HUMAN POSTURAL RESPONSES TO LEG MUSCLE VIBRATION ALTERED BY VISUAL SCENE MOTION

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Sensory interaction in posture control was investigated by postural responses to differently timed proprioceptive and visual stimulation. The aim of this study was to find out whether visual scene motion started some seconds before proprioceptive stimulation onset can modulate magnitude and velocity of the postural responses to the lower leg muscle vibration. We evaluated postural responses of 22 subjects (10 men and 12 woman, range 24-60 years) to paired proprioceptive-visual stimulation with difference in the timing of the both stimulation onsets. As proprioceptive stimulation was used both soleus muscles vibration with duration 8 s, frequency 55 Hz and amplitude 1 mm. Visual stimulation was rotating disc in diameter 125 cm, placed on the right side of subjects and moving in both direction. The visual stimulation started either together with the vibration, or there was 3 s before vibration onset. Centre of pressure (CoP) was recorded by force platform. Trunk tilts in AP direction was measured by two accelerometers attached on spinal column, one at the level of shoulder blades and on the coccyx at hip level. Subjects performed 4 series of 5 trials with duration 20 s: vibration, vibration together with scene motion forward, vibration together with scene motion backward, scene motion forward 3s before vibration, scene motion backward 3 s before vibration. The results showed that early slopes and final angles of body tilt induced by soleus muscle vibration were modified by changes in visual input activated by motion of visual scene. Soleus muscles vibration induced in early period body tilt backward followed by a slight slower body lean ended by fast body return to initial position after vibration offset. In paired visual and proprioceptive stimulations early part of postural responses were faster as in single proprioceptive stimulation. When visual stimulation started 3 s before muscle vibration, the induced CoP and Acc records of postural response to soleus muscle vibration occurred with faster slope and did not depend on direction of scene motion. Significantly different final angles occurred between situations with visual scene motion forward and backward in all values of timing. The final body tilts in backward direction induced by sensory stimulation indicated on hip level an additive effect of leg proprioceptive and visual inputs.

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CONTROLLED BEHAVIOR OF VASCULAR SMOOTH MUSCLE CELLS ON SYNTHETIC POLYMERS WITH BIOACTIVE SURFACES

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A novel approach in the field of tissue engineering, i.e. construction of bio-artificial tissue and organ replacements, requires design of materials actively promoting specific cell responses in a controllable manner. In the first set of experiments, we tried to control the adhesion strength, proliferation rate and onset of differentiation of rat aortic smooth muscle cell (RASMC*) in vitro* by changing the physicochemical surface properties of polyethylene growth support by its irradiation with O^+, C^+ or Ar^+ ions (energy 30-150 keV, dose from 10^{12} to 10^{15} ions/cm^2). The ion irradiation increased both wettability and concentration of carbon and oxygen functional groups in the polymer surface, which leaded to a higher and more homogeneous adsorption of collagen and fibronectin and more effective adhesion of RASMC. These cells displayed larger cell-material contact area, larger and more numerous focal adhesion plaques, and a higher concentration of focal adhesion proteins paxillin and α-actinin. Their proliferation phase was less pronounced, and these cells entered sooner the differentiation program, as revealed by a higher concentration of alpha-actin, SM1 and SM2 myosins and calponin. The ion-irradiation technique was also used for the micro-patterning of the polyethylene surface, i.e. creation for cell-adhesive and non-adhesive micro-domains for regionally selective cell colonization. In the second set of the experiments, GRGDSG peptides, ligands for integrin adhesion receptors on cells, were covalently bound (in 5 and 20 % concentration) against a cell non-adhesive background, represented by a copolymer of poly(D,L lactide) and poly(ethylenoxide). These constructs ensured ligand concentration-dependent integrin-mediated cell adhesion, spreading, growth and viability of RASMC even in serum-free media, i.e. without exogenously added adhesion and growth factors. These results indicate the possibility to control growth and differentiation of smooth muscle cells on vascular prostheses by extent of adhesion of these cells, which could help in prevention of excessive proliferation of these cells leading to restenosis of the vascular lumen.

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ROLE OF PROTEIN KINASE C IN CELL SWELLING INDUCED INSULIN SECRETION

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Osmomechanical stress, resulting in cell swelling, causes an immediate secretory response in various cell type. Secretion possesses some unique features (including independence from extracellular Ca^{2+}), signal transduction pathway remains unclear. In present work we have tested, whether protein kinase C (PKC) is engaged in cell swelling induced secretion. PKC potentiates an existing oscillatory secretory activity in various cells. PKC is known to induce phosphorylation of one or several proteins directly participated in exocytosis in the mechanism of glucose-stimulated insulin release. We have used specific inhibitor of PKC - bisindolyl maleimide VIII (BIM) in cell swelling induced insulin secretion from rat pancreatic islets. Isolated islets were incubated in basal and stimulated medium (30 % hypotonicity) with Ca^{2+} or without Ca^{2+}. Neither basal nor somatically stimulated secretion of insulin from pancreatic islets was inhibited by BIM. However, as awaited, islets treated with BIM showed decreased insulin secretion in response to glucose. Results suggests that the pancreatic B cells can use multiple pathways to control exocytosis of secretory vesicles in response to extracellular stimuli. Protein kinase C is necessary for glucose stimulated secretion of insulin from pancreatic islets, but it is not required for cell swelling stimulated insulin secretion.

THE EFFECTS OF CARBACHOL TREATMENT ON DEVELOPMENT OF MUSCARINIC RECEPTORS AND BETA-ADRENOCEPTORS IN EMBRYONAL CHICK HEART VENTRICLES

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When activated, G protein-coupled receptors can undergo desensitization, internalization or down-regulation of receptors. The aim of our study was to follow the changes in muscarinic receptors (MR) and beta-adrenoceptors (BAR) densities and function in developing heart tissue and after carbachol treatment (muscarinic agonist). Developing embryos of white Leghorn hens from the day 13 *in ovo* to day 21 (hatching) were exposed to continual infusion of carbachol starting at the day 13 day for 24 hours, 3, 5 or 7 days. The solutions were applied from the osmosis-based apparatus to the papyraceous membrane. The embryos were killed from 1-24 hours after the end of infusion. Radioligand binding of specific receptor antagonists with rapid filtration was applied to detect the receptors. The ligands used were [^{3}H]-quinuclidynyl benzilate for the MR and [^{3}H]-CGP 12177 for the BAR. Heart rate (deduced from acoustocardiography) served as a marker of the functional outputs. There were no changes in the MR density after 3, 5 and 7 days of carbachol treatment. The dose and decease-interval dependent up-regulation in the density of MR (about 2.2 by control level) was found after 24hour application only. The density of BAR was not changed during treatment. The differences in heart rate between control and carbachol treated animals were not substantial. In order to determine the nature of MR up-regulation we have employed the co-infusion of carbachol together with PKC inhibitor bisindolyl-maleimide. Our results show that MR in the developing chick heart ventricles does not undergo a down-regulation as in other systems. The BAR density was not changed under carbachol. Despite that the receptors were in imbalance, the functional output was not changed.

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HIGH-FREQUENCY ELECTROMAGNETIC FIELD EXPOSITION INFLUENCES AN ELECTRICAL ACTIVITY OF NORMAL AND NEURODEFECTIVE BRAIN

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The aim of the present study was to perform a direct registration of brain cortical activity during an influencing it by the HF EMF and a depiction of possible changes in the hippocampal rhythmicity. All experimental procedures were done under urethane anesthesia (20 %, 2g/kg i.p.) in Lurcher mutant mice, wild-type (healthy animals) were used as controls. This mutation represents a natural model of genetically determined olivocerebellar degeneration. Experimental animals were exposed to the HF EMF with frequency of 870 MHz. Our method is based on the use of gel electrodes (silicon tubes filled with agar) and a connection with platinum electrodes has been located out of HF EMF space. Spontaneous electrocorticogram (ECoG) was measured as 2 min segments from continuously recorded activity either without HF EMF or with it. Final calculation by the Fourier analysis and averaging were performed off-line on DISYS-system (Software for data acquisition and analysis). Hippocampal activity was recorded by teflon coated stainless steel wire (125 μm bare diameter) which was used for registration, grounding electrode was fixed by screw to bone in prefrontal area. Stereotactic coordinates - ref. point bregma: 2.0; 1.5; 1.3 (CA1) - 2.0 (hilus DG). DISYS-system for a final averaging according to frequency spectra with emphasis on the theta-oscillation has been used. ECoG evaluation showed a distinct shift to lower frequency components but clear effect only in wild - type (healthy littermates) has been observed whereas in Lurcher mutant mice gentle differences between frequency spectra were found. These findings support an idea about higher vulnerability of the CNS in Lurcher to some physical and chemical factors in comparison with controls, describing in recent studies, including ours. Measurement of hippocampal activity showed in both types of animals (healthy and mutant) changes in theta oscillations namely a location of its "dominant peak". In wild - type, main theta rhythm in CA1 was observed in "native" animals (i.e. without HF EMF exposition) whereas in "native" Lurcher mutant mice theta oscillations in dentate gyrus area were registered.

