better explain the gender differences.

EFFECT OF ARGININES ON $CA_V3.1$ CHANNEL ACTIVATION

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Positively charged arginines or lysines in S4 segments are part of a putative voltage sensor of voltage-dependent calcium channels. We have investigated contribution of S4 transmembrane segment in domains I - IV of α_1 subunit of the Cav3.1 calcium channel to the channel gating. Using site-directed mutagenesis we have constructed four channel mutants in which top arginines in each S4 segment were replaced by neutral cysteines (R180C, R834C, R1379C and R1717C). Ion currents through wild type and four mutated $Ca_V3.1$ channels transiently expressed in HEK 293 cells were measured using whole cell configuration of patch-clamp technique. 2 mM calcium was used as a charge carrier. Current-voltage (I-V) relation, maximal current density and voltage dependence of activation were compared. Each mutation reduced maximal current density measured in a maximum of I-V relation (-30 mV). Averaged current densities were 83.6±1.1 pA/pF for wild type, 27.7±0.4 pA/pF*** for R180C, 55.1±1.3 pA/pF for R834C, 53.2±0.9 pA/pF* for R1379C and 37.5±0.4 pA/pF*** for R1717C mutated channel (* $p<0.05$, *** $p<0.001$). Reversal potential was not affected by any mutation. The most significant effect on voltage dependence of channel activation was observed on the channel with cysteine substitution in the domain III (R1379C). Values for half maximal activation voltage and slope factor were: -45.0±1.2 mV and 3.9±0.2 for the wild type; -53.4±0.9*** mV and 4.6±0.3 mV for R180C; -50.8±1.3 mV** and 4.7±0.2 mV** for R834C; 53.2±0.9*** mV and 5.1±0.2 mV** for R1379C and -44.9±1 mV and 4.5±0.2 mV for R1717C (*** $p<0.001$, ** $p<0.01$).

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THE ROLE OF AXONAL DELAYS; COMPARING NETWORKS CONTAINING DYNAMIC AND STATIC SYNAPSES

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Axonal delays reflect the distances that action potentials travel between neurons. It is known that delays play very important role in network dynamics, but they are still frequently omitted by the neuronal network modelling approach. We built in the C language the model of neuronal network with implemented axonal delays and tested how they influence network activity. Several network setups containing synapses modelled by alpha-functions, called here static synapses, were compared with corresponding setups containing more complex, dynamic synapses. The dynamic synapses have four state variables and the time constants of different orders of magnitude. Response of the network to modelled stimulations was studied together with effects of axonal delays, neuronal interconnectivity and the proportion of excitatory to inhibitory neurons on the network output. We found very strong dependence between the degrees of neuronal synchronization and mean axonal delay in the network with dynamic synapses and only weak dependence in the network with static synapses. Different behaviours resulted when the delays were random and when they were fixed, both with the same mean. We found that dynamic synapses enable network to exhibit broader spectrum of responses to given input and they also make the network more sensitive to changes of axonal delays and other network parameters. As a step towards memory modelling, retention of input sequences in the network with static and dynamic synapses was studied under various delay distributions.

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EXPERIMENTAL MODEL OF MILD-DIABETES IN RATS INDUCED BY MULTIPLE LOW DOSES OF STREPTOZOTOCIN

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Diabetes mellitus is a metabolic syndrome that requires long-term medical care allowing both to limit the development of the disease and to prevent accompanying complications. Streptozotocin (STZ) is a widely used diabetogenic agent: its single high dose leads to diabetic complications occuring within a short time period and that are difficult to postpone. The main problem is the validity of the analogy of such a „severe“ experimental model with physiological human conditions. Our goal should be prevention rather than treatment of diabetic complications. In this study we tested the effect of multiple low STZ doses administered to adult rats in a dose of 20 mg/kg b. w. for three consecutive days. We compared two ways of STZ administration – intravenous (i.v.) and intraperitoneal (i.p.). After 3 months of the experiment, the rats were killed by cervical dislocation under thiopental anesthesia. We evaluated body weight, blood pressure, pre- and post-prandial plasma levels of glucose, triglycerides, cholesterol, reduced glutathione and creatinine as well as urine levels of creatinine. To quantify the oxidative stress, the activity of the lysosomal enzyme N-acetyl-β-D-glucosaminidase was studied in the liver, kidney, pancreas and plasma at the end of the experiment. Studies on rings of the superior mesenteric artery, testing the functional integrity of the endothelium, were also done. The obtained results indicated that the observed changes simulate mild-diabetes injury. The comparison of the two ways of STZ application favors i.v. administration. The new model of mild-diabetes induced by multiple low STZ doses should serve as a useful tool for further study of diabetic complications mechanisms and possibilities of their pharmacological treatment.

LAPAROSCOPIC MONITORING OF THE OVARIAN ACTIVITY IN EWE

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Individual ovulation responsiveness to superovulatory treatment is variable and little understood phenomenon. This variability originates in the genetic apparatus of an individual, season, superovulatory treatment so as selection of donor animal. There exist several prognostic markers: constitution, age, weight, selection of animals that have already lambed,

determination of progesterone and oestradiol -17β level in the blood sample. The optimal marker is the macroscopic inspection of the ovaries, oviducts and uterus. This can be performed laparoscopically. Oestrus cycle of a total of 12 Slovak merino female sheep was synchronized using a CIDR devices for 13 days during breeding and non breeding season. To induce multiple ovulations the donors received 18mg FSH i.m. in a decreasing dose level. Animals were used for experiment once a month for 3-5 consecutive months. A total of 48 treatments with CIDR and FSH led to 43 cases (89.6 %) with signs of standing oestrus. An average oestrus began 30±6 h after CIDR removal. Ovulation rate (OR) - mean number 4.5 (range 3-8) was determined from the number of CL present at the ovarian surface laparoscopically in comparison with laparotomy and exteriorization of the ovaries. There were found moderate differences between laparoscopic and laparotomic finding when the superovulatory response exceeded 5CL (82 % vs.100). A total of 130 ova were collected. Recovery rates (RR) varied among individual flushings ranging from 34-97 % (mean number 67 %). RR was similar up to the number of five CL per animal. A significantly reduced RR ($P<0.05$) was obtained when the superovulatory treatment had resulted in more than 5 CL. From a total of 130 ovas recovered 74 (56.9 %) were considered to be fertilized. The highest fertilization rate (FR) was observed in animals with a low or moderate superovulatory response. A total of 33 out of 74 (44.6 %) fertilized eggs were considered morphologically intact and suitable for further transfer. The majority of the embryos were at morula, compacted morula and blastocyst stage. We also observed a high percentage of damaged morulae and blastocyst (31.5 %, 41 out of 130; 55.4 % of the fertilized ova) and empty zonae pellucidae 9.2 % and unfertilized oocytes 43.1 % (not included to fertilized ova).

SOME ASPECTS OF LIFE QUALITY OF PATIENTS WITH DIABETES MELLITUS

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In the past twenty years substantial progress was achieved in the treatment and monitoring of diabetes mellitus. In principle diabetic people could lead a fully active life without substantial hindrances. On the other side new medication schemes, new possibilities in blood glucose monitoring, and other scientifically based procedures alone are not sufficient to ensure good quality of life of people suffering from this lifelong disease. Lifelong education of patients, their motivation through improved communication between patient and the health care providers (doctor, nurse, dietician) alongside with application of modern technologies is the only way to achieve good quality of life comparable with that of nondiabetic people. Our study is aimed to evaluate and validate the questionnares commonly used in different diseases and pathological conditions for middle aged patients with Type 2 diabetes mellitus. We present our modified battery of tests which consist of six parts:

1. Satisfaction with the current therapy
2. Patient's view of his own disease
3. Patient's attitude towards therapy
4. Overall health status
5. Knowledge about the disease and its treatment
6. Ten point overall evaluation of health

After evaluation and validation of the test battery we plan to assess the quality of life in patients treated in outpatient clinics and during hospitalisation and evaluate the results according to parameters of glycemic compensation.

SEASONAL CHANGES OF ANTIOXIDANT STATUS IN RATS

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Oxidative stress, increased lipid peroxidation and decreased activity of antioxidant systems may contribute to the accelerated development of many human and animal diseases. In this work the seasonal changes of red cell antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and plasma total antioxidant status (TAS, the integrated marker of total antioxidant activity of all plasma antioxidants) were investigated in rats. Adult male Wistar albino rats (n=113) aged 91±2 days with an average weight 308±34 g were housed in conventional conditions, without any control of temperature and light. The experiment was organised in two series. The first one was carried out in the summertime (July, August – series A) and the second one in the wintertime (December, January – series B). Antioxidant enzymes and TAS were determined in blood collected from the heart. SOD, GPX and TAS were measured by spectrophotometric methods (RANDOX, UK) on Cobas Mira automatic analyser (Roche), CAT by UV spectrophotometric method on Specord semiautomatic analyser. The GPX activity was significantly higher in the wintertime (series A: 963±127 U/g Hb; series B: 1053±116 U/g Hb; $p<0.001$). Contrary, the plasma TAS significantly decreased in the wintertime (series A: 1.01±0.14 mmol/l; series B: 0.94±0.12 mmol/l; $p<0.005$). We found out no seasonal changes in activities of red cell antioxidant enzymes SOD (series A: 1840 ± 182 U/g Hb; series B: 1905±272 U/g Hb) and CAT (series A: 2.46±0.46 mkat/g Hb; series B: 2.39±0.36 mkat/g Hb). The results demonstrate the significant seasonal differences in two very important parameters of antioxidant status GPX and TAS in rats. It may be an adaptive process of organism to changed environmental conditions. Higher activity of GPX as well as higher consumption of extracellular antioxidants may be related to the elevated needs for antioxidants in wintertime.

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INTOXICATION OF RATS WITH HEAVY METALS IN DRINKING WATER. 1. PRELIMINARY STUDY

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Chromium, cadmium and mercury are widely used industrial chemicals. The toxicity associated with these metals is well known. However, less information is available concerning the mechanisms of toxicity and reprotoxicity parameters after long-term oral administration of heavy metals. A long term-low level exposure experiment (concentration of Pb - 100 μmol/l, Hg -1 μmol/l, Cd - 20 μmol/l in drinking water) was conducted on Wistar rats to determine some physiological parameters. Results are presented in Table 1.

Table 1. Some parameters after long term low-level exposure with heavy metals in drinking water

Parameter	Control	Pb	Hg	Cd
Life time (day)	729±217	664±274	714±248	791±165
Body mass (g)	340±85	237±49	240±85	245±21
Water intake ml/rat/day	42±13	61,5±12	35±5	34±4
Food intake g/rat/day	19.8±3.3	22.6±0.9	16.9±0.6	20.3±3.3
Food intake in g/g of body mass gain	5.5±0.9	7.6±1.5	5.05±0.9	5.1±1.06
Number of partes/number of neonates	49/326	53/416	62/485	45/321
% weanlings from neonates	59	69	67	72
Tumors	-	+	-	+
Morphological malformations	-	+	+	+

The results showed some differetiations among heavy metal groups and control group, and had treatment-time related effects. Most of these changes became more expressed – and, partly, significant – by the ending of the experiment at weeks 130-143.

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THE SIGNIFICANCE OF UNCOUPLING PROTEINS (UCPs) FOR HUMAN THERMOGENESIS

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Nonshivering thermogenesis (NST) due to calorigenic effect of adrenaline was recently described in adult long-term cold adapted men (winter swimmers) (1,2). The aim of this study was to find out if this NST is caused by increase of UCP1 and UCP2 expression and if lipogenesis, respectively the fatty acid syntase (FAS) expression, is affected. The mRNA levels were measured using real time RT-PCR. The samples were obtained by biopsy from subcutaneous abdominal white adipose tissue. We found no expression of UCP1 in any group. The UCP2 expression was insignificantly increased in winter swimmers ($p = 0.09$). On the other hand, it was found that the UCP2/FAS ratio was significantly enhanced ($p = 0.01$) in adapted men, despite the expression of FAS was same in both groups. No correlation of any transcript expression with age, time of hardening, BMI (body mass index) and percentage of body fat was found, except of UCP2/FAS negatively correlated with BMI in winter swimmers. The increase of UCP2 expression can explain the high capacity of NST observed in winter swimmers only partially. It should be taken in consideration that NST can be present also in other tissues, especially in muscles. Winter swimmers are, besides of cold adaptation, also adapted to oxidative stress (3). Thus, it is possible that enhancement of UCP2 expression in cold adapted men contributes also to oxidative stress adaptation that accompanies cold adaptation.

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FLUOFRESCENCE RESONANCE ENERGY TRANSFER (FRET) IN DANSYLATED CREATINE KINASE MOLECULE

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The aim of our study is to bring more light on how conformational changes underlying the cellular control of enzyme activity should be measured. The present study follows up on the purification of creatine kinase (CK) EC 2.7.3.2 from myofibrils (1) and the search for the appropriate fluorophores for FRET. The former combination of donor - acceptor pair, ErITC-IAF blocked CK activity (Cys283 by IAF) (2) and a tested fluorophore FITC, was randomly bound at three sites of the molecule (3). Reaction of CK with dansyl chloride, under reversibly protected cysteines, was carried out as described (4), with a modification of the procedure, when dialysis was replaced by Centricons YM-30 (Millipore, 30 000 MW cut-off). The absorbances at 280 and 340 nm indicate that one mole of the dansyl group has reacted per mole of monomeric CK. The specific enzyme activity of dansylated CK dissolved in 50 mM Hepes buffer pH 8.0: 45.8 $\mu mol.min^{-1}.mg^{-1}$ confirmed a successful protection of cysteines during dansylation. Fluorescence decays and anisotropy-measuring apparatus were based on a laser excitation source and a photon counting detection (Hamamatsu), with excitation at 298 nm and fluorescence collection at 360 nm. The intrinsic tryptophans' fluorescence life time for "CK, CK-ATP and CK-ATP-creatine" decreased from 2.72, 2.38 and 2.42 ns to 2.28, 1.96 and 1.97 ns, respectively, indicating presence of an efficient FRET, being equal to 16.2, 17.6 and 13.6 %, respectively. However, in all three cases the calculated donor - acceptor distances were found to be close to 2.8 nm. Data indicates that the labelled segment of the protein does not significantly change the distance to the tryptophans after binding to both substrates. In order to follow physiological conformational changes we will identify a different place for the attachment of the label.

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ACTIVITIES OF CHOLINERGIC PROTEINS IN APP/PS DOUBLE TRASGENIC MICE

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Malfunction and decrease of number of the cholinergic neurons in central nervous system is common finding in Alzheimer´s disease. It is not clear, however, whether impairment of cholinergic transmission is involved in the early patogenesis of the disease or if it is a reflection of overall damage caused by accummulation of beta-amyloid in the terminal stage. The aim of our experiments was to disclose a possible changes of levels of proteins that play role in cholinergic transmission in mouse model of the disease. Measurements were performed on parietal and prefrontal cortical tissues derived from 9-month-old double transgenic APP/PS1 mice and non-transgenic control littermates. Samples were obtained from University of Kuopio (Finland) in the scope of EU project „Lipidiet". We determined activities of choline acetyltransferase (ChAT), acetylcholinesterase, butyrylcholinesterase, and a number of muscarinic receptors in controls and transgenic animals from two generations. None of the measuerd parameters significantly differed between males and females in control animals. However, results on transgenic animals that were thus pooled irrespective of sex indicated changes between controls and the two generations. These changes were unexpectedly different with regard to both brain region and generation. For example, ChAT activity was significantly higher in parietal cortex only in the second generation while in prefrontal cortex in both generation. The number of muscarinc receptors in parietal cortex was higher in both generations whereas in prefrontal cortex it was apparent only in the first generation. These preliminary results point to the necessity of careful selection of appropriate controls when interpreting results of an experimental treatment. Proportional comparison of the levels of measured proteins between corresponding males and females with regard to the two generations indicated a decrease of ChAT activity in female transgenic animals but not of other analyzed proteins. The decrease of ChAT activity in transgenic females which is not present in control animals point to a higher sensitivity of their central cholinerg neurons to increased level of beta-amyloid.