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ROLE OF I_{Na} AND I_{to} IN RATE-DEPENDENT EFFECT OF AJMALINE: ACTION POTENTIAL VOLTAGE CLAMP ANALYSIS

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The efficacy of antiarrhythmic drugs is significantly affected by their frequency dependent effect. This study was aimed to assess how Ca^{2+}-independent currents contribute to the ajmaline-induced rate-dependent changes of action potential (AP) configuration. The experiments were performed on isolated rat ventricular myocytes at room temperature. Ca^{2+}-dependent currents were inhibited by Co^{2+} (2 mmol/l). To bring the analysis nearer to natural conditions, we used the AP voltage clamp method. Free running APs were recorded in a sequence of regular stimuli (2.5 Hz) following a period of rest (60 s). In control (Tyrode + Co^{2+}), action potential duration measured at 75 % of repolarization (APD_{75}) gradually increased whereas its amplitude remained unaltered. In contrast, APD_{75} was not significantly affected but AP amplitude was gradually decreased under the effect of ajmaline (30 μmol/l). To reveal the underlying currents, a steady state AP recorded in control at 2.5 Hz was stored for use as a command signal in voltage clamp protocols (regular stimuli at 2.5 Hz following a 60 s period of rest). In control, the gradual prolongation of free running AP (APD_{75}) correlated with the decreased charge carried by the transient outward current I_{to} measured in the AP clamp mode. Under the effect of ajmaline, marked gradual decrease of AP amplitude correlated with gradual decrease of the charge carried by the fast sodium current I_{Na}. To understand the mechanism of these changes in more detail we studied the recovery of both currents I_{Na} and I_{to} from inactivation and from block. In control, the recovery of I_{Na} from inactivation was fast (time constant ≈ 60 ms) compared to the period of stimulation (400 ms) which prevents the cumulative inactivation of I_{Na}. The longer time constant of I_{to}-recovery from inactivation (≈ 460 ms) can lead to cumulative inactivation of I_{to} and, thus, to gradual prolongation of AP. Under the effect of ajmaline, both currents were greatly attenuated and their recovery from block was slow. The attenuated slowly recovering I_{Na} can account for gradual decrease of AP amplitude whereas the suppressed I_{to} was unable to induce significant AP changes.

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LONG-LASTING INTERMITTENT HYPOXIA IN COMBINATION WITH KAINIC ACID ADMINISTRATION – A MORPHOMETRICAL STUDY (III)

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Using an NADPH-diaphorase staining we studied effects of i.p. administration of kainic acid (KA) on individual hippocampal regions and on the auditory cortex. One day prior to the KA application, experimental animals were exposed to chronic hypoxia. The young rats from the 2nd till the 17th day of age were exposed to long-lasting repeated hypoxia in a hypobaric chamber in the simulated altitude of 7000 m, for 8 hours a day. At the age of 18 days, the animals were given a single i.p. injection of KA (2.5 mg/kg). Aged 1 year, animals were killed by transaortal perfusion of 4 % buffered paraformaldehyde. Cryostat sections were stained to prove NADPH-d positive neurons, which were then quantified in individual parts of the hippocampus (CA1, CA3, hilus, dorsal and ventral blades of the dentate gyrus) and in the auditory cortex. The results show that chronic hypoxia and KA given to the normoxic animals decreased the density of the NADPH-d positive neurons in the hippocampus, compared to the control group. In contrast, KA given to the hypoxic animals didn't change the density of these neurons in CA1 and CA 3 areas of the hippocampus, in the hilus, and ventral blade of the dentate gyrus, and increased in the dorsal blade of the dentate gyrus, and in the auditory cortex. Hypoxia and KA having influenced the young rats brought about the decrease of the number of NADPH-d positive neurons in some areas of the central nervous system. This could stand for hypoxic or eventually toxic alteration of these regions depending on different sensitivity to these factors.

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EFFECT OF SIMULTANEOUS PYRIDOSTIGMINE BROMIDE AND STRESS TREATMENT ON CARDIOVASCULAR SYSTEM IN MICE

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Pyridostigmine bromide (PB) acts by reversible inhibition of acelylcholinesterase (AChE) which is involved in the metabolism of ACh and thus in regulation of neuromuscular and autonomic function. Experiments were performed to determine the effect of simultaneous PB (10 mg/kg/day, 7 days, s.c. osmotic minipumps) and stress (shaker stress, 45 sessions/day, 2 min/session) on blood AChE, mean arterial pressure (MAP) in C57BL6/J male mice. Additionally, the structure and apoptotic markers (Bax, BCl-2) in the heart and aorta were investigated. MAP was determined before treatment, on days 1 and 7 of treatment using chronic carotid arterial catheters. The average basal 24-h MAP was 107±1 mm Hg. Stress significantly increased MAP on day 1 while PB had no effect. On day 7, no differences in MAP were observed among the groups, even though there was a significant reduction in blood AChE in PB treated animals. Stress-induced pressor reactivity was investigated in the dark (23.00 h) and light (11.00 h) period of day and no differences were revealed between PB and PB+stress treated mice. Heart-to-body weight ratio was slightly reduced in PB-treated mice; however there was no change in cardiac fibrosis. Interestingly, there was a 4-fold increase in the Bax-to-Bcl-2 ratio in the heart of PB and PB+stress treated mice. Significant reduction of the aortic wall thickness-to-diameter ratio was found only in PB-treated mice. In the aortic endothelium, Bax-to-Bcl-2 ratio was reduced in PB, stress and PB+stress groups vs the control with the lowest value in the PB+stress group. Data suggest possible antiproliferative and antitrophic effect of PB in the heart and aorta of mice that was eliminated by simultaneous stress-treatment. It may result from the elevation of ACh concentration and thus the NO production in the heart and aorta.

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METABOLIC CHANGES AT THE ACUTE HEMORRHAGIC HYPOVOLEMIA

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Hemorrhagic hypovolemia critical states are connected with high morbidity and mortality. The most expressive clinical signs of the first phase of acute hemorrhagic hypovolemia are: hypotension, tachycardia, tachypnoe and rapid reorganization of the energy - producing metabolic system. Clinical useful markers for assessment of the quantity of blood loss and state of patient after bleeding are still widely discussed with contradiction results. The aim of this study was besides cardiorespiratory changes to follow the metabolic changes during the bleeding. The acute experiments were performed on 20 cats, anesthetized by pentobarbital (i.p. 40 mg/kg). Two kind of bleeding were used, continual bleeding till to 30 % of blood volume loss and gradual bleeding each 10 % of blood volume with 5 minute time intervals between bleeding episodes. Variables as blood pressure, heart rate, breathing, acid base balance, hematological markers, plasma concentration of: glucose, lactate, pyruvate, proteins and lipids were evaluated. We found during the bleeding in addition to tachycardia also paradoxical bradycardia in spite of the hypotension and tachypnoe. Immediately after the blood loss (up to –30 %) there was paradox of metabolic acidosis in venous blood and signs of respiratory alkalemia in arterial blood. The number of erythrocytes, leucocytes, hematocrit ratio, concentration of hemoglobin and arterio-venous oxygen-saturation difference significantly increased. Markers of metabolic effects of bleeding were dependent on degree and speed of bleeding. During the gradual bleeding, the plasma lactate level significantly increased at 10 % of blood volume loss, the plasma glucose level significantly increased at 20 % of blood volume loss followed by decreasing of total protein and lipid concentrations. The most marked metabolic changes were at level of 30 % of blood loss. Our findings in experimental animals account for the fact that venous blood the most correctly reflects the acid base state in the first phase of hemorrhagic hypovolemia. The best markers are arterio-venous differences of pH, PO_2, PCO_2, oxygen saturation and lactate concentration. Higher leukocyte count and blood glucose concentration are considered as the useful nonspecific markers.