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INFLUENCE OF GESTAGENS AND EQUINE CHORION-GONADOTROPINE ON TERTIARY FOLLICLE SELECTION AND OVULATION RATE IN MILKING EWES

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The follicle selection and ovulation rate (OR) is the result of a complex interaction between endogenous gonadotropin hormones (FSH, LH), sexual steroid hormones and the sensitivity of the receptors of tertiary ovarian follicles on the trajectory of growth to the above hormones; these follicles can be modulated by intraovarian paracrine and autocrine humoral factors (1,2). In Slovak Merino and Improved Wallachian ewes folliculogenesis and the possibilities of its regulation were studied. In two experiments oestrus synchronization was carried out with preparations on the basis of synthetic gestagen and 500 IU eCG whereas in Experiment 3 a dose of 1000 IU eCG was used in order to induce multiple pregnancies. In Experiment 1 the ewes of flock B and C were treated 30 days after cessation of milk production and 5 days after stopping milking, respectively. In flock C an increased number of twin and triplet births was recorded when compared to flock B ($P<0.05$). In the second experiment 85.3 % of the ewes treated during galactopoiesis (flock E) were mated after oestrus induction whereas in flock D

(unmilked, untreated controls) only 37.5 % were mated (P<0.001). Fertility in flock E was increased when compared to that in flock D (P<0.001). Comparison of fecundity levels revealed that simultaneous induction of fertile oestrus and increased OR caused a significant increase of fecundity (P<0.001). Similarly, significantly increased natality was observed after oestrus induction and simultaneous induction of double ovulation and multiple pregnancies in the flock of ewes that were regularly milked during galactopoiesis (P<0.001). After OR stimulatory treatment estradiol-17β levels in follicular fluid of the selected dominant follicles increased significantly (P<0.001). The results obtained revealed that the galactopoiesis-regulating hormone complex facilitated the terminal phase of the process of dominance of preovulatory tertiary follicles on the trajectory of the growth wave during oestrus.

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IMPROVEMENT OF LEARNING IN WATER MAZE ELICITED BY HYPOXIC PRECONDITIONING IS PARTIALLY BLOCKED BY APPLICATION OF MELATONINE BEFORE THE HYPOXIA

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In previous experiments the impaired learning in water maze was described after one flurothyl seizure. This impairment was blocked by hypoxic preconditioning (3 d before the seizure) and also by application melatonin (1 h before the seizure). Central nervous system could be affected by hypoxia as well as epileptic seizures and they are connected with increase in reactive oxygen species (ROS) production. It may serve as one of signals for activation of preconditioning mechanisms. Therefore we tried to block the increase of ROS after hypoxia by previous (1h) application of melatonin (100 mg/kg). 3 days after hypoxia one seizure was elicited by flurothyl and 24 h later the animals were tested in water maze. Experiments were performed on adult male Wistar rats. The rats after these procedures learned worse than preconditioned rats without melatonin. This result supports our hypothesis that ROS may play some role in preconditioning as well as in impairment of learning in water maze after one flurothyl seizure.

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DETERMINATION OF MEMBRANE DEFORMABILITY AFTER EXERCISE BY MEANS OF THE HEMOLYTIC ACTION OF MERCURY IONS

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Twenty-two healthy young male between 15-19 years of age, took part in this study. They were subjected to a series of exercise on a veloergometer loaded at two submaximal levels of 1.5 W/kg and 2.0 W/kg, both at 60 steps/min for 4 min and one maximal level, which commenced at 3.0 W/kg at 70 steps/min and increased every every minute by 0.15 w/kg. The exercise was terminated when the frequency of the steps could not be regularly maintained. Blood samples were taken before and after exercise. Erytrocyte membrane deformability was studied by means of cation osmotic haemolysis (1,2). The haemolytic action of $HgCl_2$ (0.15 mmol/l) was studied in relation to the ionic strength of NaCl in the incubating medium. Ionic strength ranged from 0.0 to 154.0 mmol/l of NaCl, the isotonicity being corrected with glucose. The haemolysis was comprared with a control sample incubated in distilled water (100 % haemolysis). The degree of haemolysis in the tests being measured spectrophotometrically at 540 nm. The significance of differences was evaluated using Student´s test. The course of the haemolysis curves before and after exercise was similar, showing a non-significant decrease after exercise, with one exception of one individual where difference was significant.

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HYDROGEN PEROXIDE DOES NOT AFFECT THE METALLOPROTEINASES SYNTHESIS IN RBL-2H3 MAST CELLS

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MMPs play an important role in collagen turnover and participate on structural remodelling of peripheral pulmonary arteries. These processes result in hypoxic pulmonary hypertension. Mast cells are an abundant source of MMPs. In our previous studies we confirmed that 24 h of in vitro hypoxia increases synthesis of metalloproteinases (MMPs) in isolated rat lung mast cells (1) and in RBL-2H3 mast cells. We also showed that antioxidant N-acetylcysteine (NAC) decreases MMPs formation in RBL-2H3 mast cells exposed to hypoxia. The mechanism of mast cells activation is unknown. We hypothesized that mast cells activation during hypoxia is triggered by reactive oxygen species (ROS). Present study was designed to determine whether hydrogen peroxide in low levels affects MMPs production in RBL-2H3 mast cells. The RBL-2H3 mast cells were seeded (50 000 cells per well). The control group C and three experimental groups with descending H2O2 concentrations (100 μM, 10 μM and 1 μM) were cultivated for 24 h in air. Cell-free cultivation media were drain off and calcium ionophore A 23,187 was added. Four sets of each group were examined. MMPs activity was estimated by using fluorescent substrate. Presence of interstitial rodent-like collagenase MMP-13 in the cells was visualised by immunohisto-chemistry. Sets of 100 cells were examined. ANOVA and Fischer's PLSD were used for statistical evaluation of the data. Total MMPs activity in media and positive marked cells were similar in all tested groups, ANOVA ($p<0.05$). Our results showed that hydrogen peroxide did not affect MMPs production and that the mechanism of mast cells activation seems to be more complicated.

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11β-HYDROXYSTEROID DEHYDROGENASE AND COLONIC INFLAMMATION IN MICE

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Glucocorticoids (G) are widely used for treatment of inflammation diseases. On the other hand there is a limited knowledge of local metabolism of these hormones in inflamed tissue. 11β-hydroxysteroid dehydrogenase (11HSD) catalyzes interconversion of G to their less potent 11-oxo metabolites and thus this enzyme might influence the local level of these hormones direct in the inflammatory tissue. Whereas 11HSD type 2 (11HSD2) is only able to decrease local level of G and is expressed in mineralocorticoids target tissues including colonic epithelium, 11HSD type 1 (11HSD1) in vivo increases predominantly the local concentration of G and is expressed in nonepithelial tissues such as colonic lamina propria. Variations in the expression of 11HSD's can lead to self-control of inflammation. The aim of this study was to ascertain the changes in 11HSD1 and 2 expression during experimental colitis in mice. The second aim was to prove the presence of these enzymes in immune cells, which can infiltrate the inflamed intestine. The study was performed on Balb/c mice (DSS model of acute and chronic colitis) using real-time RT-PCR. We found that mRNA

11HSD1 was up-regulated in acute colitis and even more up-regulated in chronic colitis. 11HSD2 displayed the opposite trend. The chronic colitis led to decreased level of mRNA 11HSD2. The mRNA of inflammatory cytokines IL-1β and TNF-α were increased in both type of colitis. 11HSD1 was found in macrophages and T-lymfocytes isolated from spleen. The signal was also obvious in mesenteric nodes but no changes of mRNA 11HSD1 were found during chronic colitis in these nodes. In conclusion, our results show the significant changes of 11HSD's during colitis that might influence the control of inflammation in colon. The results from mesenteric nodes did not correlate with the observation in the colon but we cannot exclude that the up-regulation of 11HSD1 is caused by immune cells themself or by up-regulation of some cells of lamina propria origin.

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THE SUBSTRATE-DEPENDENT THREE CONFORMATIONS OF MUSCLE CREATINE KINASE

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Muscle creatine kinase EC 2.7.3.2 (CK) has been selected for studies of conformational changes that underlie the cellular control of enzyme activity. Computed force fields indicate that the molecule poses the substrate - dependent three conformations, namely a substrate free inactive "open", ATP conjugated reactive "closed" and ATP-creatine conjugated nonreactive "intermediary" forms (1). In order to confirm the three conformations experimentally, the rabbit muscle CK were dissolved at room temperatuire in 50 mM Hepes buffer (10 μM CK, pH 8.0, specific activity 38.5 $\mu mol.min^{-1}.mg^{-1}$), with and without CK reaction substrates. Fluorescence decays and anisotropies were measured using an apparatus based on a laser excitation source and a time-correlated single photon counting detection with a cooled MCP photomultiplier (Hamamatsu, R3809U-50). A second harmonics of the synchronously pumped picosecond dye-laser (Spectra Physics, model 375) served for excitation of samples at 298 nm. Fluorescence was collected at 360 nm. The intrinsic tryptophans fluorescence lifetimes for "CK, CK-ATP and CK-ATP-creatine" were 2.72, 2.38 and 2.42 ns, respectively, indicating conformational transitions with consequent changes of the Trps microenvironment, after ATP and ATP+creatine ligand bindings. Rotational correlation times reflecting overall hydrodynamic property of the protein were found to be 35, 27 and 29 ns, respectively. The correlation time values indicate the proportional changes in radii of CK-free and CK-liganded molecule gyration. The results provide evidence for triple CK conformers, which are consistent with computed data.

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HEAT SHOCK PROTEINS CONCENTRATION IN HEALTHY SUBJECTS AND IN ISCHEMIC HEART DISEASE PATIENTS

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Myocardial ischemia is a result of an imbalance between the myocardial oxygen need and actual coronary oxygenated blood supply. Heat shock proteins (Hsp) play an important role in myocardial protection against ischemic eventually reperfusion damage (1). In experimental models of myocardial ischemia an increase of Hsp in damaged tissue was noted (2). We have examined 15 healthy individuals (M=8, F=7) aged from 16 to 63 years and group of 35 patients with ischemic heart disease /IHD/ (M=18, F=17); 16 patients had myocardial infarction (MI). Venous blood was taken into Vacutainer test tubes; serum was placed into NALGENE container. Concentration of Hsp 70 was measured by using ELISA method and diagnostic kit StressGen EKS 700. Average serum Hsp 70 concentration in healthy subjects was 1484+388 ng/ml; in IHD patients the values were significantly higher – 3298+1095 ng/ml ($p<0.05$). Patients who underwent MI had lower serum Hsp 70 values (2707+668) compared to 3796+1150 ng/ml in patients without MI ($p<0.05$). Significant age influence on Hsp 70 concentration in IHD patients was noted ($p<0.05$): increased age is characterised by decrease in serum Hsp 70 values. Analogical results were obtained in animal models of ischemia (2). In IHD patients who take antioxidants regularly the Hsp serum concentration was significantly lower ($p<0.05$) than in patients without antioxidants use (2772+581 vs. 3795+1243 ng/ml). Our results in healthy subjects are similar to those of Pockley's (3). As a possible consequence of weaker heat shock proteins cardio protective effect, patients with myocardial infarction could be characterised by lower Hsp 70 concentration.

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M_2 MUSCARINIC RECEPTORS ACTIVATE DIFFERENT G-PROTEINS IN THE AGONIST SPECIFIC MANNER

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Muscarinic receptors belong to a large family of G-protein coupled receptors. There are five subtypes of these receptors denoted M_1-M_5. Odd numbered receptors couple preferentially with phospholipase C (PLC) via $G_{q/11}$ class of G-proteins while even numbered receptors preferentially inhibit adenylyl cyclase via $G_{i/o}$ class. We have found previously an unusual coupling of the M_2 receptors with G_s proteins that depended on a nature and concentration of agonist and also on receptor density (1). In presented experiments we have investigated whether M_2 receptor can also activate $G_{q/11}$ G-proteins. For experiments we have employed Chinese Hamster Ovary Cells (CHO) which express always only one subtype of muscarinic receptors. Stimulation of M_2 receptors by carbachol, acetylcholine and oxotremorine-M increased the accummulation of inositol phosphates about four-fold, by furmethide and methylfurmethide about two-fold, and oxotremorine had no effect. Inactivation of $G_{i/o}$ proteins by pertussis toxin only slightly attenuated carbachol stimulation while $G_{i/o}$ mediated inhibition of adenylyl cyclase was abolished. Pertussis toxin had no effect on PLC activity in CHO-M_1 cells but increased stimulation of cAMP synthesis indicating inhibitory coupling of M_1 receptors with adenylyl cyclase via $G_{i/o}$ proteins. Altogether these findings confirm that stimulation of PLC was not mediated by preferential Gi/o proteins and indicate that both M_1 and M_2 receptors can couple with all investigated classes of G-proteins. An increase of density of M_2 receptors resulted in an increase of inositol phosphates accummulation and cAMP synthesis while preferential inhibition via $G_{i/o}$ proteins of cAMP synthesis did not change. The increase of M_2 receptor density also led to a decrease of EC_{50} of carbachol for all these responses. Our results indicate that muscarinic M_1 and M_2 receptors are able to activate also other than their preferential G-proteins. Different efficacies of tested agonists to stimulate various responses suggest that individual agonists induce different conformational changes of receptors and in this way the interaction with other G-proteins and their functional outcome.

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EFFECT OF THE PERINATAL ALCOHOL ABUSE ON THE DEVELOPMENT OF NEURONAL POPULATION IN THE HIPPOCAMPUS

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This study could contribute to explication of neurotoxic substance, in this case alkohol, onto CNS of laboratory rats in the prenatal period. Next project goal of this experiment is morfological analysis of the hippocampus after prenatal exposure to alkohol and detection of the most vulnerability areas of the hippocampus to alkohol effect. Male Wistar rats of our own breed were use for these experiments. There were 24 animals in the experimental group. Experimental group of pregnant Wistar rats was exposed to 20 % alkohol solution effect. Alkohol was applied every day, i.p., from 1th day to last day of the gravidity (21 days) in dose 2 g/1000 g live weight. Kontrol group of pregnant Wistar rats was exposed to appropriate dose of normal saline solution. After birth from 1th day till the 34th of the age the young animals were together with their mother and weren't exposed to alcohol effect. The 35th days of postnatal age were young animals perfused under deep thiopenthal anesthesia with buffred solution of the paraformaldehyd. Brains of these young animals were sliced in the frontal plane into 40 μm thin section with a cryostate. Then were sections stained by combinations DNA staining Hoechst and Fluoro – Jade B (This staining makes possible identification extinctive neurons). Preparatives were examined under a epifluorescent microscop Olympus AX-70. In the area CA1 of the hippocampus we could observe groups of degenerative cells. In the area CA3 we could observe groups of degenerative cells also. There are very interesting cells with fine granulated nucleus and a lot of glial cells. From our results clearly arise neurotoxic activity of the alcohol and very hight vulnerability of progressive CNS. Very noteworthy was detection extentive cells 35th day after the final day of the last exposition to alcohol. It is possible discuss about starting of long-term process in the juvenile tissue, probably apoptosis. The identification of cells with fine granulated nucleus can testify to apoptotic mechanism of the cell deth.

QT DISPERSION ESTIMATED FROM BODY SURFACE IS DETERMINED BY DIPOLAR CHARACTER OF ELECTRICAL FIELD OF THE HEART

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Standardized markers for detailed analysis of cardiac repolarization from surface ECG are still not available. This study was aimed to determine relative contribution of dipolar (global) as opposed to non-dipolar (local) sources of cardiac electrical field to the constitution of ECG signals on body surface, specifically the duration of QT interval (QTi). Body surface potential mapping (BSPM) was used to obtain QTi in surface ECG signals from 80 unipolar leads distributed regularly around the chest. Simultaneously, 12 lead ECG and vectorcardiogram (VCG) were recorded. In a simple calculation, VCG-obtained dipole was used as a source of simulated cardiac field and the distribution of QTi minima in the system of measurement electrodes analogous to the BSPM system was estimated. The theory and thus calculated data imply that QT intervals of shortest durations appear in the leads that are perpendicular to the terminal vector of T wave. In all cases of measured QTi (n=18 healthy volunteers), the distribution of shortest intervals correlated well with the localizations predicted by the calculation. All the QTi absolute minima fell into the predicted area. Variable duration of electrical activity in different leads may indicate local inhomogenities in cardiac repolarization. However, in all the cases that were examined, short and the shortest QT intervals were identified in the region where minimal durations are expected due to dipolar (global) character of the field. Thus we assume that repolarization times estimated from body surface are not sensitive enough to discover local disturbances of electrical field of the heart. Detailed analysis of VCG and T wave morphology by means of computation model is believed to provide further insight.

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SURFACTANT LUNG LAVAGE AND ASYMMETRIC HIGH-FREQUENCY JET VENTILATION IN THE TREATMENT OF MECONIUM ASPIRATION SYNDROME

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Surfactant lung lavage improving meconium removal and lung functions becomes a method of choice in the treatment of neonatal meconium aspiration syndrome (MAS). On the other side, asymmetric high-frequency jet ventilation (HFJV) removing materials from the lungs, has not been tested in MAS. Aim of our study was to assess effects of surfactant lung lavage (SLL) combined with asymmetric HFJV on meconium removal and lung functions. Forty-one rabbits were anesthetized and ventilated conventionally. Animals were intratracheally given suspension of human meconium (25 mg/ml, 4 ml/kg). When respiratory failure developed, 4 groups of animals were lavaged with saline or diluted exogenous surfactant (Curosurf, Chiesi Pharm.) during conventional or asymmetric HFJV. After the lavage, animals were ventilated for the next 1 hour with the same type of ventilation than during the lavage. Two more groups were lavaged with saline or diluted Curosurf during asymmetric HFJV and after the lavage were ventilated conventionally for the next 1 hour. Surfactant lung lavage removed significantly more meconium than saline in both surfactant groups. SLL combined with asymmetric HFJV was more effective in meconium removal than SLL with conventional ventilation. SLL significantly improved lung compliance and gas exchange, and reduced right-to-left pulmonary shunts and requirements for ventilation, however, oxygenation was better during conventional ventilation after the lavage. Combination of SLL and asymmetric HFJV was superior to SLL combined with conventional ventilation in meconium removal. In the post-lavage period, conventional ventilation was more suitable type of ventilation than HFJV.

CHANGES OF PULMONARY, CARDIOVASCULAR AND INFLAMMATORY PARAMETERS IN ADULT RABBITS AFTER THE MECONIUM ADMINISTRATION

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Meconium aspiration causes airway obstruction, inactivation of surfactant, inflammation and pulmonary vasoconstriction. Our goal was to evaluate changes of several pulmonary, cardiovascular and inflammatory parameters after meconium administration in adult rabbits. Twelve rabbits were anesthetized and ventilated with room air. Initial values of parameters were recorded and blood samples were taken. Then 3 animals were sacrified and used as controls. Other animals were administered 4 ml/kg of saline (n=4) or meconium (25 mg/ml, n=5) and were further ventilated with 100 % oxygen. When respiratory failure developed, parameters were recorded and animals ventilated for next 5 hours. Animals were then sacrified and lungs were excised. Right lungs were dried at 60 °C 24 hours to determine wet/dry ratio, left lungs lavaged with saline (3x10 ml/kg). Lavage fluid was centrifuged and supernatant used for biochemical analysis. Differential WBC count from the blood and sediment of lavage fluid was evaluated microscopically. Instillation of meconium led to significant worsening in dynamic lung compliance, ventilation efficiency index and gas exchange in comparison with saline group. Mean blood pressure, heart rate and central venous pressure were without significant differences

between groups. Right-to-left pulmonary shunts increased after both meconium and saline, with significantly higher shunting in meconium group. Meconium caused higher edema production and neutrophil accumulation in the lungs with changes in biochemical inflammatory parameters. Changes of evaluated parameters indicate that the rabbit model of meconium aspiration syndrome may be used for testing various medicaments, e.g. influencing inflammation.

PHARMACOLOGICAL MODULATION OF URINARY BLADDER SMOOTH MUSCLE REACTIVITY IN VITRO

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Nowadays, new therapeutic approaches are sought for treatment of functional disorders of urinary bladder, which could prevent the decrease of patients` quality of life (1). The aim of presented study was to assess the mechanisms participating in urinary bladder smooth muscle reactivity (UBSM), its inter-species differences and age dependence, as well as the possibilities of its pharmacological modulation. In vitro method of organ bath was used in experiments (2,3). The UBSM strips were prepared from guinea pigs, rabbits and humans. Contractile response curves were evaluated after administrating the contractile mediators in cumulative manner. 15 minutes incubation with tested agent was done before repeated administration of contractile mediator. The author confirmed that the UBSM reactivity could be evaluated in in vitro conditions in different kinds of laboratory animals and in different conditions. Comparing to humans and rabbits, the UBSM reactivity to acetylcholine and carbachol in guinea pigs was significantly increased. The acetylcholinesterase inhibition resulted in increased UBSM reactivity to acetylcholine in guinea pigs. However, the reactivity to carbachol was not significantly influenced. In adult rabbits, the UBSM reactivity was significantly higher than that of neonate rabbits. Oxybutynin as selective antagonist of muscarinic M_3 receptors (4) caused significant diminution of UBSM reactivity in guinea pigs, which was amplified by adding of propranolol and indomethacine; propantheline and dicyclomine caused decrease of UBSM reactivity, which, however, did not reach the effect of oxybutynin. Selected xanthine derivatives produced decrease of UBSM reactivity in rabbits; caffeine and teophylline had similar efficiency, which was significantly higher than that of aminophylline.

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MELATONIN ACTION IN THE GASTROINTESTINAL TRACT OF RAT

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Melatonin similarly to its precursor 5-hydroxytryptamine occurs both in the central nervous system and the gastrointestinal tract (GIT). However, his role in the latter has been only partially characterised. Smooth muscle contractions, blood flow, ion transport, proliferation of cells and scavenging of free radicals are the processes modulated by melatonin in the GIT (1). The aim of the present study was to assess the effect of melatonin on the ion transport of rat distal colon and to determine the mRNA expression of melatonin receptor MT1 in the rat intestine both at control and fasting conditions. Distal colons were isolated from adult rats, partially stripped, mounted in Ussing chambers and bathed in Krebs & Ringer solution. Short-circuit current (Isc) was measured in the absence or presence of various concentrations of melatonin and several secretagogues (PGE_2, bethanechol, serotonin and histamine). Real-time RT-PCR was used for quantification of MT1 mRNA expression using total RNA isolated from epithelial as well as sub-epithelial layer of several intestinal segments. Melatonin applied from both serosal and mucosal side influenced Isc. In approximately 50 % experiments melatonin increased Isc but the increase did not exhibit classical sigmoidal dose-response relationship and thus it is possible that melatonin affects different systems at different concentrations. In spite of this ambiguous response elicited by melatonin itself, the hormone significantly inhibited the secretory response of PGE_2 but not the response of bethanechol or histamine. Preliminary results suggest that MT1 mRNA is present in epithelial as well as sub-epithelial layer with higher expression in the latter. The expression pattern of the MT1 in rostro-caudal axis is homogenous in sub-epithelial layer but not in epithelial layer where the major signal was found in duodenum and colon. Fasting increased the expression of MT1 mRNA in sub-epithelial but not epithelial layer in the distal part of the intestine.

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MUSCARINIC RECEPTORS AND ADRENOCEPTORS DISTRIBUTION IN THE RAT HEART

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The autonomic nerves release the transmitters that could activate a number of adrenoceptor subtypes and muscarinic receptors. As there exist some obscurities in the expression of mRNAs and their receptors in the heart, we have investigated the expression of adrenoceptor (α_{1A}, α_{1B}, β_1, β_2, β_3) and muscarinic receptor (M_2) mRNAs and their binding sites (β_1, β_2, α_{1A} α_{1B}, muscarinic receptors) in rat left and right atria with or without neuronal ganglia, left and right ventricles and septum. For detection of receptor binding sites we have used radioligand binding experiments with ^{3}H-QNB (specific ligand for muscarinic receptors), ^{3}H-CGP (specific ligand for β-adrenoceptors), and ^{3}H-prazosin (specific ligand for α_1-adrenoceptors). β_1, β_2-adrenoceptors were distinguished using antagonists CGP20712A (for β_1-adrenoceptors) and ICI 118.551 (for β_2-adrenoceptors), α_{1A}-, α_{1B}-adrenoceptors were distinguished using antagonists RS 17053 (specific ligand for α_{1A}-adrenoceptors) and l-765,314 (specific ligand for α_{1B}-adrenoceptors). The mRNA levels were determined using RT-PCR. We have found that mRNAs for muscarinic receptors and α_{1B}-adrenoceptors differ in their distribution in above mentioned heart regions. The differences were also evident at the level of the binding sites. On the other hand, the mRNAs for β_1- and β_2-adrenoceptors were almost equally distributed throughout the heart regions, but their transcripts (determined using ligand binding experiments) significantly differ in the region distribution. The remaining subtypes (α_{1A}, and β_3-adrenoceptors) mRNAs were also expressed in all heart regions, but there were significantly lower than the main subtypes (α_{1B} and β_1-adrenoceptors). Taken together, our results have shown that: 1) muscarinic receptors and α_{1B}-adrenoceptors differ in the expression in heart regions both on mRNA and receptor level, 2) although the mRNA for β_1, β_2-adrenoceptors do not differ in the distribution throughout heart region, the receptor expression of these receptors do, 3) we were able to find the β_3-adrenoceptor mRNA in all heart regions (including ganglionic and non-ganglionic tissue), that is the new finding according to our knowledge.

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IMMUNOHISTOCHEMICAL DETECTION OF MnSOD FOLLOWING ISCHEMIC PRECONDITIONING AND POSTISCHEMIC REPERFUSION IN THE RAT BRAIN HIPPOCAMPUS

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We studied MnSOD immunopositive neurons occurrence in a rat model of cerebral ischemia (1) and reperfusion under conditions of ischemic tolerance induced by a short-time (4 min) sublethal ischemia followed by 2-day interval, after which the lethal (20 min) ischemia was performed. Results were collected after different reperfusion time intervals (5 hours, 1, 2, and 4 days) and compared to sham-operated controls. We found from the previous studies, that 2-day intraischemic interval is the most appropriate because of the greatest induction of SOD as an effective endogenous free radicals scavenger (2) in the postischemic nervous tissue. Sections from rat brain were incubated with the primary MnSOD antibody overnight, and visualized by the avidin-biotin-peroxidase complex ABC and DAB staining. Sections were scanned by digital camera system (Olympus DP-50), analysed by Image Tool software and positive results are evaluated as a percentage of dark stained area in 1 mm^2 of whole rat brain hippocampus and in the CA1 pyramidal layer, which is known as the selective vulnerable region to ischemic insult. Comparison among obtained results showed that the highest number of MnSOD positive neurons was found in CA1 hippocampal sections with 1-day postischemic reperfusion (4i+2dR+20i+1dR = 7.88±1.42 %) and this increase was statistically important at the level $p<0.01$ to sham-operated control (SOC = 3.44±0.88 %). Other postischemic reperfusion time intervals (5hR, 2dR, or 4dR) did not show important differences, although interval 5h of reperfusion (4i+2dR+20i+5hR = 4.06±0.68 %) gave increased results and they were decreased in time course 2 (4i+2dR+20i+2dR = 3.15±0.47 %) or 4 (4i+2dR+20i+4dR = 2.04±0.50 %) days, respectively. Results obtained from whole hippocampus did not show so important differences and they were at low percentual level (from 0.44±0.17 to 0.93±0.18 %). MnSOD plays a key role in scavenging superoxide, which is generated during ischemia-reperfusion. The enzyme is nucleus encoded and localized in mitochondria, which is protected by this way from superoxide mediated damage.

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EFFECT OF SIGMA RECEPTOR LIGAND HALOPERIDOL ON CARDIAC EXCITABILITY

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Sigma receptor ligand haloperidol is a psychotropic drug used in the treatment of various psychiatric disorders. Severe cardiovascular side effects (mostly ventricular arrhythmias) have been reported. Thus, we have investigated the effects of haloperidol on the sodium current I_{Na} and potassium currents, the transient outward current I_{to}, the current at the end of 250ms-impulse $I_{K,end}$ (that is mainly composed of the delayed rectifier current I_K) and the inward rectifier current I_{K1}. Experiments were performed on enzymatically isolated rat ventricular cardiomyocytes by whole cell patch clamp technique at room temperature. Haloperidol inhibited reversibly and in concentration-dependent manner amplitudes of all tested ion currents with 39 % inhibition of I_{Na}, 39 % inhibition of I_{to} and 14 % inhibition of $I_{K,end}$ in the presence of 1 μmol/l and 95 % inhibition of I_{Na}, 80 % inhibition of I_{to}, 37 % inhibition of $I_{K,end}$ and 29 % inhibition of I_{K1} in the presence of 10 μmol/l haloperidol. Inhibition of I_{to} was voltage-independent, with a small (-1.4 mV; $P<0.05$) hyperpolarizing shift of the steady state inactivation curve. The apparent inactivation of I_{to} was accelerated in the presence of haloperidol (τ = 27.4±3.3 ms in the absence and 6.9±2.3 ms in the presence of 10 μmol/l haloperidol). Inhibition of both other tested potassium currents $I_{K,end}$ and I_{K1} did not depend on membrane voltage as well. We conclude that haloperidol causes reversible and concentration-dependent inhibition of sodium and potassium membrane currents in rat ventricular cardiomyocytes with the highest effectivity on I_{Na} and I_{to}. In the case of potassium currents, the inhibition was voltage-independent. The observed acceleration of apparent inactivation of I_{to} after aplication of haloperidol is a typical sign of interaction with I_{to}-channels in the open state. Simultaneously, the negligible effect of haloperidol on the steady state inactivation curve of I_{to} implies no interaction with the channels in the inactivated state. However, further examination with lower haloperidol concentrations is needed in order to explain frequent ventricular dysrythmias in haloperidol-treated patients.

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SEX DEPENDENT CHANGES IN MUSCARINIC, α_{1A}, α_{1B}, α_{1D}-, β_1- and β_2-ADRENERGIC RECEPTORS IN LUNG TISSUE OF CRH KO MICE AFTER IMMOBILIZATION STRESS

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The biosynthesis of catecholamines, mainly adrenaline, is the crucial event in the develompment of stress reaction. Glucocorticoid hormones play the important role in this process because of their importance in activation of PNMT (phenylethanolamine N-methyltransferase) transcription, the enzyme that catalyzes the transformation of noradrenaline (NA) to adrenaline (A). We investigated whether the absence of CRH (corticotropin releasing hormone) affects the densities of muscarinic (MR), α_1- (AAR) and β-adrenergic receptors (BAR) in the mice lungs during stress reaction and if there exist any difference between males and females. Lung tissue can serve as a model of tissue where the effects of sympathetic neurotransmitters and parasympathetic neurotransmitter display antagonistic effects on lung function. Therefore, it is possible to suppose the existence of heterologous regulation between these receptor systems. [^{3}H]QNB, [^{3}H]CGP12177 and [^{3}H]prazosin were used as specific markers of MR, BAR and AAR, respectively. β_1-, β_2-AR were distinguished using antagonists CGP20712A (for β_1-AR) and ICI 118.551 (for β_2-AR), α_{1A}-, α_{1B}- and α_{1D}-AR were distinguished using antagonists RS 17053 (specific for α_{1A}-AR), L-765,314 (specific for α_{1B}-adrenoceptors) and BMY7378 (specific for α_{1D}-AR). The amounts of receptor binding sites in CRH KO mice were decreased in comparison to their wild type counterparts. This decrease was more prominent in males, strikingly in AAR densities. The immobilization stress brings about profound decreases in MR, BAR and AAR both in WT and KO animals, especially females. The changes in BAR densities were more expressed in CRH KO females, the changes in AAR and MR densities were more pronounced in WT mice. In CRH KO males there was not significant decrease in AAR. The stress have decreased the amount of BAR and MR, but it was not as prominent as in CRH KO females. We can conclude that: 1) CRH knock-out is able to change the densities of MR, AAR and BAR in the lung tissue; 2) reaction to stress differs in CRH KO animals and WT mice; 3) the receptor densities as well as reaction on stress treatment in CRH KO animals depends on sex. Our results showed that lacking of CRH influence the development of stress reaction on the level of receptors.