INTEGRATION OF MITOCHONDRIAL UNCOUPLING PROTEIN 3 INTO SKELETAL MUSCLE METABOLISM

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Our previous results demonstrated postnatal induction of uncoupling protein 3 (UCP3) gene expression in skeletal muscle of human newborns, involvement of nutritional lipids in the induction, and impairment of the induction in neonates delivered before approximately 26 weeks of gestation. Aim of this work was to elucidate the control of UCP3 gene expression and the role of UCP3 in the muscle metabolism. Human studies were performed on autopsy samples of skeletal muscle, obtained from 30 newborns (who mostly died during the first postnatal month) and two aborted fetuses. Samples of skeletal muscle were also obtained from 3 day-, 10/20 day-, and 5.5 month-old C57BL/6J mice. Expression of the genes for UCP3, transcription factors controlling the expression of UCP3 (PPARα and MyoD, respectively), and selected genes of glucose and energy metabolism was characterized by real-time RT-PCR. Content of UCP3 and AMP-activated protein kinase (AMPK) was evaluated by immunoblotting. Activity of AMPK was estimated from the content of the phosphorylated form of the kinase. Levels of adenine nucleotides in muscle extracts were measured by HPLC. Neither of the transcripts studied in human samples showed a similar postnatal recruitment as UCP3. Both PPARα and MyoD transcription factors were present regardless of gestational age, i.e. deficit of any of them was not responsible for the observed impairment of UCP3 gene induction in extremely premature newborns. On the other hand, a strong correlation between UCP3, PPARα, and glucose transporter 4 (GLUT4) was found. Study in newborn mice documented a similar postnatal expression pattern of UCP3, PPARα and GLUT4, but also ALAS1 (5-aminolevulinate synthase) and AMPK. AMPK, known as a regulator of lipid and glucose metabolism and also mitochondrial biogenesis, is activated by the increase in the AMP/ATP ratio. By comparison of glycolytic and oxidative muscles from adult mice, we observed an association between the expression of UCP3 and AMPK, and between the activity of AMPK and AMP/ATP ratio, respectively. Our results suggest that UCP3 may be involved in the changes in the cellular energy charge (i.e. ATP/AMP ratio) and AMPK cascade activity and demonstrate that UCP3 is linked to both lipid and glucose metabolism.

EFFECT OF PENTOXIFYLLINE ANALOGUES ON P-GLYCO-PROTEIN MEDIATED MULTIDRUG RESISTANCE OF MURINE LEUKEMIA CELLS l1210/VCR

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In our previous papers (1-3) we described the ability of methylxanthine pentoxifylline (PTX) to depress the P-glycoprotein (P-gp) mediated multidrug resistance (MDR) of the mouse leukemic cell line L1210/VCR. In the present paper we have analysed the capability of twenty-five methylxanthines to depress MDR of L1210/VCR cells. These methylxanthines structurally differ in substituents located in positions N1, N3, N7 and C8. The results indicate that for an effective reversal of P-gp mediated MDR of our cells the existence of a longer polar substituent in the position N1 plays a crucial role. The elongation of the substituent in the positions N3 and N7 (from methyl to propyl) increases and in the position C8 (from H to propyl) decreases the efficacy of xanthines to reverse the vincristine resistance of L1210/VCR cells. The multiple linear regression for effectiveness of methylxanthines in reversal of P-gp mediated MDR of L1210/VCR cells (expressed as respective IC50r values) has been computed, with molar weight - Mw, molar volume - VM, molar refractivity - RM, crystal density - d and partition coefficient n-octanol/water - log P as descriptors. A high intercorrelation of MW, VM and RM was found for the tested group of methylxanthines indicating that only one of these parameters is necessary for testing a potential correlation. The best fit in the multiple linear regression was obtained for RM applied together with d and log P and resulted in a QSAR model given by the following equation: $IC50r=-[(32.3\pm7.2)\times10\text{-}3\times RM] +[(10.1\pm2.3) \times d]+[(0.74\pm0.10) \times \log P]-[10.5\pm3.2]$ Model revealed that: i) the molar refractivity influences the effectiveness of xanthine positively; ii) the crystal density and partition coefficient influence the MDR reversal effectiveness of xanthine negatively.

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COLONIC INFLAMMATION ALTERS LOCAL METABOLISM OF GLUCOCORTICOIDS

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Glucocorticoids are used as usual drugs for treatment of inflammatory bowel disease for many years. However, a certain proportion of patients respond poorly or is unresponsive to these steroids. The absence of response was attributed to the increased expression of a truncated spliced variant of glucocorticoid receptor. However, the biological activity of glucocorticoids depends not only on the number of receptors and responsiveness of the target cells but also on the local metabolism of glucocorticoids that is catalyzed by the enzyme 11β-hydroxysteroid dehydrogenase (11HSD). The isoform 1 (11HSD1) is an enzyme that operates predominantly as a reductase and increases local concentration of glucocorticoids (cortisol, corticosterone) by reduction of their 11-oxo derivatives (cortisone, 11-dehydrocorticosterone). In contrast, the isoform 2 (11HSD2) appears to function exclusively as dehydrogenase that inactivates cortisol and corticosterone to their 11-oxo derivatives. To assess whether the local metabolism of glucocorticoids is changed during intestinal inflammation, we studied mRNA expression and enzyme activity of both isoforms of 11HSD in two models of ulcerative colitis (UC) in rats and in bioptic samples taken from patients with UC and in healthy controls. In both models of colitis (TNBS and DSS model) we found down-regulation of corticosterone oxidation to 11-dehydrocorticosterone and reciprocal stimulation of 11-dehydro-corticosterone reduction to corticosterone. The expression of 11HSD1 mRNA was up-regulated whereas 11HSD2 mRNA was down-regulated. Similar pattern of both mRNA transcripts was found in biopsies of patients with UC. In conclusion, intestinal inflammation is associated with increased local activation of glucocorticoids (cortisol in man, corticosterone in rat) from their 11-oxo derivatives. We propose that the observed changes in local metabolism of glucocorticoids could contribute to the control of an overshoot of inflammation process in the colon.

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IMPACT OF GLUTAMATE AND *N*-ACETYL-*L* -ASPARTATE ON NEUROTOXIC EFFECT OF *N*-ACETYL-*L*-ASPARTYL-*L*-GLUTAMATE IN NEONATAL RATS

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N-Acetyl-L-aspartyl-L-glutamate (NAAG), a potential neurotransmitter, acts as an agonist at metabotropic glutamate receptors Group II and also activates the *N*-methyl-D-aspartate type of ionotropic glutamate receptors (NMDA-Rs) at high μM concentrations. Acting through the NMDA-Rs, NAAG displays neurotoxic effects in adult (1), but mainly in neonatal rat brain (2). However, brain NAAG can also be hydrolyzed by glutamate carboxypeptidase II (GCP II; EC 3.4.17.21) into its constituents, *N*-acetyl-L-aspartate (NAA) and L-glutamate (GLU). It means that high extracellular concentrations of NAAG should activate ionotropic glutamate receptors either by a direct action of NAAG on a subset of NMDA-Rs, and/or indirectly, by L-GLU and NAA. Verifying these possible interactions, we infused equimolar doses of NAAG, L-GLU and NAA (0.25 μmol/0.25 μl in saline) into the both lateral cerebral ventricles of rat on postnatal day 12. We evaluated neuronal damage in Nissl-stained brain sections (cut in the distance of 105 μm between two consecutive slices) 24-h and 96-h after the infusion of corresponding compound. We found pronounced neurodegeneration in both blades of granule neurons in the dentate gyrus and in neurons of the dorsal thalamus and retrosplenial cortex 24-h after NAAG infusion, but not after the infusion of L-GLU. However, infused NAA induced a limited degeneration in the dentate gyrus, but much less pronounced than NAAG did. For further analysis of the findings, we administered 2-(phosphonomethyl)pentanedioic acid (2-PMPA; an inhibitor of GCP II) to increase the brain interstitial levels of NAAG without its possible conversion to L-GLU and NAA. We injected 2-PMPA (i.p.) 1 x 50 mg/kg b.w, 0.5-h after the intra-ventricular infusion of NAAG and/or 2 x 50 mg/kg b.w. of 2-PMPA (30 min before and after the NAAG infusion). Similar and even more appreciable damage of granule neurons in the dorsal part of dentate gyrus was observed 24-h after NAAG/2-PMPA injection then after NAAG alone. We conclude that NAAG damages brain neurons directly and 2-PMPA does not protect against the NAAG-induced injury.