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SOCIAL STRESS AND ULTRASTRUCTURE OF CARDIO-MYOCYTES AND AORTIC WALL CELLS OF BORDERLINE HYPERTENSIVE RATS

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Genetic predisposition to hypertension can represent important trigger in etiology of heart disease. The aim of our study was to examine effect of social stress produced by crowding on ultrastructure of myocardium and aorta of rats with family history of hypertension. Male adult borderline hypertensive rats (BHR) were exposed to 6-week crowding stress (5 rats /cage, 200 cm2/rat). Control rats were kept 4 rats /cage (480 cm2/rat). Blood pressure was determined non-invasively. Basal blood pressure of BHR was 132.2 mm Hg. Crowding stress increased significantly blood pressure ($p<0.01$ vs. basal value) and relative weight of right ventricle, while without alterations in the relative weight of left ventricle. Crowding stress had no influence on NO synthase activity in the heart however, in aorta it was reduced by 52 % ($p<0.005$ vs. control). The heart and aorta were perfusion fixed with 2 % glutaraldehyde and tissue from left and right ventricle and aortic rings were routinely processed for electron microscopy. Experimental stress in BHR rats induced moderate ischemia-like subcellular alterations in cardiomyocytes of both ventricles. A part of cardiomyocytes in the right ventricle revealed structural markers of hypertrophy and capillaries revealed structural markers of angiogenesis. In aorta, stress resulted in serious injury of endothelial cells: they were edematous, contained enhanced amount of vacuoles, lysosomes and Weibel-Palade bodies. Most smooth muscle cells had normal architecture, but some contained vacuoles with degraded membrane structures. Our results showed that social stress induced subcellular alterations in myocytes of heart and aorta of BHW rats suggesting injury and activation of adaptation processes and reflecting different vulnerability of cardiovascular system on stress.

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THE IMPACT OF NONINVASIVE VENTILATION ON BLOOD PRESSURE AND HEART RATE VARIABILITY IN PATIENTS WITH COPD

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Noninvasive pulmonary ventilation (NIPV) emerged as an effective treatment procedure of acute respiratory failure in patients with chronic obstructive pulmonary disease (COPD). The objective of our study was to determine the effect of NIPV on blood pressure (BP), heart rate (HR) and oxygen saturation (SaO_2), and furthermore on high frequency (HF) component of heart rate variability in patients with COPD. We studied 15 patients with an acute exacerbation of COPD at the baseline, and after 15, 30, 60, and 120 min of NIPV application. NIPV was applied using bi-level positive airway pressure ventilation. Heart rate variability was assessed by VarioCard. NIPV increased SaO_2 ($p<0.05$), and reduced BP ($p<0.001$) and HR ($p<0.001$). HF component of heart rate variability increased significantly in the subgroup of hypoxemic patients with COPD (median, 25 and 75 %: 573, 140 and 1528 before BiPAP versus 3107,109 and 18548 ms^2/Hz while on BiPAP, $p<0.05$). NIPV is a potentially effective treatment modality of acute respiratory failure that improves cardiorespiratory parameters and parasympathetic regulation of heart rate in patients with an acute exacerbation of COPD. Further studies are needed to assess the effects of NIPV on the long-term outcome in patients with respiratory failure.

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SALIVARY TESTOSTERONE LEVELS IN CHILDREN WITH DIVERSE MENTAL CAPABILITIES

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Psychophysiological research of recent years has focused on existing relations between the levels of sex hormones and cognitive functioning. What the data imply is that there are a very large number of factors that have more than small effects on IQ. Testosterone seems to play important role. The organizational effects of testosterone in early stages of life are central for understanding of sex differences in man. Geschwind and Galaburda (1) in their well known theory proposed the increased concentrations of testosterone in utero to be responsible for alterations of brain development. The prenatal and permanent effects of hormonal levels, which are called also organizational, may be studied in young children in the silent period of their childhood, before puberty occurs. During puberty and adulthood the cognitive performance may reflect the relatively immediate effects of currently circulating levels of sex hormones (2). In our study salivary testosterone levels in preadolescent children of both sexes were determined. The youngest child was 6 years old, the oldest 10 years old. Among 320 subjects, 122 were intellectually gifted children, whose general IQ was at least 130 in two independent standard tests on general intelligence (mean IQ=142). Their salivary testosterone levels were compared with those of 93 control children from general population (mean IQ=114), and 115 mentally challenged children whose IQ was lower than 75 (mean IQ=58). In general, statistical analysis did not prove changes in salivary testosterone concentrations throughout the period of preadolescence in previous studies (3). Intellectually talented as well as control and mentally challenged children were divided into subgroups according to sex. The results proved significant differences between studied groups in boys as far as testosterone levels in saliva were concerned.

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CHANGES IN THE CATECHOLAMINE IN FOLLICULAR FLUID AFTER SUPEROVULATORY TREATMENTS OF SHEEP

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There is less knowledge on the function in metabolism, and catecholamine levels in follicullar fluids in sheeps after administration of exogenous hormonal preparations. The aplication of the superovulatory hormones is connected with a marked increase in estrogen in blood plasma. High concentration of estrogens act specifically on the adrenergic receptors both peripheral and central nervous system (1, 2). The present study has been aimed at the radioenzymatic investigations of the effect of various combination of serum gonadotrophins (PMSG, Antex Leo, Denmark), choriongonadotrophins (hCG, Praedyn, Léčiva Praha), follicle – stimulating hormone (FSH, Folicotropin, Spofa Praha) and anti-PMSG (goats antiserum against PMSG, Bioveta, Ivanovice) administrated in oestrus period after synchronization (12 days) by Agelin vaginal sponges (20 mg chlorsuperlutine) on the levels of catecholamine in the follicular fluid. Administration of PMSG (1000 IU) resulted in significant decrease of dopamine (DA) levels ($p<0.01$) and epinephrine (EPI) $p<0.05$ in ovine follicular fluid. Anti PMSG serum application partially modified the DA ($p<0.01$) and EPI ($p<0.05$) levels to those of the control group. After hormonal stimulation by PMSG and hCG

different effect has been observed. Antisergon application after the given combinations of hormones had effect on the EPI and NE levels in ovine follicular fluid but resulted in the increase of DA connect ($p<0.05$). The must conspicious effect on the catecholamine levels in follicular fluid was induced by FSH after which follows a marked increase DA ($p<0.01$) and NE ($p<0.01$) levels. Similar significant effect of FSH in the change catecholamine levels in reproductory organs of sheep and regulatory regions of reproduction were recorded in our previous contributions.

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THE MECHANISM OF ANTIHYPERTENSIVE ACTION OF MELATONIN IN SPONTANEOUS HYPERTENSIVE RATS: THE EFFECT ON REACTIVE OXYGEN SPECIES AND NO FORMATION IN THE KIDNEY

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The excess of reactive oxygen species (ROS) formation may interfere with vasodilatative mechanisms like the L-arginine-NO pathway and thus contribute to arterial hypertension development. We aimed to investigate weather the pineal hormone melatonin that has anti-oxidative and anti-inflammatory effects is able to reduce hypertension in spontaneous hypertensive rats (SHR), in relation to modulation of NO-synthase (NOS) activity, NOS expression and ROS concentration expressed as nuclear factor κB (NFκB) expression. Two groups of adult SHR were investigated: Control group (placebo, n=7) and melatonin group (10 mg/kg/24 h melatonin, n=8). During the five weeks of treatment, the systolic blood pressure (SBP) was measured by the tail-cuff plethysmography. The NOS activity was investigated on the base of L-citruline formation from radioactive L-arginine, the protein expressions were determined using the Western-blotting. In the control group, SBP increased progressively (177±3 mmHg). The melatonin administration caused a reduction of SBP (74 % of the control), increase in NOS activity (121 %) and a decrease in NFκB expression (71 %). There were significant changes neither in the endothelial (109 %) nor in the inducible (98 %) NOS expression. Melatonin administration caused a reduction of systolic blood pressure and an increase in NO-synthase activity, without modulating its expression in SHR. These changes were accompanied by decreased reactive oxygen species concentration. We suppose that the increased availability of nitric oxide and the stabilization of the enzyme NO-synthase may play an important role in the antihypertensive action of melatonin.

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RELATIONSHIP BETWEEN THE OXIDATIVE STRESS AND SYSTEMIC INFLAMMATION IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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The purpose of our study was to establish the relationship between antioxidant activity and levels of proteins of the acute phase of inflammation in patients presenting with an acute exacerbation of chronic obstructive pulmonary disease (copd). We investigated a study group of 126 patients with a history of cigarette smoking and fev1/fvc lower than 70 % predicted values, who were divided into two subgroups depending on the activity of catalase (cat) in red blood cells. In patients with higher than upper level limit of normal values of cat activity (4.3 ukat/ghb) we found significantly higher plasma values of orosomucoid (orm) than in patients with normal cat activity (0.84±0.06 versus 1.02±0.05 g/l, $p<0.031$). no differences were seen between between the two groups in the plasma values of ceruloplasmin (0.36±0.02 versus 0.39±0.01 g/l, p=ns) or transferin (2.48±0.16 versus 2.74±0.24 g/l, p=ns). the concentrations of superoxide dismutase and glutathione peroxidase in the two groups were as follows: (1008±28 versus 952±35 u/ghb, p=ns; 52.30±3.16 versus 57.56±2.66 u/ghb, p=ns, respectively). the most striking findings was a significant linear correlation ($r=0.234$; $p=0.013$) between cat activity and plasma orm concentrations. Our results indicate that in patients presenting with chronic obstructive pulmonary disease there is a close relationship between some prameters of antioxidant activity in erythrocytes and plasma concentrations of proteins of the active phase of inflammation.

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TRANSPORT PROTEINS AND VITAMIN A IN THE ROMANY CHILDREN

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The socio-economical as well as healthy information about the Romany population living in Slovakia are only partially mapped. The aim of the study was to evaluate some anthropological parameters, serum concentrations of vitamin A, transtyretine (Prea) and retinol-binding proteins (RBP) in four age categories of the children Romany population (children to 2 years, from 3 to 6, from 7 to 10, and from 11 to 15 years) in the East Slovakiam region. Within more extensive projects 130 Romany children, at the age of 0-15 years from the East Slovakian region were examined 100 children of the majoroty population from the town Bardejov formed the control group (CG). Concentrations of vitamin A were determined using the HPLC method, transtyretine by the method of radial immunodiffusion, and concentration of RBP by the ELISA method, using the commercial sets of IMMUNDIAGNOSTIK company. Of the total amount of examinations, the attention was focused on the selected anthropometrical parameters (body weight, height, body mass index – BMI). The mean values of the serum concentrations of vitamin A and RBP were not statistically influenced by the sex. Of the anthropometrical parameters, the statistical significance was found at the comparison of the body height between the Romany and majority population in the second group (3-6 years) on the significance level of $p<0.001$, and in the third group it was $p<0.005$. The statistical signif. was also recorded in the values of body weight in the second group on the signif. level of $p<0.001$. For BMI a signif. difference was found between the Romany and majority population in the fourth group on the signif. level of $p<0.005$. The concentration of vitamin A in the first group of children ranged within 0.8-6.28, in the second 1.64-10.37, in the third 2.27-8.32, in the fourth group 2.4-7.96 μmol/l in the Romany children compared to the CG (21.6-10.5 μmol/l), and it considerably differed in all the age categories, where the deficiency of vitamin A was recorded. The statistical signif. was recorded at comaprison of Prea in the Romany population against the CG on the signif. level of $p<0.005$. The RBP concentrations in all the age categories ranged from 20.93 to 67.5 ng/ml. Our results lead us to deeper understanding of the mechanism of influencing the metabolism of vitamin A and transport of proteins. Concentrations of Prea and vitamin A ranged within the risk values that point to the slight protein carency. In most of the children malnutrition has not been not caused only by the shortage of food, but also by various infections and qualitatively incorrect nutrition in combination with other risk factors to which the Romany children are exposed.

EFFECT OF SELECTED CHALCONES ON CANCER CELLS SURVIVAL

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We tested newly synthesized chalcones with code names: Q-510, Q-705 (methoxychalcones) and Q-701, Q-797 (hydroxychalcones) for their antiproliferative effects on Jurkat cell line (human acute T-lymphoblastic leukemia cells). Effects of these compounds were tested by emploing MTT cytotoxicity assay, FACS analysis of cell cycle and apoptosis by using PI and annexin V/PI staining, and DNA fragmentation by gel electrophoresis. Our data indicate that methoxychalcones are more effective than hydroxychalcones with Q-510 being the most effective with efficacy higher than 80 % in concentration 10^{-6} mol.l^{-1}. Cell cycle analysis revealed significant effects of methoxychalcones after 24h incubation. Q-510 caused redistribution of DNA content to sub G_0/G_1 phase in concentration 10^{-6} mol.l^{-1}. Similar effect but in higher order concentration was described by Q-705. Hydroxychalcones had significant effects only in the highest concentration (10^{-4} mol.l^{-1}). Detection of apoptosis using PI and annexin V staining achieved significant increase in late stage of apoptosis. Proapoptotic effects of tested compounds was also exhibited by DNA fragmentation. In conclusion, newly developed Q-510 and Q-705 have high anticancer activity and could be considered as potential new anticancer drugs.

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RELATIONSHIPS BETWEEN CALCIUM RELEASE ACTIVATION AND CALCIUM CURRENT INACTIVATION IN CARDIAC MYOCYTES

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Cardiac excitation-contraction coupling is the process by which electrical excitation triggers a cardiac myocyte to contract. Depolarization of plasma membrane activates L-type calcium channels (DHPRs). Calcium influx through DHPRs activates calcium release from the sarcoplasmic reticulum (SR) through ryanodine receptors (RyRs). Inactivation of the L-type calcium current (I_{Ca}) has a dominant fast component, dependent on calcium release. Nevertheless, this aspect of I_{Ca} kinetics is not well understood. Here we studied the relationship between activation of calcium release from the SR and the release-dependent inactivation (RDI) of I_{Ca}. We used whole-cell patch clamp to record I_{Ca} in isolated rat ventricular myocytes in response to two-pulse protocols, composed of a prepulse from a holding potential of –50 mV with constant amplitude (50 mV) and variable duration (0.5-10 ms) and a test pulse of constant amplitude and duration (50 mV, 70 ms). The global increase of Ca in the cytoplasm was buffered with EGTA. Calcium flux from individual dyadic junctions was observed as spatially and temporally localized fluorescence increase, using confocal microscopy and the fluorescent calcium indicators fluo-3 or fluo-4. I_{Ca} kinetics was described using our model of RDI (1). The prepulse-induced calcium release and I_{Ca} inactivation increased with the duration of the prepulse and were highly correlated. The prepulse-evoked decrease of calcium release during the test pulse was manifested as a decrease in the number of activated release sites, highly correlated to the decrease of test I_{Ca}, and as a decrease in release synchronization. The fraction of test I_{Ca} inactivated by RDI and the rate of RDI were proportional to the fraction of activated Ca^{2+} release sites, while the delay of RDI was proportional to Ca^{2+} release latency and the synchrony of RDI decreased with the standard error of Ca^{2+} release latency. We conclude that measurements of spatially resolved calcium release provide evidence of local control of RDI, resulting in direct relationships between calcium release activation and calcium release-dependent inactivation of calcium current.

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LEARNING IN MORRIS WATER MAZE AFTER PRENATAL EXPOSURE TO METHAMPHETAMINE

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Aim of the present study was to examine the effect prenatal methamphetamine (MA) exposure on cognitive function in adult male rats. Rats (postnatal days 60-80) were tested for learning in Morris water maze. There were 3 groups of animals: prenatally MA- (5mg/kg) exposed, prenatally saline-exposed and absolute controls without any prenatal exposure. Rats were tested in 5 consecutive days. In each day animals were exposed to 8 consecutive trials with 4 different start positions. Two experiments were conducted using two different types of learning tests: “Place navigation test” and “Short-term memory test”. The Place navigation test used fixed platform position during the entire time of experiment. The platform position changed daily in the Short-term spatial memory test. Data of both experiments were analyzed separately using a One-way ANOVA with Repeated Measure with Bonferoni post-hoc test. Male with prenatal exposure to MA learned slowly in Place navigation test than both control groups. There were no differences in Short-term spatial memory test between groups. Thus, our data suggest that treatment of MA during pregnancy has long-term negative effect on learning of adult offspring that is test-specific.