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HYDROLYTIC DEGRADATION OF GLYCOLIDE AND L-LACTIDE COPOLYMERS

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Polyglycolide, polylactides and their copolymers are of growing interest in the field of temporary therapeutic applications such as osteosynthesis devices, scaffolds for tissue engineering and drug delivery systems. In all these applications susceptibility to degradation and degradation kinetics must be carefully controlled. Copolymers of glycolide and L-lactide (PGLA) of different chemical composition (18:82 and 50:50) were synthesized by ring opening polymerization in the presence of zirconium acetylacetonate as a biocompatible initiator. The copolymers were processed by injection molding to obtain the samples in the form of paddles suitable for mechanical testing. The mechanical properties of copolymers (tensile strength and Young's modulus) were analyzed by Zwick 1435 universal testing machine. Hydrolytic degradation was realized in distilled water at 37 ºC for 10 weeks; incubation medium was exchanged every week. The samples were studied by viscosity, gel permeation chromatography, ultrasound wave rate and mass change as a function of degradation time. Moreover, the degradation process was monitored by pH and conductivity measurements of incubation medium. Mechanical test show that PGLA 18:82 was more durable and less stiff (σ = 43.6±5.4 MPa, E = 1.33±0.18 GPa) than PGLA 50:50 (σ = 21.3±9 MPa, E = 1.52±0.1 GPa). PGLA 50:50 exhibited significantly faster hydrolytic degradation than PGLA 18:82, as shown by decreases in molecular mass, relative mass and ultrasonic wave velocity. In addition, PGLA 50:50 started to release low molecular weight degradation products after 1 week of incubation, and after 6 weeks the sample commenced to disintegrate. On the other hand, PGLA 18:82 started to release degradation products after 4 weeks of incubation and it was dimensionally stable up to 10 weeks. These results confirm that the copolymer composition has a significant effect on the degradation kinetics of bulky PGLA devices.

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VARIOUS LEVELS OF D_1 DOPAMINE RECEPTOR BLOCKADE IN THE PROCESS OF SPATIAL LEARNING IN WILD TYPE AND LURCHER MUTANT MICE OF THE C3H STRAIN

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Lurcher mutant mice represent a natural model of genetically determined olivocerebellar degeneration (1). The degeneration is caused by a mutation of δ2 glutamate receptor gene (2). The aim of the work was to assess the effect of various doses of D1 receptor antagonist SCH 23390 on spatial learning in healthy wild type and Lurcher mutant mice of the C3H strain. In the C3H strain Lurcher mutants are usually light gray colored, most of wild type mice are dark gray colored. Adult mice of the C3H strain were used. Spatial learning was tested using the Morris water maze method (3). The spatial learning task was repeated 4 times a day for 6 consecutive days. The mice were treated with SCH 23390 in the doses 5.0, 0.5 or 0.1 mg/kg of the body weight each day of the experiment 20 minutes before the first trial. To controls was given physiological saline. A group of atypical light colored wild type and dark Lurcher mutant mice was also collected. These atypical mice were treated only with the dose of SCH 5.0 mg/kg or physiological saline. There were no significant differences between dark-colored wild type mice and light Lurchers in spatial learning ability. Their results were poor in general. Light colored wild type mice reached significantly better results in the Morris water maze as compared with the other types of mice. SCH 23390 had a negative effect in wild type mice in all doses. In Lurcher mutants it did not change spatial ability significantly. The experiments showed a negative effect of the D1 antagonist only in wild type mice. Spatial learning ability in mice of the C3H strain is dependent on the coat color more than on the presence of olivocerebellar degeneration.

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EUROPEAN RESOURCE CENTRE FOR ALTERNATIVES TO USING ANIMALS IN HIGHER EDUCATION – ESSENTIAL INFORMATION FROM A NATIONAL CONTACT

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The EUropean Resource Centre for Alternatives to using animals in higher education (EURCA) was established in 1998 during a workshop devoted to the use of animals in higher education and sponsored by ECVAM (European Centre for the Validation of Alternatives in Medicine). The main aims of EURCA are to promote the use of alternatives to using animals in higher education and provide a mechanism for effective dissemination of relevant information about alternative approaches to using animals in higher education. These aims will be achieved by establishing a Resource Centre, which is responsible for collection and dissemination of electronic alternatives comprising computer modelling – computer aided learning, films, videos, CD ROMs, multimedia, mannequin models. EURCA also offers alternatives based on use of invertebrate organisms, materials from slaughterhouses, mammalian cells cultured *in vitro*, human volunteer studies. One of the important activities of EURCA is National Contacts Programme. The role of national contacts is to disseminate information about alternatives within their own countries, participate on EURCA activities and share experience and good practice. Currently, 11 countries are represented in this programme. Since 2003 Zuzana Červinková (wolff@lfhk.cuni.cz) has been appointed as a national contact for the Czech Republic. The first National Contact Meeting was held in Konstancin, Poland in October 2003. The meeting, which was organised by the promoters of EURCA Dr. David Dewhurst (UK) and Dr. Jan van der Valk (NL) attended 13 participants. The fruitful discussion during the meeting was focused mainly on the role of national contacts in their countries. Following activities for the national contacts were proposed: to be a local point for dissemination of information and refer teachers, students and members of Animal Ethics Committees to the EURCA database to promote EURCA through, e.g., being cited from other web sites (http://www.eurca.org/) to search actively for potential authors on new alternatives, get them involved and make locally developed alternatives available and accessible to attract people who are engaged in similar work using serious scientific evidences to convince teachers that the quality of education could be improved when scientifically based alternatives to animal experiments will be used.

EFFECTS OF SINUSOIDAL MAGNETIC FIELD 50 HZ (0,5 MT) ON CELL-MEDIATED IMMUNITY OF HUMAN LEUKOCYTES

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The leukocyte surface properties manifests the cell mediated immunity (CMI). The response of the CMI to external magnetic field was examined by observing leukocytes adherence to solid state surfaces. Leukocytes taken from immunized humans (n=14) with pharyngeal cancer have diminished adherence if corresponding antigen is present in the suspension (specific antigen prepared from human ca pharyngis and non-specific antigen prepared from blood of inbred C3H/H2K strain mice infected by a mice LDH virus (LDV). In the presence of antigens, leukocytes taken from cancer patients exhibit decreased adherence in contrast with adherence of leukocytes from healthy humans. After 1 h exposure to a sinusoidal magnetic field of 50 Hz and of 0.5 mT, adherence of leukocytes taken from cancer patients is strongly increased. To alter the adherence properties, the antigens has to bind the corresponding receptor in the cell membrane. We will examine the effects of sinusoidal magnetic field on CMI of leukocytes by investigating their adherence. The experimental findings may help us to understand the signaling mechanism in leukocytes and the electromagnetic field around the cell (1,2,3).