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TRANSFER OF EMBRYOS IN FALLOW DEER

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We monitored in our work multiple ovulations and embryo transfer (MOET) in fallow deer in latitude of middle Europe in mild climatic region. Thirteen does and four males (dama dama) were used in our experiment performed in two consecutive years during an estrus period in October and November. Thirteen European fallow deer (n=13) (Dama dama dama) 5-7 years old and 30-35 kg of liveweight were allocated into embryo donor (n=8) or embryo recipient (n=5) groups. Animals in the emryo donor group were divided into two subgroups (n=4). Animals in the donor group were treated with follicle-stimulating hormone - 24 mg of FSH i.m. in 12 hours intervals in six doses respectively starting 48 hours before the CIDR withdrawal at the time of pregnant mare serum gonadotropin PMSG administration - 300 I.U i.m. - in animals of embryo recipient group. All donor animals were mated naturaly (two males for each experiment) – 24 hours after CIDR devices withdrawal at the onset of estrus which culminated 12 hours later. Embryo recovery and transfer of embryos were performed surgically at day five since the onset of estrus. The overall recovery from donor animals was 57 transferable embryos in morula stage. Transfer of embryos was performed in five recipient animals, four of which became pregnant.

ALLOSTERIC INTERACTIONS AT MUSCARINIC RECEPTORS: GRÖBNER BASES AND THE DESIGN OF EXPERIMENTS

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Muscarinic acetylcholine receptors (MR) belong to a large family of G protein-coupled receptors (1). Allosteric ligands interact with binding sites on the receptor molecule that are topographically distinct from the orthosteric site. Numbers of compounds displaying high structural diversity were found to modulate the orthosteric ligand binding at the muscarinic receptor in positive or negative manner (2, 3). Most of them decrease both association and dissociation rates of the competitive antagonist N-methylscopolamine at M2 subtype of muscarinic receptor. Mechanisms of allosteric modulation of MR remain to be resolved in detail. Until recent years, the primary experimental set-up has been radioligand binding. Ternary complex model (TCM) can explain equilibrium experiments well. Unfortunately, no measurement technique is available to measure an arbitrary rate constant relevant to the model. And besides, the fractions of radioligand bound to the binary (RL) or ternary complex (ARL) with receptor cannot be discriminated (where L, A, R denote competitive orthosteric radioligand, allosteric ligand, and receptor, respectivelly). An important question is whether the observed parameters uniquely determine the model parameters. Blind identification of the rate constants in TCM need to investigate the model at least with respect to observability and identifiability . An application of Gröbner basis method is an approach to answering these questions and effectively design experiments to obtain sufficient data set. Effective Gröbner basis algorithms are implemented in computer algebra systems such as Maple or Mathematica (4).

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C-PEPTIDE ASSAY IN OBESE TYPE 2 DIABETIC PATIENTS

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In this study we investigated the concentration of plasma and urine C-peptide (Elecsys C-peptide Roche, Switzerland) in 33 obese Type 2 diabetic patients (16 men and 17 women, age 21-76 years) treated by oral antidiabetics or diet only. Body mass index was in the range 24.2-50.4 (32±5.7) kg/m^2. postprandial blood glucose 9.2±3.5 mmol/l and Hb A1c (Unimate Roche. DCCT units: 7.04±1.95 %; IFCC units: 5.35± 2.13 %) The results of C-peptide assay are summarised in Table. Most results were above the reference range (0.25-0.6 nmol/l in blood and 24.7±8.7 nmol/l in urine). We did not find any correlation between the markers of glycaemic compensation (blood glucose. Hb A_{1c}) and the concentration of C-peptide in plasma or urine. On the other side we found a statistically significant direct association between plasma C-peptide and BMI (r = 0.54; p = 0.0011; BMI = 4.21*C-peptide + 26.08) Our results indicate that C-peptide assay can be a valuable tool in the assessment of insulin secretion in obese hyperinsulinaemic patients.

Group N=	C-peptide. Plasma (nmol/l)	C-peptide. urine (nmol/l)	Ratio plasma/urine
All	1.41± 0.73	15.6 ± 6.9	13.3 ± 3.8
33	0.56 – 3.92	5.3 – 35.2	3.4 – 46.1
men	1.50± 0.82	13.6 ± 5.8	10.7 ± 5.8
16	0.56 – 3.92	5.3 – 26.1	3.4 – 21.9
women	1.31 ± 0.61	17.7 ± 7.3	16.2± 10.5
17	0.56 – 2.73	6.0 – 35.2	4.3 – 46.1
on OAD	1.42 ± 0.62	15.8 ± 7.42	12.1± 5.3
17	0.56 – 2.73	5.3 – 35.2	3.4 – 21.9
on diet	1.40 ± 0.83	15.3 ± 6.2	14.7±11.2
16	0.56 – 3.92	6.0 – 28.1	3.2 – 46.1

ERYTHROCYTE ION TRANSPORT IN RATS SUBJECTED TO CHRONIC AND ACUTE HYPOBARIC HYPOXIA

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Erythrocytes are susceptible to oxidative damage due to the high content of polyunsaturated fatty acids in their membrane and the high cellular concentration of oxygen and hemoglobin. Under normoxic conditions reactive oxygen species (constantly generated intracellularly) are mostly neutralized by antioxidant defense systems. However, under the conditions of hypobaric hypoxia, autooxidation of hemoglobin is facilitated and an increased flux of superoxide radicals is produced (1). The aim of our study was to examine some selected parameters of erythrocyte ion transport after acute and chronic hypobaric hypoxia. In the first experiment erythrocytes were isolated from 21-day-old Wistar rat, which showed the low resistance to a lack of oxygen (2). Experimental rats were exposed to acute hypoxia, i.e. 30 min hypobaric hypoxia simulating the altitude of 9 000 m. The significantly increased level of thiobarbituric acid-reactive substances in cerebral cortex in acute hypoxia in comparison with controls (32.14±0.30 vs. 25.51±0.35 ng/mg w.w., $p<0.0001$) was found but not in erythrocytes (16.34±1.26 vs. 14.86±1.09 μM, n.s.). As concerns ion transport, no significant differences of Na^+ content or the activity of Na^+-K^+ pump, Na^+-K^+ cotransport and cation leaks were observed. In the second experiment 5-month-old Wistar rats were exposed to chronic hypobaric hypoxia (intermittent hypoxia simulating the altitude of 5 000 m in a hypobaric chamber for 8h/day, 5 days a week with the total number of exposures 30). Chronic hypoxia significantly increased hematocrit (73 % vs. 49 %, $p<0.0001$). Erythrocyte Na^+ content was higher in chronic hypoxia in comparison with controls (5.981±0.180 vs. 4.952±0.255 mmol/l ery, $p<0.01$), but no significant differences in the activity of Na^+-K^+ pump (5.570±0.249 vs. 6.191±0.357 mmol/l ery/h, n.s.), Na^+-K^+ cotransport (1.598±0.221 vs. 1.561±0.171 mmol/l ery/h, n.s.) or monovalent cation leaks were found. Although no significant differences in the activity of Na^+-K^+ pump were observed, it was evident from the relationship between Na^+-K^+ pump activity and intracellular Na^+, that the affinity for intracellular Na^+ was decreased in hypoxic rats. We concluded that chronic but not acute hypoxia modified ion transport in rat erythrocytes.

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ATRIAL ACTIVATION RELATED TO AGE DEPENDENT SOMATOMETRIC CHARACTERISTICS OF BODY CONSTITUTION

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The increased occurrence of atrial arrhythmias necessitates improved quantification of normal variability of electrocardiologic characteristics of atrial activation. The aim was to identify the somatometric parameters of body composition, body constitution and thoracic configuration affecting significantly the electrocardiologic measures of atrial activation, i.e. vectorcardiographic data. The study group consisted of 211healthy boys and men without signs of cardiac disease, with normal ECG and VCG, aged from 10 to 60 years, (median 19 years). The maximal spatial vector of atrial activation (sPmax), the orientation of the integral P vector (azimuth, declination) (were obtained

by the Frank lead system in seated persons in mid respiration from a single heartbeat using the computer system Cardiac 128 - PC (METE, Prague). The sample of subjects was stratified into subgroups according to the pecentil values of indices computed from 11 body and chest parameters as well as fat percentage estimated from subcutaneous skinfolds. (Body Mass Index, Rohrer Ponderal and Conicity Index), body circumferences (Relative Abdominal Circumference, Pignet Index), anteroposterior and transversal dimensions (Thoracic index, Relative Sagittal Diameter. Differences in magnitudes of sPmax and orientations of integral P vectors between these subgroups were tested by ANOVA and by. regression analysis. Body and chest configuration parameters had a greater impact on the average magnitudes of sPmax than on the orientation of integral P vectors ($r=-0.2$-0.5, $p<0.001$). Median values o sPmax were more than 60 % higher in subjects with linear body constitution and low adiposity. With increasing anteroposteriorl and circumferencial dimensions of the chest integral P vectors were shifted backwards and more horizontally($r=-0.3$, $p<0.01$). Tightest relationships were found in subjects younger than 30 years and they decreased with age. The variability of the magnitudes of maximal spatial vectors of atrial activation was significantly influenced by the proportionality of body constitution. The influence of anthropometrical parameters was most pronounced in younger adulthood and should be taken into account when evaluating electrocardiograms and vectorcardiograms.

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REPEATED KAINIC ACID ADMINISTRATION AND HIPPOCAMPAL NEURONS DEGENERATION

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The aim of our study was to analyse structural alterations in the hippocampus of adult rats, after the repeated kainic acid (KA) administration. In the first group KA was given i.p. repeatedly in six consecutive daily doses (10mg/1000g). In the second group KA was administered i.p. repeatedly in six lower doses (5mg/1000g) every second day. Two days after the last injection animals were transcardially perfused (neutral paraformaldehyde) and their brains were processed for neurohistological examination (Fluoro-Jade B and Hoechst). In the first group the following neuropathological changes were found: In the CA1 area many degenerating cells were observed, the pyramidal cells layer being the most affected structure. Neuronal population was replaced by numerous glial cells. The CA2 area was not that much deteriorated as the CA1 area and gliosis was not so prominent. In the CA3 area no degenerating cells were observed, all cells were already extinct. In the second group, the most prominent effect was the damage both in the CA3 area and in the hilus of the dentate gyrus. Almost all pyramidal cells were destroyed and a massive gliosis was observed. Some degenerating cells in the CA1 area were found. The most vulnerable region of the hippocampus to the neurotoxic effect of KA is the CA3 area, as its neurons were almost completely extinct already after the administration of reduced KA doses. The CA1 area appears to be more resistant to KA than CA3 area. Its neurons were largely destroyed only after the longer period of KA administration.

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DISTRIBUTION AND KORELLATION OF LIPID PROFILE OF ROMANY CHILDREN IN EAST SLOVAK

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The data about incidence of aterosclerosis in the Romany minority is not properly known, that is why we have paid attention to the children population of this minority. The aim of this study was to evaluate some anthropological parameters, serum concentration of total cholesterol (TC), HDL and LDL cholesterol, including triacylglycerols (TG) in various age groups of the Romany children in the Eastern region of Slovakia. Monitoring of the main risk factors (blood pressure, obesity) that condition occurrence of cardiovascular diseases; statistical procession and comparison of the results with the data of the monitoring group were also parts of the study. We examined 400 children between the ages of 0 and 15 in different parts of the Eastern Slovakia. In the studied group there were 250 Romany children and in the monitoring group 150 children of the majority population. The differences in values of the monitored serum lipid parameters in the 3 age groups of the Romany boys and girls were not confirmed. However, there were significant differences between the values of body weight, body height, blood pressure and BMI comparing the Romany and non-Romany children. In the studied group, lower values of the monitored parameters were observed, which points out the specific disparity of this ethnic group in comparison with the monitoring group formed by the majority population. The concentrations of the serum TC in 3 age groups were outside the zone of tolerable values. Hypercholesterolemy was evaluated by means of the following criteria: 5.0-5.2 for the children and 5.2-5.8 for the adolescents. To compare the 3rd group of Romany children with the monitoring group, the concentrations were less favourable with the values of 0.82-3.05 mmol/l. Different values were also observed in monitoring of HDL and LDL cholesterol concentrations.

COLOCALIZATION OF NEURONS IN THE PHEASANT ILEUM

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Using double enzyme histochemical methods was investigated the colocalization of nitrergic and cholinergic neurons in the pheasant ileum. Neuronal nitrergic and cholinergic activities in the pheasant ileum were demonstrated in the nerve fibres and bodies of nerve cells. Nitrergic and cholinergic nerve fibres entered into the wall of the pheasant ileum at its serosal surface frequently in the vicinity of the ileal arterial branches. Nerve fibres formed either thicker nerves and nerve plexuses in muscular, submucosal and mucosal layers. Both fine nerve fibres from the lamina propria mucosae were distributed in colocalization into the intestinal villi and surrounding the crypts they reached the epithelium. The nitrergic and cholinergic bodies of nerve cells polygonal in shape were located in the muscular and submucosal layers they were solitary or arranged in groupe forming the ganglia. We conclude that the nitrergic and cholinergic neurons colocalize in several layers in the pheasant ileum and may play a significant role in the small intestinal functions.

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TWO METHODS OF EMBRYONIC CEREBELLUM TRANSPLANTATION IN LURCHER MUTANT MICE OF C3H AND C57BL/7 STRAINS

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Lurcher mutant mice (+/Lc) represent a natural model of olivocerebellar degeneration, which is caused by a mutation in the δ2 glutamate receptor gene (1). They suffer from postnatal complete loss of Purkinje cells resulting in cerebellar ataxia (2). Similar cerebellar degeneration occurs also in human pathology. In our laboratory we use Lurcher mutant mice of two strains. We transplanted embryonic cerebellar tissue

to adult Lurcher mutant mice of C3H and C57Bl/7 strains. As donors we used 12-13 days old GFP expressing embryos without δ2 receptor mutation. The tissue was applied through the trepanation in the midline of the occipital bone either in form of a solid graft or as a cell suspension. To control animals only vehiculum was administered by the same procedures. Motor skills were examined before and at week intervals after the operation. Finally the mice were sacrificed and then the cerebella were examined histologically. The graft was identified according to the green fluorescence of GFP. Detailed morphology was assessed in Nissl stained preparates. After cell suspension application the graft-derived cells were detected in 12.5 % of C57Bl/7 and 63 % of C3H mice. Solid graft was present in 75 % of C57Bl/7 and in 64 % of C3H mice. The success rate was not dependent on the age of animals. The mass of graft-derived tissue was different in various individuals. In some of them the graft created nerve fibres sprouting into the host tissue and the graft-derived cells colonized the host cerebellar cortex. The cells also created abundant dentritic trees. Motor tests showed slight insignificant differences between experimental and control mice. In the C3H mice was the success rate of both methods comparable, while in the C57Bl/7 strain the solid graft transplantation was more effective than the cell suspension application.

(1) Zuo J et al.: Nature 388: 769-773, 1997.
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EFFECT OF BISPHENOL A AND DI (2-ETHYLHEXYL) PHTHALATE ON STEROIDOGENESIS IN PORCINE GRANULOSA CELLS

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Bisphenol A (BPA) and di (2-ethylhexyl) phthalate (DEHP) are chemicals widely used as plasticizers in the manufacture of polycarbonate and PVC plastics. They can leach out of food and medical product covers and thus enter the human body. These coumpounds are supposed to affect the reproductive system, cognitive functions, neurodevelopment in prenatal life and to be linked to hormone dependent cancers. We have investigated the effect in vitro of BPA and DEHP in primary porcine ovarian cell culture system. Granulosa cells (GC) isolated from porcine ovarian follicles (4-6 mm) were incubated with the tested compounds in the presence or absence of gonadotropins (FSH, LH) (1 $\mu g.ml^{-1}$), forskolin (10 $\mu mol.l^{-1}$) and cyclic AMP (100 $\mu mol.l^{-1}$) for 72 h. At the end of the incubation, progesterone (P_4) and estradiol (E_2) levels released by GC to the culture media were measured by radioimmunoassay. BPA significantly stimulated basal and FSH-induced P_4 production by GC at 10^{-5} and 10^{-6} $mol.l^{-1}$ concentrations, respectively. While BPA failed to stimulate P_4 synthesis induced by the action of LH, forskolin and cAMP, a significant inhibitory effect of BPA at 10^{-4} $mol.l^{-1}$ concentration was observed after each stimulus, except cAMP treatment. BPA (10^{-8}–10^{-4} $mol.l^{-1}$) significantly suppressed FSH-stimulated E_2 production by GC. DEPH (10^{-6}–10^{-4} $mol.l^{-1}$) induced a stimulation of basal P_4 synthesis by GC, but slightly inhibited FSH-stimulated P_4 levels at the same concentrations. The results indicate that the action of selected plasticizers could interfere with FSH signaling pathway in the stimulation of progesterone production by porcine granulosa cells and might induce the changes in estradiol synthesis. We suppose that enzymes involved in steroidogenesis might be implicated in the action of these agents.

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ANTIOXIDANT SYSTEMS IN ISCHAEMIA-REPERFUSION MYOCARDIAL INJURY

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Oxidative stress plays an important role in the pathogenesis of many diseases. Reactive oxygen species (ROS), the products of univalent reduction of oxygen are the key mediators of ischaemia-reperfusion injury of heart muscle, too. Living systems possess different defence mechanisms against ROS, which are called as antioxidant systems. Myocardial injury occurs in the case of disturbed balance between reactive oxygen species formation and antioxidant system activity. A suitable clinical model of ischaemia-reperfusion myocardial injury is acute myocardial infarction (AMI) treated by percutaneous coronary intervention (PCI) with angioplasty and eventually application of stent. PCI leads to instantaneous opening of closed coronary artery and reperfusion of previously ischaemic myocardium. In this study we followed the effect of PCI on total plasma antioxidant capacity and the levels of malondialdehyde, a marker of lipid peroxidation. We studied 14 patients with AMI. The blood samples were taken from cubital vein before (t0) and 15 minutes (t15), 12 hours (t12) and 24 hours (t24) after coronary artery opening by PCI. Plasma total antioxidant status (TAS) was assessed by a spectrophotometric method (Total antioxidant status, Randox laboratories, UK). The results of our pilot study are in the table:

TIME (hours)	Total antioxidant status (mmol/l)				Distribution of TAS (n)		
	mean	SD	min.	max.	<1.30	1.30-1.77	>1.77
0	1.22	± 0.36	0.7	2.17	9	4	1
1/4	1.23	± 0.29	0.71	1.66	7	7	0
12	1.27	± 0.25	0.82	1.68	7	6	0
24	1.28	± 0.19	0.89	1.6	9	4	0

These results are lower as compared by the reference range (1.30-1.77).From the second part of the table is evident that more than 50 % (32 out of 62) of all results are below the reference range. We did not observe any change in this integrated parameter of extracellular antioxidant activity before and after PCI.

VISUALIZATION OF BUTYRYLCHOLINESTERASE (BUCHE) – POSITIVE NERVE COMPONENTS IN THE SPLEEN OF RABBITS

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The occurrence of BuChE-positive components of the spleen in rabbits was investigated by the direct thiocholine method. BuChE-positive nerve components enter the spleen in a common bundle with arteries. In the organ they form characteristic periarterial and periarteriolar plexiform arrangements, which are especially conspicuous around aa. centrales running through the white pulp. Nerve fibres extend away from these plexuses into adjacent layers of trabeculae, further into marginal layers of periarterial lymphatic sheath (PALS), as well as into mantle zone of follicles. Several scattered periarteriolar and individual nerve fibres can be seen in the marginal sinuses and cords of the red pulp. In the fibrous capsula BuChE-positive nerve fibres can also be seen which have an evident connection with trabecular and parenchymal nerves of the organ. Microscopic findings support the notion that BuChE-positive nerve components supply as vascular as parenchymal components of the spleen, and they participate in the regulation of the immune processes in this organ.

GLUTAMATERGIC ASPECTS OF PSYCHOSIS IN ANIMAL MODELS: WHAT CAN THEY TEACH US ABOUT SCHIZOPHRENIA?

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Elevated levels of neurotoxic substances like quinolinic acid (QUIN) have been found in post-mortem frontal cortices of patients with schizophrenia. Evaluation of existing experimental and clinical data suggest that there are following relationships: (a) synthesis of QUIN is induced by viral/bacterial infections; (b) released QUIN acts preferentially on a subset of ionotropic glutamate receptors of NMDA-type containing NR2B subunits; (c) these receptors are particularly abundant in developing brain tissue and (d) QUIN-induced activation of this receptor type may have neurotoxic consequences. Having in mind these facts we have proposed a schizophrenia-like animal model to disclose a role of NMDA receptor in pathophysiology of this devastating disorder. The QUIN treatment on postnatal day (PND 12) resulted in a reduction of the receptor-specific L-[^{3}H]glutamate binding to synaptic membranes isolated from the cerebral cortex, striatum and hippocampus of rat males on PND 50. In all brain regions, glycine (10 μM GLY) but not spermidine (1 mM SPD), potentiated the effect of NMDA as a displacer. It suggests that agonist acting at the glycine site may have a therapeutic benefit in the treatment of schizophrenia. As functional activity and number of NMDA-Rs can be regulated by concentrations of GLU, we determined levels of glutamate (GLU) and aspartate (ASP) in the hippocampus of young adults neonatally-treated with QUIN. The treatment significantly decreased (15 %, $p<0.001$, ANOVA) the level of GLU (but not ASP) in QUIN-treated animals. Moreover, using immunoblots, we measured protein expression of ubiquitous NR1 subunit and family of NR2 subunits (NR2A-D) of the NMDA receptors in synaptosomes isolated from the hippocampi of young adult rat males (PND 50). Besides the observed differences in the protein expression of NR2A/2B subunits in the hippocampus of adult rats [1], we have also found differences in the expression of remaining NMDA-R subunits. The results derived from this animal model suggest that the glutamatergic system in schizophrenia can be dysregulated and NMDA-Rs can operate at reduced activity. Moreover, the dysfunction of glutamatergic system may represent an important target for pharmacological stabilization of patients with schizophrenia.

(1) Skuba I, Šťastný F: Physiol Res 53: 34P, 2004.

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STRESS AND NUTRITIVE VALUE OF POULTRY MEAT

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A conventional method of column ion-exchange chromatography was used to determine the level of amino acids in the breast muscles of chickens and ducks exposed to experimentally simulated stress. The results showed that generally higher levels of amino acids were found in the breast muscles of chickens in comparison with ducks. Highly significant differences ($P<0.001$) were observed in essential valine, isoleucine, leucine and phenylalanine. After exposure of chickens to stress, a statistically significant decrease ($P<0.05$) in amino acids glycine, alanine, arginine, essential threonine, isoleucine, leucine, and lysine was recorded in their breast muscles. A significant decrease ($P<0.001$) in amino acids histidine and essential phenylalanine was observed in the breast muscles of ducks but only after exposing them to more intensive simulated stress.

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VIRAL INFECTIONS AND ALLERGIES IN CHILDREN

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Viral infections belong among the most frequent agents inducing respiratory infections in children. In infants there are mainly respiratory-syncytial viruses (RSV), and in children of school age there are rhinoviruses. Viruses act on the respiratory tract by two mechanisms – non-immunological and immunological. The immunological mechanism has been confirmed by the findings on the mucosa of airways (submucous infiltratiuon of lymphocytes, neutrophils and monocytes, elevation of eosinophils (Eo) and eosinophilic cationic protein). In the blood, the lymphopenia, increase in eosinophils, activation of granulocytes and increased secretion of proinflammatory cytokines were found. In the bronchoalveolar lavage, there were Eo, increase in IL 5, INF gamma and findings of CD8. These changes lead to blocking of cationic proteins and M2 muscular receptors, increasing of acetylcholine protection, which is the cause of the rise of bronchospasm and bronchial hyperactivity. In literature two different opinions on the effect of viral infection on allergy have appeared: 1. Protective effect on allergy; 2. The onset of allergic disease later after overcoming infection. In our retrospective study we wanted to find out the incidence of viral infections in children with asthma. Seventy eight patients with bronchial asthma were randomly chosen. The mean age was 5.71 years, the ratio of boys to girls was 1.8:1. In 56 children (71.8 %) the atopic disease was found in the family. 38 children (48.7 %) overcame viral infections to their 3 years of life. RSV infection was confirmed serologically in 8 children (21 %). 29 children (37.1 %) had HCD infection end 6 (20 %) had laryngites or recurr. laryngites. Obstructive bronchites and recurrent obstructive bronch. occurred in 41 children (52.5 %). In 11 children (12.8 %) no disease was found.. 49 children (62.8 %) attended the pre-school establishments. In 37 children (47.5 %) who attended nursery school, asthma arose at the age of 6,7 years and in 37 children (38.4 %), who do not attend nursery school the rise of AB was at the age of 5.03 years. Viral infections participate with a great deal in the diseases of respiratoty organs also in the children with later rise of asthma. Further studies will be needed for confirmation of controversial literary data in the sense of positivity or negativity of the effect of viral infections on the rise of allergic diseases.

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□ 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. 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 superovulation became evident also in the changes of the oviducts as in the natural oestrous period.

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INFLUENCE OF THE OPIOID ANTAGONIST ON RESPIRATORY BURST IN PHAGOCYTIC CELLS AFTER VARIOUS STRESSORS IN RATS

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Physical and emotional stress may cause changes in immune system responses. Immune system reactions are also modified by analgesic effects of endogenous opioids produced in organism during the stress. Our aim was to observe the influence of stress conditions on phagocytic cells, which play a key role in non-specific immunity. We were also interested in the role of naloxone, the opioid antagonist, and its possible protective function in stress response. In our experiments rats (Sprague-Dawley) were exposed to a series of various subsequent stressors: immobilization (1x, resp. 7 x 45 min.), whole body irradiation (6 Gy), injection, hot plate test (55 °C, max. 2 min.) and frequent handling. Single dose of naloxone was injected i.p. 8 mg/kg b. w. Peritoneal exudate cells (PEC) and bone marrow cells were isolated. The effect of stressors and naloxone on phagocytes was determined by micro – INT – test reflecting their respiratory burst activity. We have found that the stressors induced the changes of metabolic burst in zymozan stimulated bone marrow and peritoneal cells. Injection of naloxone significantly eliminated the effects of stressors. Our data are consistent with results of other authors and indicate the protective role of naloxone through the stress induced endogenous opioids inhibition.

CIRCADIAN PROFILE OF MELATONIN CONCENTRATION IN PLASMA, PINEAL GLAND AND PERIPHERAL TISSUES OF RAT

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Melatonin is a hormone synthesized primarily in the pineal gland. The substantial part of melatonin action is to coordinate endogenous rhythms in different physiological functions. Melatonin production in the pineal gland is under circadian control and is directly inhibited by light. During the nighttime melatonin concentrations in the pineal gland and plasma are several times higher than during the daytime. In addition to the pineal gland high melatonin levels were measured in the retina, Harderian glands and gastrointestinal tract (GIT). While endogenous melatonin synthesis in retina and Harderian glands was definitely proven, direct evidence for melatonin synthesis in GIT is still missing. In our study we measured the levels of melatonin in the plasma, pineal gland, duodenum, colon, pancreas, kidney and spleen of rats during 24-hour cycle in 4-hour intervals (starting at 13.00 hour). The aim of our study was to find out if melatonin levels in peripheral tissues follow a similar pattern than in the pineal gland and circulation. Wistar rats were kept in LD cycle (12:12). Hormone concentration was determined directly in plasma and after solvent extraction in different tissues by radioimunoassay. An expected daily rhythm of melatonin was found in the pineal gland and plasma. In kidney and spleen tissues melatonin concentrations were determined in range 80-100 pg/g of tissue during the day and 120-145 pg/g during the nighttime. In the pancreas concentrations of the hormone were low during the day (100-120 pg/g) but increase during the night (200-210 pg/g). A partially different pattern was found in gut where daytime melatonin concentrations were higher than in other organs and a transitory increase was measured at the end of the darktime. Colon exhibited higher melatonin content (165-177 pg/g) than duodenum (120-130 pg/g). Rhythmic pattern of melatonin concentrations in the spleen, kidney and pancreas reflect rhythmic changes in circulation while different pattern in gut may reflect another source.

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COMPARISON OF INFLUENCE OF EIGHT QTc INTERVAL REGRESSION EQUATIONS IN SLOVAK YOUNG HEALTHY WOMEN

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The electrocardiographic QT interval depends on the heart rate and gender, with increasing rate it shortens. For comparison of QT duration among several humans a corrected QT interval (QTc) is used. There were tens of corrected QTc interval regression equations from different groups of humans proposed. The aim of this study was to compare using eight of them in a larger sample of Eastern Slovakia young healthy women. In 112 non-obese resting women from our previous studies the QTc interval duration was calculated from manually measured QT intervals and heart rate or RR intervals of the Frank XYZ lead electrocardiograms by the correction formulas of Bazett, Fridericia, Sagie, Sarma, Rautaharju, Arrowood, Lecocq, Malik, and compared to each other. The average measured QT interval (mean ± SD) was 368.59±24.49 ms, heart rate 72.23±8.89 per min, RR interval 844.16±112.87 ms. The average QTc duration decreased in the following order: Bazett (402.33±16.95 ms), Fridericia (390.58±16.22 ms), Sagie (368.62±24.48 ms) = Rautaharju (368.62±24.48 ms) = Arrowood (368.62±24.48 ms), Lecocq (368.54±24.50 ms), Sarma (368.51±24.51 ms), Malik (344.69±39.26 ms); differences among all corrections used were highly statistically signifficant ($p<6.33xE^{-17}$ to $2.1.E^{-78}$). The longest average QTc interval was calculated by the Bazett`s formula, the shortest QTc by Malik`s formula, the largest variance (1541.70 ms^2) and standard deviation (39.26 ms) by Malik`s formula. Our results indicate that probably none of these eight formulas is the most appropriate for young healthy Eastern Slovakia women. Every formula has some limitations (pacing, drug intake, tachycardia, exercise, etc) and should be usually used during spontaneous changes of QT interval and heart rate during resting conditions. Using an incorrect formula can produce the signifficant differences of QTc duration in larger samples. Some informations on very individual QT-RR relation indicate that the applied correction formula should always be derived from the data of our healthy women and not from other people.

PRENATAL METHAMPHETAMINE EXPOSURE INCREASES SUSCEPTIBILITY TO FLUROTHYL SEIZURES.