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EFFECT OF MELOXICAM AND DEENDOTHELISATION ON VASCULAR RESPONSES IN RABBIT RENAL AND EAR ARTERIES

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are used to treat acute and chronic inflammatory disease and pain. The use of conventional NSAIDs is frequently limited by adverse effects on kidneys and GIT resulting from inhibition of prostaglandins synthesis. These drugs inhibit both cyclooxygenase (COX) isoforms leading to increase of vasoconstrictor activity (1). Therefore, the aim of present study was to evaluate effects of selective COX-2 inhibitor meloxicam on vasoconstrictor responses to noradrenaline (NA) in rabbit renal artery as a model vessel and in ear artery as a peripheral vessel under in vitro perfusion conditions. Responses to standard NA doses were measured in controls, after meloxicam [10^{-5} mol.l^{-1}] and deendothelisation by air bubbles (DE). In both, renal and ear arteries meloxicam did not significantly enhance vasoconstrictor responses to NA when compared with controls. Responses to NA in both arterial preparations were not significantly affected by DE comparing with meloxicam but these were significantly potentiated if compared with controls (values expressed as medians in mmHg): *renal artery – 0.1 μg:* 5.25 vs. 9.00; *1 μg:* 28.5 vs. 31.5*; 10 μg:* 42 vs. 75; *ear artery – 0.05 μg:* 42.75 vs. 111.75*; 0.1 μg:* 57.75 vs. 129.38; *0.5 μg:* 128.63 vs. 194.25; *1 μg:* 176.63 vs. 243.38. No significant differences between renal and ear artery responses were found. The results of our experiments did not reveal neither unfavorable effect of meloxicam on vasoconstrictor activity in both types of vessels nor selectivity on different vascular beds.

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PLASMA GHRELIN LEVELS IN ANOREXIA NERVOSA PATIENTS

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Ghrelin is a novel GH-releasing peptide initially isolated from the stomach and subsequently found in other tissues, including the brain. Studies in rodents have consistently shown that ghrelin plays a major role in energy homeostasis, adipogenesis and modulation of feeding behavior. However, little is know about the influence of food consumption on ghrelin plasma levels in humans. Therefore, we investigated the short-term response of plasma ghrelin to food intake, meal volume and meal nutritional value in healthy volunteers and women suffering from anorexia nervosa (AN). After overnight fasting, all subjects received either a standardized breakfast or fibers. Ghrelin plasma levels were measured before and after the meal. Fasting plasma ghrelin was significantly higher in patients with AN than in controls (1800.6±24.4 *vs* 795.9±24.3 pg/ml, $P<0.001$) and correlated negatively with percentage of body fat in both groups. Ghrelin levels markedly fell after consumption of both a standardized meal and fibers in healthy women, but did not change significantly in anorexic women. The acute response of plasma ghrelin to food intake, which in healthy individuals is independent of meal caloric value, is impaired in women with AN. This abnormality may be part of a chronic adaptation to the prolonged food restriction, which tries to restore a normal feeding conduct by maintaining the drive to eat.

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DIABETES-INDUCED FUNCTIONAL CHANGES IN MEMBRANES OF RAT HEART MITOCHONDRIA

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It was well documented that diabetes (DIA) induces non-enzymatic glycation of proteins (NEG) and glycooxidation (GO)-mediated remodeling of the rat heart sarcolemma. This remodeling was accompanied with significant decrease in fluidity of the sarcolemmal membrane (SLM). The aim of present study was to reveal the character of the DIA-induced functional changes in mitochondrial membranes (MM) particularly in respect to our earlier finding that DIA hearts exhibit increased formation of energy transferring contact sites in the MM. Following the former goals contents of coenzymes Q9 and Q10 in the respiratory chain, mitochondrial ATPase activities as well as the fluidity of MM were investigated in DIA- and healthy heart mitochondria. Male Wistar rats were made DIA by a single dose of streptozotocin (ST, 55 mg/kg i.v.)- Hearts were removed, the mitochondria isolated (differential centrifugation, proteinase treatment) and investigated on the 8th day after ST administration (aute DIA). The metabolic status of rats was checked by estimation of blood glucose, triacylglycerols and cholesterol. Mg-dependent and 2,4-dinitrophenol (DNP)-stimulated ATPase activities were measured by estimating the amount of Pi liberated during ATP splitting. MM fluidity was determined spectrofluorometrically using the fluorescence probe 1,6-diphenyl-1,3,5-hexatriene (DPH). Coenzyme Q9 and Q10 contents were estimated by means of HPLC. Total mitochondrial ATPase activity (Mg-dependent + DNP-stimulated) was slightly elevated in cardiac mitochondria with acute diabetes. In contrast to SLM, MM from acute DIA- hearts show significantly ($p<0.05$) increased membrane fluidity. The concentrations of coenzymes Q9 and Q10 in DIA in DIA-heart MM were found increased in comparison to the controls. In conclusions, remodeled mitochondria in hearts with acute DIA exhibit features different from those reported in SLM. In contrast to the latter they exhibited increased membrane fluidity and mitochondrial ATPase activities as well as elevated concentrations of coenzymes Q9 and Q10 as a sign of certain prevention of oxidative damage to the respiratory chain. These changes in the mitochondria seem to reflect the presence of mechanisms of endogenous protection, which either have compensatory or adaptive meaning for function of the DIA-heart. In contrast to SLM, NEG and GO seem to modulate the function and properties of MM to minor extent.

EFFECT OF 2-DEOXYGLUCOSE AND ITS DERIVATIVES ON GLUCOSE ACCUMULATION AND VIABILITY OF SENSITIVE AND MULTIDRUG RESISTANT CELL LINES L1210

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Multidrug resistance (MDR) of murine leukemic cell line L1210/Vcr, predominantly mediated by drug efflux activity of P-glycoprotein (Pgp), was obtained by adaptation of drug sensitive L1210 cells to vincristine. In such a model, overexpression of Pgp is associated with several changes of cell metabolism. 2-deoxy-D-glucose (2DG) causes a decrease of ATP and phosphocreatine levels and an increase in 2-DG-6-phosphate accumulation much more in resistant cells of several human cell lines (1, 2). 2DG has shown to be more toxic to MDR variants of both MCF7 breast cancer and KB carcinoma cell lines (2, 3). Some deoxy-fluoro-derivatives glucose were evaluated for their antineoplastic properties (4). The plasma membrane level of the facilitative glucose transporter GLUT-1 is progressively reduced in a series of MDR human KB carcinoma cell lines, expressing elevated levels of Pgp (3). We found that L1210 cell lines are not hypersensitive to neither 2DG nor x-deoxy-x-fluoro-glucose (x=2,3,4). Interestingly, 2DG is more toxic for sensitive cells, unlike described for KB cell lines (2, 3), allowing us to speculate if the level of GLUT-1 is reduced in L1210 cells. Resistant cells accumulate more [^{14}C]-glucose (*G) than sensitive cells. Short-time (<4hrs) *G accumulation, following 3hrs preincubation of cells with 2DG, is depressed by 2DG more in resistant cells (45 to 35 %). The same goes for *G accumulation by cells growing with 2DG within 20-30 hrs but, note worthily, after this time more *G is accumulated in cells affected by 2DG. Again, resistant cells (2-fold increase of accumulation comparing unaffected cells) are affected more than sensitive cells (1.5-fold increase). It is possible that in L1210 cells 2DG is modified becoming a substrate for Pgp and being pumped out of the cells. Or there may be another mechanism, remaining obscure to us.

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DEVELOPMENT OF NEUROPATHIC PAIN IN ANIMAL MODEL OF SCHIZOPHRENIA

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Intracerebroventricular (i.c.v.) infusion of quinolinic acid (0.25μmol QUIN/0.25μL PBS/ventricle) to 12-day-old rat pups (QUIN group) evokes psychosis-like behavior in early adulthood. Quinolinic acid, pro-inflammatory metabolite of tryptophan, is a weak NMDA receptors agonist, overproduction of which unleashes glutamatergic neurotransmission leading to a selective loss of neurons in vulnerable rat brain regions. The control group (SHAM) was operated exactly in the same way as QUIN-treated pups but QUIN was not infused. Since the involvement of NMDA receptors in the development of neuropathic pain syndrome is very well known, the aim of this study was to investigate a development of hyperalgesia and allodynia in this animal model. In order to induce peripheral neuropathic pain in younger adult animals (50-day-old rats), four loosely constrictive ligatures of sciatic nerve were placed (1). One week after the constriction development of thermal hyperalgesia and mechanical allodynia was tested using plantar test and von Frey filament respectively. In thermal nociception test, there were no significant differences between SHAM and QUIN groups. Thermal hyperalgesia developed in both groups in a similar manner. When the animals were tested using von Frey filaments, two significant differences were found as follows: 1. mechanical pain thresholds decreased in both hind limbs of QUIN group compared to SHAM, and 2. there was a lack of development in a gap between ligated and intact hind limb in QUIN group. The present results show that the i.c.v. infusion of QUIN during vulnerable neonatal period of rat brain development is responsible for bilateral mechanical allodynia after the sciatic nerve constriction observed in younger adults.

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ATTENUATION OF PROLIFERATION OF VASCULAR SMOOTH MUSCLE CELLS BY A CELL CYCLE INHIBITOR CVT-313

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Excessive proliferation of vascular smooth muscle cells (VSMC) is a major complication of vascular surgery including introduction of artificial vascular prostheses or stents. One of the possibilities how to control the proliferation of VSMC is a local delivery of antiproliferative agents, e.g. by its controlled release from the prosthesis or stent material. In our preliminary experiments, we tested the effects of CVT-313, an inhibitor of cyclin-dependent kinase 2 (1), on the number of VSMC *in vitro* after addition of this compound into the culture medium. The VSMC were isolated from the thoracic aorta of newborn or adult male Wistar SPF rats and used in passages 2 to 40. CVT-313 was added to the DMEM medium with 10 % FS at the concentration range 3.9-1000 ng/ml. An excellent growth inhibition was observed in cultures of newborn rats. After 2 day- or 6 day-incubation in medium with CVT-313, the number of newborn rat VSMC decreased by about 54-79.0 % and 91 %, respectively, compared to the control value obtained in the medium without CVT-313. Adult rat VSMC were inhibited less markedly. The number of cells incubated 2 days in the inhibitor-supplemented medium dropped by 10-41 % in comparison with the control, while after 6 days, the decrease in cell number even by 24-58 % compared to control was observed. Proliferation of VSMC after 48-hour-synchronization in serum free medium with 1 % ITS (insulin, human transferring, sodium selenite; SIGMA, USA) followed by 48 hours of cultivation in CVT-313 supplemented medium was attenuated in a similar manner than that after 6 day of incubation with CVT-313 in serum-supplemented medium. Our results indicate that CVT-313 inhibition on proliferation of VSMC depends on the cell type, the time of application and the fraction of cell population entering the cell cycle.

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DIETARY INTAKES, FOOD PREFERENCES AND BLOOD LIPIDS IN COLLEGE STUDENTS IN SLOVAKIA

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This study was designed to identify the beliefs, motivation and personal and environmental influences shaping eating habits of a group of college students in Slovakia. We studied 167 students of medical faculty- 43 men and 124 women, average age 21.5 years, who provided information on demographic and socio-economic variables, responded to an interviewer-administered, food-frequency questionnaire that assessed the consumption of more than 100 food items and 24-hours recalls of food intake. Study included measuring of blood pressure, anthropometric and lipid parameters as well as lipid peroxidation levels- conjugated dienes. Higher level of total cholesterol has been found in 26 % of students, high levels of LDL cholesterol in 13.6 %. Almost a half of students did not take food regularly, in most of the cases they replaced the main meal with the fast food. One third of the investigated group, takes vitamin and mineral supplements. However, there were variations between individuals, with specific practices being influenced by personal food preferences, time availability, health beliefs and concern, food availability, and the physical and social environment. Results indicate that, in general, the study group was reasonably well nourished. However, fat consumption was 30% higher than the recommended intake, for both males and females. The percentage of energy derived from carbohydrates was below the guideline value in both sexes. Relatively low iron and fiber intakes were found for females. Based on these results, some concern about the dietary habits and the related health consequences in medical students appears justified.

RILMENIDINE PREVENTS DEVELOPMENT OF HYPERTENSION IN NO-DEFICIENT RATS WITHOUT AFFECTING NO SYNTHASE ACTIVITY

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Imidazoline receptors (IR), are supposed to be localized in vasomotor centers in brain stem and modulate sympathetic outflow (1); however, IR are also present in vascular smooth muscle. Question was posed (i) are the IR involved in the nitric oxide (NO) defective hypertension (NODH), (ii) does the IR agonist affect NO synthase activity and expression? Three groups of 7 rats, were used: 1) controls, 2) rats with inhibition of NO synthase by N^G-nitro-L-arginine methyl ester (L-NAME 40 mg/kg/day for 4 weeks in tap water), and 3) rats with inhibited NO synthase as in second group, plus agonist of imidazoline receptors rilmenidine (3 mg/kg/day for 4 weeks by gavage). Systolic blood pressure, measured weekly, increased significantly in the group with inhibited NO synthase. Relaxation to acetylcholine of aortic rings was significantly attenuated. Rilmenidine, administered simultaneously with L-NAME for the period of 4 weeks, prevented the increase of blood pressure and did not change significantly heart rate. The finding indicated that in NO defective hypertension the imidazoline receptors, very probably in rostral ventrolateral medulla, have a remarkable role maybe by inhibiting the sympathetic outflow from these centers. Moreover, the acetylcholine relaxation of thoracic aorta in L-NAME + rilmenidine treated rats, that did not differ from controls, also suggested the effect of rilmenidine on vascular smooth muscle in aorta. However, the prime cause of hypertension was not affected by rilmenidine: NO synthase activity (left ventricle and brain) remained in L-NAME + rilmenidine treated rats as low as in the group treated with L-NAME alone. No change in NO synthase expression (aorta and left ventricle) was found in either of the three groups studied.

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EFFECT OF LIPID DIET ON PROTEIN KINASE C EXPRESSION IN CHRONICALLY HYPOXIC RAT HEARTS

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Adaptation of rats to chronic hypoxia increases the expression of protein kinase C (PKC) isoforms δ and ε in the myocardium (1). It is known that the translocation of inactive PKC from the cytosol to the particulate fraction and its activation depend on fatty acid (FA) composition of membrane phospholipids (2). The aim of this study was to analyze effects of diets with different FA composition on the expression of PKC δ and ε in normoxic and chronically hypoxic rat hearts. Adult male Wistar rats were fed non-fat diet enriched by 10 % of lard (saturated FA, SFA), fish oil (n-3 PUFA, n-3) or corn oil (n-6 PUFA, n-6) for 10 weeks. After 4 weeks on diets, each group was divided into two subgroups that were either exposed for 6 weeks to intermittent high altitude hypoxia of 7000 m in a barochamber for 8 h/day, 5 days/week or kept under normoxic condition for the same period of time. The immunoanalysis of PKC isoforms was performed in particulate and cytosolic fractions (differential centrifugation, 105×10^3 g) from the left ventricles, followed by Western blotting and chemiluminescent ECL technique with the aid of Image Quant software. In normoxic tissue, the diet composition had no effect on relative amounts of PKC δ in any fraction. The proportion of PKC ε was higher in the particulate fraction of n-6 group as compared with SFA and n-3 groups. Chronic hypoxia increased the relative amount of PKC δ in the particulate fraction of SFA and n-3 groups (by 40.0±2.6 % and 82.7±11.3 %, respectively) but not in n-6 group. In contrast, chronic hypoxia did not influence PKC ε of SFA and n-3 groups but it decreased the relative amount of this isoform in the particulate fraction of n-6 group (by 41.4±2.5 %. In conclusion, the diet lipid composition significantly modulates the effect of chronic hypoxia on cardiac PKC isoform expression and subcellular distribution.