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Previous studies demonstrated that seizure susceptibility may be altered in offspring of drug-abusing mothers. Additionally, it has been shown that methamphetamine (MA) affects the γ-aminobutiric acid (GABA) system of the CNS. Flurothyl is a convulsant ether that is suggested to interfere with GABA receptor-mediated neurotransmission. The purpose of the present study is to examine the hypothesis that prenatal MA exposure (5 mg/kg daily throughout the entire gestation period) alters susceptibility to flurothyl seizures. Adult male rats (prenatally MA-exposed, prenatally saline-exposed and controls) were challenged with flurothyl at a constant flow rate (30 µl/min) in an air-tight chamber until tonic–clonic seizures occurred. Flurothyl produces two types of seizures that occur sequentially: clonic seizures occur first, and tonic–clonic seizures follow. Clonic seizures consist of facial and forelimb

clonus with preservation of the righting reflex. Tonic–clonic seizures consist of loss of righting reflex, tonic flexion or extension of all four limbs, followed by long clonus of all four limbs. The seizure threshold was analyzed by a one-way ANOVA (prenatal drug exposure) with Bonferoni post-hoc test. Prenatally MA-exposed rats had decreased threshold of first fasciculations and clonic seizure threshold. The tonic-clonic seizure threshold was not altered by prenatal MA exposure. Thus, the present study suggests different sensitivity of several brain regions that are associated with different types of seizures to prenatal MA exposure. It seems that the seizure-sensitive target of flurothyl in prenatally MA-exposed rats is the forebrain region (clonic seizures) rather than the brainstem (tonic-clonic seizures).
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SECOND MESSENGERS (cAMP, cGMP AND IP3) IN RAT SKELETAL MUSCLE AS INFLUENCED BY THE PERFUSION FLOW RATE

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On the isolated perfused and superfused rat skeletal muscle (musculus gracilis anticus) a stimulation of metabolic turnover was induced by the increasing medium flow rate under relaxed conditions (1). In the recent study we have measured the tissue concentrations of second messengers cAMP, cGMP and IP3 under similar experimental conditions to analyse their potential role in the described stimulation of metabolic rate by changes of perfusion flow rate. Second messengers were analysed in muscles frozen in situ in anesthetized rats (control 1), in muscles slowly isolated with similar ischemia as muscles prepared for perfusion experiments (control 2), and in muscles under different perfusion flow rates in vitro (perfusion 1 – low perfusion flow, perfusion 2 – increased perfusion flow for 5 minutes and perfusion 3 – increased perfusion flow for 60 minutes). After finishing the isolation or perfusion, the muscles were quickly frozen by tongs precooled in liquid nitrogen and were stored in – 75 °C until analysis of second messengers by commercial kits (Enzymeimmunoassay Biotrak System for cAMP and cGMP, and D-myo-Inositol 1,4,5-trisphosphate [3H] Biotrak Assay System for IP3). The tissue levels of the two second messengers cAMP and cGMP had not any significant change after the increase of perfusion flow rate and they probably have no transduction role in the induced alteration of skeletal muscle metabolism. The exact IP3 analysis was possible in control 1, control 2 and perfusion 1 groups; the IP3 concentration in perfusion 2 and perfusion 3 groups was so reduced (after increased flow rate), that it was lower than RIA calibration curve (less than 40 pmol IP3/g tissue) and arbitrary it may be considered as zero concentration (in comparison with in vivo level). This decrease in the tissue concentration of IP3 induced by the increasing flow rate is significant ($P < 0.05$). It indicates the possible role of IP3 in this signal transduction, leading to changes in the cellular metabolic pathways.
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EFFECT OF APNOE ON SOME ELECTROPHYSIOLOGICAL PARAMETERS OF ECG IN WISTAR RATS.

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Prolongation of PQ a QT intervals indicates myocardial predisposition to the development of ventricular arrhythmias resulting of conduction impuls disorders or enlarged dispersion of myocardial refractoriness. Evaluation of dynamics of PQ and QT interval changes, the ventricular arrhythmia threshold (VAT) and heart rate (HR) after the surgical interventions, 2 min apnoe and 20 min reoxygenation in in vivo rat experiments was the goal of this study. Experiments were performed on female Wistar rats in ketamine/xylazine anesthesia (100 mg/kg + 15 mg/kg, i.m.) after adaptation on the light-dark cycle 12:12 hours, with the dark part from 6.00 to 18.00. The animals were ventilated by artificial respirator at ventilatory parameters: 1 ml/100 g of body weight and respiratory rate 40-50 breaths/min. ECG was recorded in the single steps of experiment (intact animal, tracheotomy, artery preparation, thoracotomy, the end of 5 min stabilization, after 30, 60, 90 and 120 s of apnoe and after 5, 10, 15 and 20 min of reoxygenation), HR in each minute of experiment and VAT at the end of stabilization, after 2 min apnoe and after 5, 10, 15 and 20 min of reoxygenation. Significant PQ interval prolongation ($p<0.02$) was seen only after thoracotomy vs. initial value. More expressive PQ interval prolongation was during apnoe with significant ($p<0.001$) prolongation after each 30 seconds. QT intervals were shortened after tracheotomy ($p < 0,001$), thoracotomy ($p<0.024$) and stabilization ($p<0.022$) vs. initial value. The significant prolongation ($p<0.001$) was detected already after 30 s of apnoe. Two minutes of apnoe was without influence on duration of QT interval. Reoxygenation returned QT interval to the level of initial value. VAT was significantly decreased ($p<0.009$) at the end of apnoe but reoxygenation returned VAT. HR was significantly decreased only after thoracotomy and during apnoe. The marked HR increase was recorded during the first 3 min of reoxygenation. It is concluded that the surgical interventions influence mainly impuls conduction. Apnoic PQ and QT interval prolongations are probably linked also with gradual VAT decrease increasing so assumption that onset of ventricular arrhythmias is not only result of impuls conduction disorders but also enlarged dipersion of myocardial refractoriness.
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INTERACTION OF HALOPERIDOL WITH l-TYPE VOLTAGE DEPENDENT CALCIUM CHANNELS

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Haloperidol is neuroleptic agent, effective in the treatment of various psychiatric disorders, such as schizophrenia or mania. Cardiac arrhythmias and other severe cardiovascular side effects have been associated with this therapy. We have investigated effects of haloperidol on currents through cardiac calcium channels in native tissue, i.e., in cardiomyocytes, and in expression system, i.e., in HEK 293 cells. Cardiomyocytes were enzymaticaly isolated from adult rat ventricles. HEK 293 cells were transiently transfected with cDNAs encoding a and b isoforms of the α_1 subunit of $Ca_V1.2$ calcium channel together with β_{2a} and $\alpha_2\delta$ subunits. $\alpha_{1C\text{-}a}$ isoform is expressed mostly in cardiac tissue, while $\alpha_{1C\text{-}b}$ is prevalent in smooth muscles. Currents were measured using whole cell configuration of patch-clamp method. In HEK 293 cells transfected with $\alpha_{1C\text{-}b}$ subunit 10 μmol/l haloperidol inhibited approximately 58 % of inward current amplitude measured in peak of IV relation. The inhibition was reversible and voltage – dependent. Approximately 55 % of current through cardiac $\alpha_{1C\text{-}a}$ subunit isoform expressed in HEK 293 cells was blocked by the same concentration of haloperidol. Shift of the reversal potential was observed in both splice variants of $Ca_V1.2$ calcium channel in the presence of haloperidol. This change may be induced by alteration of ion selectivity of the expressed channel. 10 μmol/l of haloperidol inhibited also current through the L-type calcium channels in rat cardiomyocytes. This block was rapid and reversible. In conclusion, inhibition of inward currents through $Ca_V1.2$ calcium channels in model HEK 293 cells and in native cardiomyocytes by 10 μmol/l haloperidol was observed. Used concentration was one order higher than therapeutic plasma concentration, therefore its expected effects in patients will be more subtle. Nevertheless, inhibition of cardiac calcium current still may contribute to observed side effects of haloperidol.
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CARDIOVASCULAR EFFECTS OF MELATONIN IN RATS WITH EXPERIMENTAL HYPERTENSION

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Melatonin, a hormone synthetized in the pineal gland, has been shown to exert antioxidant and antihypertensive effects (1), indicating potential therapeutic application in the treatment of some cardiovascular disorders. The aim of this study was to evaluate a long lasting administration of melatonin in nitric oxide (NO)-deficient hypertensive rats. The animals were divided in four groups: 1) control (normotensive) rats; 2) rats treated with melatonin (12 mg/kg/day); 3) rats treated with N^G-nitro-L-arginine methyl ester (L-NAME, 50 mg/kg/day); 4) rats simultaneously treated with L-NAME (50 mg/kg/day) and melatonin (12 mg/kg/day). The substances were given in tap water for 5 weeks. Systolic blood pressure was measured by a non-invasive tail-cuff plethysmography. Rings of isolated rat thoracic aorta were mounted in isolated organ baths for measurement of isometric contractile force. Systolic blood pressure in L-NAME + melatonin treated rats (159±1 mmHg) was significantly lower than in L-NAME-treated rats (178±1 mmHg, $P<0.001$) but was stil higher than in age-matched melatonin-treated (121±1 mmHg) or untreated control rats (123±1 mmHg, $P<0.001$). Increase in systolic blood pressure in NO-deficient hypertensive rats was accompanied by an increase of the left ventricle/body weight ratio to 1.34±0.04 in comparison to controls (1.10±0.03, $P<0.001$). Melatonin decreased this ratio to 1.25±0.04 ($P<0.05$). Five weeks of treatment of rats with L-NAME significantly inhibited endothelium-dependent relaxation of the isolated thoracic aorta induced by acetylcholine. The inhibitory effect of L-NAME was partially reversed by simultaneous treatment of rats with melatonin. The present results showed that melatonin exerted an antihypertensive action in NO-deficient hypertensive rats. Beneficial effect of melatonin on the acetylcholine-induced relaxation in the thoracic aorta could be explained by its involvement in vascular NO pathway activity.

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THYROID HORMONES AND EXPRESSION OF CARDIAC GAP JUNCTION PROTEIN, CONNEXIN-43 IN AGED MALE AND FEMALE RATS

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Down-regulation of myocardial gap junction connexin-43 (Cx43) channels was thought involved in the development of life threatening arrhythmias. We have previously shown that short-term treatment with thyroid hormones (TH) down-regulates Cx43 (expression and phosphorylation) in young rats (1) and increases susceptibility of young while not in old female rats to ventricular fibrillation (2). The aim of this study was to examine effect of TH on Cx43 in old rat hearts. 20-month-old male and female Wistar rats were feed with l-thyroxine (T_4) 50 µg/100g/day during two weeks. Age-matched controls were used as well. Rats were anaesthetised with ether and the heart was rapidly removed to ice-cold physiological solution, weighed and ventricular tissues were frozen and storage in liquid nitrogen. Cx43 and its phosphorylated isoforms were analysed by western blot using 10 % SDS-PAGE. Separated proteins were transferred to PVDF membranes followed by incubation with monoclonal antiCx43 antibody (Chemicon, Inc. USA). Secondary antibody conjugated with alkaline phosphatase was detected by NBT/BCIP. The results showed that left ventricular weight to body weight ratio was increased due to T_4 treatment. Compared to untreated rats, neither Cx43 expression nor its phosphorylated isoforms were significantly altered in old T_4-treated male or female rat ventricles. However, unless the treatment the expression of Cx43 and its phosphorylation were significantly decreased in male compared to female old rats. In conclusion, these results suggest that there is no apparent effect of l-thyroxine on Cx43 in old rat hearts that can explain in part that susceptibility of old female rats to VF was not increased by TH. There are gender-related changes in Cx43 expression that likely may contribute to the higher susceptibility of males versus females to life threatening arrhythmias.

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THE IMPORATANCE OF THE MODEL OF „THOUSANDS OF MICROCLIPS“ IN UNDERSTANDING HYPERTENSION

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This presentation summarizes recent evolution of the proposition made by F. Henke, O. Lubarsch, and H. Goldblatt almost a century ago that the primary renal microvascular disease may be responsible for the development of hypertension. Since hypertension has repeatedly been diagnosed without clinical evidence of severe renal damage the concept of primary, or essential, hypertension has been introduced. This primary hypertension is now considered to be strictly distinct from “renovascular” or “renal parenchymal” hypertension that is thought to account for less than 5 % of all hypertensive cases. During decades, ample evidence, both experimental and clinical, has been accumulated corroborating that one type of abnormal resistance, namely a resistance situated between the heart and the kidney glomeruli, and not any abnormal resistance situated elsewhere, can cause hypertension. It is the pressure drop along the pre-renal or, obviously, intra-renal circulation that can activate renal defence mechanisms and trigger hypertension. There is no other organ or tissue besides kidney able to force the whole circulation to enhance blood pressure in front of an arterial stenosis. According to the principles unraveled by H. Goldblatt and A. C. Guyton and their colleagues rise in blood pressure emerges as a compensatory process to overcome the structural limits imposed on the altered vasculature and restore the renal perfusion pressure. The blood pressure is sacrificed to achieve body water and salt equilibrium. Sodium and water retention are the key instruments through which the kidney influences mean circulatory filling pressure and long-term systemic arterial blood pressure. Obviously, kidney arteriolopathy, afferent arteriolosclerosis and a number of similar conditions that can collectively be represented by a model of “thousands of microclips” diffusely distributed on vessels within renal parenchyma comply functionally with the “two-kidney, one clipped”, high renin model originally described by H. Goldblatt. At present, techniques for assessing structure of afferent arteriolar tree in human kidney in vivo are lacking, but urgently required since structural or functional stenoses in preglomerular segments of the kidney can emerge as an important link in the pathogenesis of essential hypertension.

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SURFACE TERTIARY FOLLICLES OF EWES OVARIES AFTER LAMB WEANING AND THEIR CHANGES DURING INDUCED OESTRUS IN CONTROLLED REPRODUCTION

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This work was based on the evaluation of the surface tertiary follicles with the exploitation of ultrasound technique and digital photo-documents. We have observed the surface tertiary follicles presence of Improved Wallachian ewes during galactopoesis (non-breeding season) three days after lamb weaning and after oestrus induction and ovulation rate (OR) stimulation. We have valued the ovaries of six ewes in the winter after slaughter milk lambs production. Ewes were in middle body condition (2.5-3.5) and fed packed grass silage, meadow hay, halite and

mineral licks. Flushing was used to increase the OR: 750 g/head/day of maize grit. Synchronization for oestrus induction and OR stimulation was carried out by the application of vaginal sponges (40 mg FGA-fluorogeston acetate) for twelve days. Immediately after sponge withdrawal ewes were i.m. treated with 1000 I.U. eCG (equine chorial gonadotropin). 48-72 hours after treatment was practise laparotomy with USG investigation (5 MHz) and digital phototographs getting of ovaries. USG pictures and photographs were analyzed quantitatively and qualitatively. Results were statistically evaluated by Student t-test. The maximal follicle diameter was larger in the right ovary after both the lamb weaning (5.34±1.16- USG; 4.23±0.95- photo) and hormonal administration (4.12±2.23- USG; 3.76±1.05- photo). Results show at the process of pholliculogenesis after parturition during sucking and milking, after lamb weaning and during galactopoesis of Improved Wallachian breed. Identification and result of the tertiary follicle quantitative and qualitative evaluation on the ovary surface with the presence of follicular fluid as the non-echogen formation is comparable with the literature statements (1; 2). For better qualitative USG evaluation would be more operative to use 7.5-8.0 MHz sound.

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EFFECT OF PHOTODYNAMIC THERAPY WITH HYPERICIN ON HUMAN ENDOTHELIAL CELLS

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Traditional cancer therapies such as surgery, radiation therapy and chemotherapy involve a delicate balance between removing or destroying diseased tissue and sparing surrounding normal healthy cells. These treatments result in serious side effects caused by the loss of normal cell functions as a result of having indiscriminate cytotoxic properties. Therefore, the development of new treatment protocols with more selectivity for diseased tissue is very important. Photodynamic therapy (PDT) is promising new treatment that involves delivery of light sensitive drug, which after accumulation in tumor tissue and activation by light can induce tumor destruction via the production of reactive oxygen species. Hypericin is promising naturally occurring photosensitiser, which is isolated from plants of Hypericum genus. The antitumoral efficacy of PDT with hypericin is related to the circulating drug level, which suggested that tumor vascular damage is primarily responsible for the antitumoral activity of this photosensitizer. Endothelium, lining the inner side of blood vessels, is the primary target population of cells for drugs occurring in plasma. Targeting the tumor vasculature by PDT with hypericin is a promising way to tumor eradication. The aim of our study was to compare the sensitivity of endothelial cells and two types of malignant human gliomas in vitro to PDT with hypericin. The first step in this study was general MTT test of viability of these cells after 72-hours incubation. Results of MTT did not prove higher sensitivity of endothelial cells if compared with U-87 MG and U-373 MG. Significant effect on viability was observed only after highest concentration of hypericin (10^{-6}M). DNA fragmentation was observed thought over lower concentration (10^{-7}M) after 48-hours incubation. However, apoptosis detection of EC by flow cytometry analysis with annexin-V FITC demonstrated the efficacy of hypericin after 4 hours incubation in concentrations that were absolutely inefficient in MTT tests. Our results compose basis for other research on PDT influence in tumor vasculature.