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CHANGES OF ZINC AND VITAMIN A IN RATS AFTER CADMIUM INTOXICATION

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Cadmium (Cd) is one of the most widespread environmental pollutants known to cause serious problems due to its accumulation and toxicity in living organisms. Zinc (Zn) is an antagonist of cadmium that inhibits its accumulation. This inhibition of cadmium toxicity is a difficult phenomenon. The main aim of this work was the study of changes blood plasma concentration of Zn and vitamin A and measurement of Cd and Zn content in the liver of Wistar rats after acute (24 h and 72 h) and chronic (90 days) application of medial lethal dosis (LD_{50} - 225.0 mg/kg b.w.) of Cd as cadmium chloride in drinking water. Experimental and control rats (n=60; 12 per group) were housed in conventional condition on a normal laboratory diet and supplied with drinking water. After Cd administration rats were anaesthetized with sodium pentobarbital and blood samples were taken from heart using heparin as an anticoagulant. The vitamin A was determined by HPLC and zinc using commercial kits WAKO, Chemicals GmbH, DE. Content of Cd and Zn in the liver were analyzed using an atomic absorption spectrophotometer (Unicam Solar 939). Acute and chronic exposure to Cd significantly ($p<0.001$) increased content od Cd and Zn in the liver of rats. During chronic exposure was plasma concentration of vitamin A (non-significantly) and Zn significantly increased. The changes of vitamin A during acute, but also in chronic intoxication, copy the changes of Zn in blood plasma. Vitamin A and Zn contribute by their antioxidant function to the maintenance of optimal values of total antioxidant plasma capacity or to the prevention of its decrease. The results of this experimental work indicate that Cd induced the formation of Zn and/or activated Zn pool in organism and partly reduced negative consequences of toxic effects of cadmium by preventive (antioxidant) function of zinc.

HOW DOES GLUTAMINE ADMINISTRATION AFFECT PROTEIN METABOLISM?

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Several studies have reported improvements in clinical outcome and in nitrogen balance when glutamine (Gln) itself, Gln-containing dipeptides, or α-ketoglutarate were given to critically ill patients. However, the mechanism by which Gln administration affects protein balance is not clear. Because of a tight relationship between Gln synthesis and branched-chain amino acid (BCAA; Val, Ile, Leu) catabolism and characteristic changes in BCAA and Gln metabolism in severe illness, it can be suggested, that favorable effect of Gln administration on protein metabolism is related to its effect on BCAA. Using male Wistar rats two separate studies were performed in which the effect of Gln on leucine oxidation, protein synthesis and proteolysis was estimated. At *in vivo* study, alanyl-glutamine or saline solution were infused to endotoxemic or intact (control) rats. At *in vitro* study, m. soleus and m. extensor digitorum longus were incubated in medium containing 0, 500 or 2,000 μmol Gln/L. The parameters of protein metabolism and leucine oxidation were measured using L-[1-^{14}C]leucine and/or according to the rates of tyrosine release. Statistical comparisons were performed using ANOVA, Bonferroni test, and Student's t-test. The significance level was set at 0.05. Infusion of Gln (alone or as alanyl-glutamine) induced a decrease in plasma BCAA levels, in leucine oxidation and an improvement of protein balance (higher decrease in whole-body proteolysis than in protein synthesis) both in intact and endotoxemic rats. In an *in vitro* study; supplementation of incubation media with Gln in concentration of 2,000 μmol/l decreased leucine oxidation by isolated muscles. It is concluded that positive effect of Gln exogenous supply on protein balance can be explained by its effect on metabolism of BCAA.

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REPRODUCIBILITY OF BAROREFLEX SENSITIVITY IN YOUNG ADULTS

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Baroreflex sensitivity (BRS) can be determined by many methods, laboratory or spontaneous techniques, in time or in a frequency domain. The aim of the study was to test reproducibility of BRS determined by a spectral method at 0.1 Hz frequency range with respect to coherence between the variability in systolic blood pressure (SBP) and pulse intervals (PI) in man (1). SBP and PI were recorded for 5 min (Finapres, controlled breathing at the frequency of 0.33 Hz) in 32 subjects (20-22 years of age) at rest in sitting position five times in regular periods of one week. The power spectra of SBP and PI, cross-spectra and coherence were calculated. BRS was determined by spectral method at 0.1 Hz frequency range. BRS values were evaluated in the range of 0.0667-0.133 Hz (7 values) in each recording. Six different methods for individualization of BRS were used: the value at the frequency of 0.1 Hz ($BRS_{0.1Hz}$); the value at maximum coherence (BRS_{COHmax}); weighed value with respect to cross-spectral values in the whole frequency range (BRS_{Wcs}) or for partial frequencies with coherence above 0.5 (BRS_{WPcs}); weighed value with respect to coherence values in the whole frequency range (BRS_{Wcoh}) or for frequencies with coherence above 0.5 (BRS_{WPcoh}). Intraindividual and interindividual variability were evaluated by MANOVA. The third square root of each BRS value was used as transfer function for normalization of distribution of BRS values. Significant correlation ($p>0.001$) was found between average BRS and range of individual BRS values for all six methods. Spearman's correlation coefficients: $BRS_{0.1Hz}$, $r=0.65$; BRS_{COHmax}, $r=0.75$; BRS_{Wcs}, $r=0.73$; BRS_{WPcs}, $r=0.79$; BRS_{Wcoh}, $r=0.78$; BRS_{WPcoh}, $r=0.78$. Each parameter revealed significantly lower intraindividual variability than interindividual ones. The lowest intraindividual variability was found for $BRS_{Wcoh.}$ Reproducibility of BRS is the greatest at low values of BRS, i.e. low BRS is an individual characteristic feature. On the other hand, increased dispersion of resting BRS values at their higher mean values could reflect sensitive reactivity of a high central gain.

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DIETARY INTAKE OF CALCIUM, MAGNESIUM AND PHOSPHORUS IN THE INTERRELATIONSHIP WITH NUTRITIONAL, BIOCHEMICAL AND BIRTH PARAMETERS OF PREGNANT WOMEN: LONGITUDINAL STUDY

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Pregnancy is a time of increased need for calcium and magnesium. Insufficient calcium supply during pregnancy and lactation could result in maternal bone loss, reduced breast-milk calcium secretion or impaired infant bone development (1). Calcium salts may reduce hypertensive disorders in pregnancy (2). This longitudinal study evaluated dietary calcium and phosphorus intake in the interrelationship between nutritional, biochemical and gynecological parameters. 692 pregnant women (in age of 26±4 years) in the third trimester were studied. For determination of nutritional parameters was used program Nutricon. Calcium, magnesium and phosphorus were measured in serum. Weight gain in pregnancy, duration of pregnancy, extent of blood loss during delivery, and weight of a newborn were observed. General evaluation demonstrates low dietary calcium intake (1013 mg/day, 68 % RDA). Consumption of phosphorus was 1428 mg/day (97.4 % RDA). Concentration of calcium and phosphorus in serum were within normal range. Serum magnesium (0.72 mmol/l) decreased throughout pregnancy. A significant positive correlations ($p<0.05$) were observed between dietary calcium intake and total plasma cholesterol, LDL lipoproteins and plasma triglycerides, between plasma calcium and plasma triglycerides, between plasma magnesium and lower LDL lipoproteins. The most conclusive correlation ($p<0.0001$) was found between dietary calcium and lipids intake. Other observed negative correlations: between plasma calcium and dietary intake of lipids, and vitamin A, full-term weight of a newborn. These results suggest that low dietary calcium intake may lead to change in the biological activity of the skeleton and to possible involvement in the pathogenesis of the osteoporosis.

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EFFECT OF ACUTE AND CHRONIC AMPHETAMINE TREATMENT ON BEHAVIOR OF RATS IN THE MORRIS WATER MAZE

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Amphetamine (AMPH) is frequently misused drug, which has pronounced behavioral effects that are dependent on the dose and chronicity of its application. The aim of this study was to test the effect of acute and chronic administration of AMPH on spatial learning and memory of rats in the Morris Water Maze (1). We used three male rat strains, namely Wistar (Velaz, Prague), Sprague-Dawley and Lewis rats (Charles River Laboratories, Germany); the latter are known to have a deficient HPA axis activity. We used a typical water maze with an automatic registration of rat movement. Rats were trained to find an invisible escape platform from 4 cardinal points. The rat performance was expressed as mean latency and distance to reach the platform; more advanced parameters (path length efficacy, heading accuracy, bearing accuracy, tortuosity, thigmotaxis, segments and rings used) were analyzed by using computer program TRAM. AMPH (as sulfate) was administered i.p. in a single dose 1 or 8 mg/kg (one hour before the test) or for 14 days in a dose 4mg/kg (last dose 24 hours before the test). In Wistar rats AMPH in a dose 1 mg/kg slightly decreased the mean latency and distance to reach the platform; this effect disappeared after next 4 hours. AMPH in a dose 8 mg/kg produced impaired performance. 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 4 and 24 hours. In a test lasting for 12 days, chronic application of AMPH revealed only slight effects. In Lewis rats, which showed slow learning, the performance was improved by AMPH. In Wistar rats AMPH slightly impaired the performance and in Sprague-Dawley rats the very good learning was not influenced by AMPH. Our results demonstrate that pronounced effects of AMPH on behavior in water maze was observed only after higher dose of AMPH, and even this effect on spatial memory disappeared in several hours.