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THE DEPENDENCE OF ION TRANSPORT AND MEMBRANE LIPIDS ON MATURITY OF RAT ERYTHROCYTES

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In our previous study on erythrocytes (ery) of Dahl rats with salt hypertension significant relationships were found between ion transport and membrane lipids (cholesterol, total phospholipids and sphingomyelins). Furthermore, mean cellular hemoglobin content (MCHC) correlated negatively with activity of the Na^+-K^+ pump and Na^+ leak and positively with activity of the Na^+-K^+ cotransport in the study. Moreover, it is generally accepted that immature erythrocytes exhibit lower MCHC than mature erythrocytes. Thus the aim of present study was to find a relationship between the activity of ion transport, the lipid composition of erythrocyte membranes and erythrocyte maturity. Male Wistar rats aged three months were used in this study and the half of these rats was subjected to repeated hemorrhage (blood loss 2 ml/day for 6 days) to enrichment of erythrocytes by immature forms. Erythrocytes of control and hemorrhaged groups were divided into immature and mature fractions by centrifugation. Hemorrhaged rats had significantly enhanced amount of reticulocytes (267.3±22.3 vs 23.3±4.1 ‰, $p<0.00001$), reduced hematocrit (34.0±0.9 vs 41.8±0.4 %, $p<0.00001$) and MCHC (4.386±0.040 vs 4.863 ±0.046 mmol/l ery, $p<0.00001$) in comparison with control rats. Immature erythrocytes differed from mature erythrocytes in the hemorrhaged group by elevated activity of the Na^+-K^+ pump (9.307±0.449 vs 8.413±0.424 mmol/l ery/h, $p<0.05$), reduced activity of the Na^+-K^+ cotransport (0.639±0.065 vs 0.715±0.059 mmol/l ery/h, $p<0.05$) and increased Rb^+ leak (0.946±0.032 vs 0.833±0.026 mmol/l ery/h, $p<0.001$). Differences in the activities of the ion transport parameters were accompanied by higher concentrations of total cholesterol (immature vs mature ery: 4.205±0.045 vs 4.004±0.040 µmol/g ery, $p<0.01$) and total phospholipids (4.691±0.068 vs 4.339±0.078 µmol phosphorus/g ery, $p<0.01$) in the membrane of immature erythrocytes. Thus certain abnormatities of erythrocyte ion transport and membrane lipid composition detected in hypertensive animals might be caused by higher incidence of immature cells.

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THE EFFECT OF PRENATAL METAMPHETAMINE AND PRENATAL STRESS ON NOCICEPTION IN ADULT MALE AND FEMALE WISTAR RATS

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Metamphetamine (MET) is a drug with psychostimulatory effect. When abused during pregnancy, MET shortens gestation period and alters growth of fetuses resulting in lower body weight and retarded intrauterine development. Functional changes are related to increased dopaminergic and serotonergic neurotransmission. Serotonin significantly influences maturation of dendrites and synaptogenesis. Children from abusing mothers have insufficiently developed descending pain modulating pathways and therefore they could be more sensitive to pain. Information about the nociception in adulthood is missing. The aim of the present study was to test gender differences in nociception in adult Wistar rats whose mothers were daily exposed to stress (injection of saline) or to stress with MET (injection of MET) during the entire pregnancy. Moreover, in female rats, the effect of estrous cycle was assessed, to determine the effect of estrogen on nociception and dopaminergic function. Nociception was tested during 85-90 postnatal day in three group of males (N=30) and females (N=28). The MET group consists of animals whose mothers were daily

treated with MET (5 mg/kg; s.c.); in the stress group, mothers were daily treated with saline and the control group (INT) consists of intact animals without any prenatal injection. Nociception was tested in afternoon hours using the tail-flick and the plantar tests; in male rats once, in female rats repeatedly in five consecutive days. The estrous phase was evaluated from vaginal smears daily in morning hours. Prenatal stress and prenatal MET influenced nociception in sex-dependent manner. Prenatal MET did not change nociception in males. In females, it increased nociceptive sensitivity of forelimbs and hindlimbs, but not of the tail. In both genders, prenatal stress decreased nociceptive sensitivity of the tail, but not of the limbs. MET females had in 40 % irregular estrous cycle. Estrous cycle had no effect on nociception in the plantar test. In the tail flick test, the highest sensitivity was observed during estrus comparing to proestrus. In conclusion, female rats prenatally exposed to MET have increased nociception and they are more sensitive to the effect of stimulatory drugs.

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DETECTION AND CHARACTERIZATION OF DYADIC CALCIUM RELEASE EVENTS

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During excitation-contraction coupling in mammalian cardiac myocytes, membrane depolarization activates voltage-dependent L-type calcium channels. The calcium influx activates calcium release channels (RyRs), clustered in the sarcoplasmic reticulum (SR) membrane at the dyadic junctions, which in turn release calcium from the SR. This release of calcium ions can be observed by means of confocal microscopy as transient increases of calcium concentration, represented by fluorescence changes of calcium sensitive indicators. Use of slow calcium chelators such as EGTA along with calcium indicators allows measurement of local calcium fluxes – calcium spikes – that represent activity of elementary calcium release units. Functionality of calcium release units, important for efficiency of excitation-contraction coupling, was assessed from the amplitude and kinetic parameters of calcium spikes evoked by calcium currents in isolated rat ventricular myocytes. Calcium currents were activated by voltage pulses to 0 mV from a holding potential of -50 mV, using whole-cell patch clamp technique. Calcium spikes, measured using the Leica TCS SP2 confocal microscope, 0.1 mM fluo-3 as calcium indicator, and 1 mM EGTA to limit Ca^{2+} diffusion, were analyzed by fitting the fluorescent intensity profiles, centered on individual dyads, with a phenomenological function. We have observed two distinct categories of Ca spikes. The early Ca spikes occurred near the peak of calcium current, had a Gaussian distribution of latencies, and their time to peak and time to half-maximum were negatively correlated to their amplitude. The late Ca spikes had significantly lower amplitudes, occurred when the calcium current was already substantially inactivated, and their kinetic parameters were independent of their amplitude. Based on simulations of spatio-temporal profiles of calcium spikes using the program CalC (1), we propose that in the early spikes, the negative correlation between spike amplitude and its time to peak arises because of differences in distances between the plane of spike origin and the focal plane. The presence of a subpopulation of late Ca spikes with low amplitude cannot be explained by this mechanism and suggests the existence of a subpopulation of "lazy" RyR clusters.

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PHYSIOLOGICAL VALUES OF FINGER ARTERIAL PRESSURE AND BAROREFLEX SENSITIVITY FOR THE AGE BETWEEN 11-21 YEARS

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Physiological limits of blood pressure measured in brachial artery were determined for causal and 24-hour measurements. The disadvantage of these methods is the impossibility of determining blood pressure (BP) beat-by-beat. Determination of baroreflex sensitivity (BRS) in children is based on spectral analysis of beat-by-beat fluctuations of BP. BRS is newly studied in children with respect to an increasing prevalence of obesity and the risk of hypertension in youth. The aim of our study was to quantify limits for BRS and BP recorded in finger arteries (FAP) at the age between 11 and 21 years. Four hundred and sixty-seven healthy subjects (11-21 years) were examined. Continuous 5-min recordings of FAP (Finapres) were taken in sitting, resting position (controlled breathing 0.33 Hz). Beat-to-beat values of inter-beat intervals (IBI), or instantaneous heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured, and their power spectra calculated. Baroreflex sensitivity in ms/mmHg (BRS) and mHz/mmHg (BRSf) were determined at a frequency of 0.1 Hz as a quotient between the power spectrum of IBI (or HR) and the cross spectrum of SBP and IBI (or HR). BRS was age independent, and therefore limits for the whole group were estimated: 5th percentile -3.9 ms/mmHg, 50th percentile - 9.0 ms/mmHg, 95th percentile 19.4 ms/mmHg. BRSf decreased significantly with age (r = -0.35), the average decrease was 0.9 mHz/mmHg. BRSf limits were estimated for each age group, and percentile graphs were constructed. The average BRSf limits for the whole group were 5th percentile -7.3 mHz/mmHg, 50th percentile -16.0 mHz/mmHg, 95th percentile 33.7 mHz/mmHg. We calculated percentiles for mean values of SBP and DBP of FAP in each age and sex group. These values did not significantly differ from those used as standard for the Riva-Rocci method. Determination of physiological standards (age 11-21 years) of BRS, BRSf, and FAP brought additional information: BRS is age independent, BRSf decreases with age; in 5 % of healthy subjects BRS is below the value associated with hypertension in adults; FAP corresponds to standards generally used.

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CIRCADIAN PROFILE OF PINEAL AND PLASMA MELATONIN IN HYPERTENSIVE TGR (mREN-2)27 RATS WITH AN INVERSE CIRCADIAN BLOOD PRESSURE PATTERN

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The transgenic TGR (mREN-2)27 rat, carrying an additional mouse Ren 2 gene can serve as an animal model of human hypertension while they express an inverse daily blood pressure (BP) profile (non dipper). Severe hypertension develops in these rats between 5-10 weeks of age and during this period they develop an inverse BP profile with higher values during the daytime and low during the nighttime. The aim of our study was to investigate circadian rhythms in concentrations of hormones melatonin and aldosterone that play a role in the circadian organization and hypertension, respectively. Animals were obtained from the Institute of Clinical and Experimental Medicine (Prague). Male rats (280-340 g) were kept under controlled environmental conditions with a free access to food and water. They were entrained to LD 12:12 with lights on at 8.00 and were killed by decapitation in 4 hour intervals. Concentrations of melatonin and aldosterone were measured by radioimmunoassay. Melatonin concentrations in both the pineal gland and plasma expressed an expected daily pattern with high concentrations during the darktime and low during the lighttime in both control and TGR rats. There were no significant differences in maximum darktime melatonin levels. However, the evening rise of pineal melatonin was phase advanced in comparison with controls.

Plasma aldosterone concentrations peaked before the beginning of the nighttime in both groups but concentrations were more than 10-times higher in TGR (968.6±192.7 ng/ml) than in control (83.4±30.9 ng/ml) rats. An opposite pattern was seen during morning hours (10.00) when higher levels were found in control (35.2±15.7 ng/ml) than in TGR (15.3±3.2) hypertensive rats. High aldosterone concentrations in TGR rats suggest its role in this type of hypertension. Our results demonstrate that the non-dipping profile of BP in TGR hypertensive rats with an over expressed renin-angiotensin system exhibit a partially disturbed circadian system but the exact place where the disturbances occur is not known.

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ALTERED VASOACTIVE BALANCE IN EXPERIMENTAL HYPERTENSION

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It is evident that increased peripheral resistance in human or experimental hypertension is partly caused by the dysbalance of vasoconstrictor and vasodilator systems. Our studies were aimed to the evaluation of vasoactive balance in three different forms of experimental hypertension – salt hypertension of Dahl rats, NO-deficient hypertension induced by chronic L-NAME administration and spontaneous hypertension in SHR animals. Using a consecutive blockade of principle vasoactive systems (renin-angiotensin system, sympathetic nervous system, nitric oxide) we have demonstrated that all three forms of experimental hypertension are characterized by augmented sympathetic blood pressure component. The magnitude of this component is also decisive for blood pressure changes elicited by various chronic antihypertensive interventions. This is even true for targeting of renin-angiotensin system (captopril-treated SHR) or antioxidant administration (Dahl rats treated with N-acetylcysteine). It should be noted that NO-dependent vasodilation fails to compensate enhanced sympathetic vasoconstriction in SHR or Dahl rats. In NO-deficient rats (L-NAME hypertension) the reduction of NO synthesis is compensated by considerable enhancement of endothelium-derived hyperpolarization factor so that vasodilator mechanisms are less altered than it was expected in this hypertensive model. It can be concluded that in most forms of experimental hypertension the augmented sympathetic vasoconstriction prevails over the existing vasodilation, leading thus to a relative vasodilator deficiency and increased vascular tone.

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CHEMICAL CONTROL OF BREATHING IN HYPOVOLEMIC RABBITS DURING EXPERIMENTAL HYPERTHERMIA AND ITS PHYSICAL TREATMENT

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The anesthetized rabbits were divided into two groups: normovolemic (NV; n=14) and hypovolemic (HV; n=16). Hypovolemia was induced by administration of furosemide. The body temperature (initial T_b=38 °C) was gradually elevated to 42 °C by body surface heating. Subsequently, T_b was lowered by gradual cooling. Recordings were all made at normothermia (T_N =38 °C), during heating at 39.5-40.5 °C (T_{H1}) and at T_b = 42 °C (T_{H2}). In the course of cooling: at 40.5-39.5 °C (T_{C1}) and at T_b recovered to the initial value (T_{C2}). Hypercapnic ventilatory response (HCVR). The animals breathed a gas mixture of 40 % O_2, balanced with N_2. For continuous rise of P_{ETCO2} CO_2 was added to inspiratory gas. The HCVR was estimated as the slope of ventilation-ET_{CO2} curves. Hypoxic ventilatory response (HVR). Four hypoxic mixtures (11, 9, 7, 5 % O_2) were inhaled for 2 minutes. The HVR was estimated as the percentual change of ventilation (V_E) during episodes of hypoxia regarding V_E in normoxia. HCVR: In NV group, heating caused an increase in the slope for V_E vs. P_{ETCO2}, but not in HV rabbits. Recovery of T_b was accompanied with significant change of HCVR neither in NV nor in HV group. Between-group comparison revealed significant decrease in HCVR at T_{H2} and T_{C1} in HV vs. NV: 100±16 $ml.min^{-1}.kPa^{-1}$ vs. 162± 20 $ml.min^{-1}.kPa^{-1}$ ($P<0.05$); 159±22 $ml.min^{-1}.kPa^{-1}$ vs. 93±11 $ml.min^{-1}.kPa^{-1}$ ($P<0.05$). HVR: In normothermia, during 5 % O_2 run V_E rised in NV by 85 %, while in HV by 80 % ($P<0.05$). Elevation of T_b to 42 °C during 7 % and 5 % O_2 runs led in HV group to 5 % and 6 % increase in V_E comparing to 31 % and 30 % increase in NV group (both $P<0.05$). During exogenous hyperthermia HCVR was augmented in normovolemic, but not in hypovolemic rabbits. Attenuation of HVR was present in both groups during heat stress, it persisted also in the course of T_b recovery and it was more prominent in hypovolemic animals.

THE INTERACTION OF MUSCLE CREATINE KINASE WITH MYOFIBRILS

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The proposed essential physiological component (1) that controls muscle creatine kinase (MM-CK) activity besides the substrate induced energy minimizing conformational changes turns attention toward the nature of the interaction of MM-CK with myofibrils. The unique interaction involves two parts, namely the molecular origin for MM-CK binding to the myofibrillar M-line and the exchange of MM-CK between the M-line and its surroundings, the both of which are not well understood. In order to evaluate the rate of the latter process, in situ exchange assays with iodoacetamidofluorescein (IAF) labelled MM-CK has been studied by confocal microscopy. The purified myofibrils from rabbit psoas muscle (20 mg prot/ml) were incubated for 10 min in the total volume of 0.1 ml relaxing solution, pH 7.1 with added 2,5 µl of IAF-MM-CK (4.5 mg prot./ml). Visualisation of the H-band by 3-D reconstruction confirmed IAF-MM-CK binding at the M-line and excluded the possibilities of outside surface absorption and axial fluorescence distribution. Thus, the real exchange time of IAF-MM-CK was determined in longitudinal two-dimensional sections, using Fluorescence Lost in Photobleaching (FLIP) method. The exponential decay of fluorescence intensity relative to the background, into the photobleached surrounding medium was followed in three compartments, i.e. at the H-zone, in the actomyosin zone overlapping the A- and I-bands and on the longitudinal myofibrillar surface. The average time constant of the intensity decrease at the H-zone (57 M-lines examined from 8 myofibrils) equalled 20.4±5.3 s. The time constants of the decreases in the other two compartments were the same and equalled to 6.5 s. The FLIP experiments confirmed a specific MM-CK interaction within the H-zone structure, with a weak but significant affinity. Since the mechanical response during the isotonic twitch by fast, as well as slow muscles, and even the power peak during an isometric tetanus (2) take up the times three order shorter, the duration of binding is long enough for a possible myofibrillar control of MM-CK conformational changes during contractions.

(1) Mejsnar J et al.: Physiol Res 51: 35-41, 2002.
(2) Mejsnar J et al.: J Physiol Lond 511: 155P, 1998.

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