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ANTIOXIDANT ENZYMES IN PATIENTS WITH SEPSIS

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Oxidative stress has been implicated in the manifestations of critical illness, including systemic inflammatory response syndrome in severe sepsis (1). We measured activity of superoxide dismutase with copper and zinc (EC 1.15.1.1, CuZn-SOD) and activity of serum paraoxonase (EC 3.1.8.1, PON1) as markers of antioxidant capacity. Blood samples were obtained from septic patients (n=13, age 26-80 years), from same individuals after recovery (n=4, age 39-65 years) and from healthy controls (n=13, age 26-80 years). Inclusion criteria: clinical signs of sepsis, hyperpyrexia or hypotermia, APACHE II score>10, CRP>10 mg/l; exclusion criteria: antioxidant intervention. CuZn-SOD was measured in erythrocytes, based on superoxide generation by xanthine oxidase and tetrazolium reduction. Activity of PON1 in serum with paraoxon and phenylacetate and concentration of total and HDL cholesterol were measured. There was higher CuZn-SOD activity (30938.9±2653.2 U/gHb) in septic patients in comparison with healthy controls (22723.2±1139.4 U/gHb; $P<0.05$). On the other hand, PON1 activity measured with paraoxon (5.25±0.79 U/ml) resp. phenylacetate (17585.9±1819.7 U/ml) was lower in sepsis when compared with controls (9.28±1.01 U/ml resp. 29182.4±2539.2 U/ml; $P<0.05$ resp. $P<0.001$). After recovery there was no difference in activity of both CuZn-SOD and PON1 of patients and healthy controls. Moreover, we found positive correlation between activity of PON1 and level of total and HDL cholesterol in sepsis and after recovery. We observed no age and sex dependence in activity of both enzymes in patients and healthy controls. Our results confirm the presence of oxidative stress in septic patients. Recovery led to return enzymatic activities to healthy population. We conclude, that measure of CuZn-SOD and PON1 activities may be a good marker of antioxidant capacity in sepsis.

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ORTHOSTERIC AND ALLOSTERIC PROPERTIES OF METHOCTRAMINE BINDING AT M_2 MUSCARINIC ACETYLCHOLINE RECEPTOR ARE DETERMINED BY PROLINE 415 AND ASPARAGINE 419 IN THE THIRD EXTRACELLULAR LOOP

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Five subtypes of muscarinic acetylcholine receptors are known and their genes were cloned (1-3). They have orthosteric binding site for acetylcholine and one or more binding sites for allosteric ligands (4). Methoctramine at nanomolar concentrations is a competitive antagonist of muscarinic acetylcholine receptors manifesting selectivity for M_2 receptor subtype. At micromolar concentrations it also slows down dissociation of the radiolabeled N-methylscopolamine ([^{3}H]NMS) from the M_2 but not from the M_3 receptors, indicating allosteric interaction at M_2 but not at M_3 receptors. In the attempt to reveal molecular domains involved in high affinity orthosteric binding and allosteric interaction of methoctramine at M_2 receptors we genetically modified the M_3 receptor at the second (o2) and the third (o3) extracellular loops known to be involved in binding of muscarinic allosteric ligands, e.g. gallamine or alcuronium. Experiments have shown that: 1) Double-mutation of serine 519 to corresponding proline 415 in M_2 sequence (S519P) and lysine 523 to corresponding asparagine 419 in M_2 sequence (K523N) in o3 region of M_3 receptor is sufficient to acquire methoctramine high-affinity binding. 2) Single mutation K523N in o3 region of M_3 receptor is sufficient to elicit methoctramine allosteric binding to M_3 receptors. We conclude that o3 loop of M_2 receptor represents a second site for methoctramine attachment to the receptor and that N419 is essential for methoctramine interaction with this site. We speculate that a role of P415 is to give suitable position to N419 enabling methoctramine to interact simultaneously with o3 loop and classical binding site resulting in high affinity binding.

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CONSTRUCTION OF VIRTUAL MODELS OF BIOLOGICAL OBJECTS

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Spatial models of biological structures are used for visualization of spatial relationships or for measurements of geometrical quantities (1, 2). Wax models or stacks of glass desks with drawings are used for a long time. Now personal computers with 3D graphic libraries and accelerators enable easy visualization of surface models (1). The virtual models are easier to manipulate than the real ones, but their creation requires intricate software environment. Source data for construction of surface models are 3D images obtained either directly by special device, e.g. confocal microscopy, or constructed by automatic or interactive registration of 2D images of physical slices of the objects, consisting in restoration of mutual position of adjacent slices or of deformation due to slicing. The registration is a nonstandard task and its success depends on the quality of slices and prior information on the object under study (4). An indispensable step in the construction of the models is identification of objects of interest either by automatic image analysis, requiring good quality images and 3D image processing environment, or by more straightforward but laborious interactive drawing of contours (3). Objects of various degree of complexity, from single cell (2) and placenta vilus (3) to early embryo (4) were reconstructed.

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EFFECTS OF SINUSOIDAL MAGNETIC FIELD 50 HZ ON LEUKOCYTE ADHERENCE

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Exposure to the magnetic field (1) 0.5 mT increases (absolutely) adherence of T lymphocytes taken from healthy humans as well as those from cancer patients before and after medical treatment. Exposure to the magnetic field 1 and 10 mT decreases adherence of T lymphocytes taken from healthy humans and cancer patients before medical treatment in comparison with adherence for 0.5 mT, and decreases adherence of T lymphocytes taken from healthy humans and increase of adherence of T lymphocytes from cancer patients before medical treatment in comparison with adherence without exposure (2). The specific antigen (human ca pharyngis) and nonspecific antigen (lactate dehydrogenase-elevating virus – LDV) have similar effects on adherence of T lymphocytes (3, 4). Correlation of the LAI assay (Leukocyte Adherence Inhibition assay) results with the cell-mediated immunity suggests that magnetic field 50 Hz alters the cell-mediated immunity of T lymphocytes.

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HUMORAL MODULATION OF CYTOKINE RELEASE FROM HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

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There is increasing amount of evidence in the literature showing that activation of the sympathetic nervous system (SNS) or application of noradrenaline (NA) influence release of cytokines both under in vivo and in vitro conditions, indicating a role of the SNS in modulation of the activity of the immune system. Further it is well documented that cytokines mediate induction of fever and the SNS is activated during the febrile process. Our recent work demonstrated that releases of IL-1β, TNFα and IL-6 are dramatically increased after endotoxin (LPS) application (1, 2) and that different cytokines, when applied intrahypothalamically induce a similar kind of the febrile response (3). In order to specify the role of the SNS and of individual cytokines in mediation of the febrile response and during stress, in general, this paper was aimed to disclose the role of NA in mutual interactions among different cytokines in nonstimulated human peripheral blood mononuclear cells. Data obtained indicate that NA in concentration 10^{-7} g/ml of incubation medium increases release of TNFα and of IL-6, while higher concentrations of NA (10^{-4} g/ml - 10^{-5} g/ml) inhibit the release of these cytokines. Release of IL –1β is not influenced by NA in concentrations ranging from 10^{-4} g/ml to10^{-5} g/ml. Further it was found, that IL-1β, (2pg to 4000 pg/ 10^6 cells), has no effect on spontaneous release of TNFα and IL-6. It is concluded that noradrenaline in physiological concentrations may activate immune system by increasing TNFα and IL-6 release. This effect may not be mediated by IL-1β.

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HYDROGEN PEROXIDE PRODUCTION BY MITOCHONDRIAL GLYCEROPHOSPHATE DEHYDROGENASE (MGPDH) AND ITS ACTIVATION BY FERRICYANIDE

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Mitochondrial glycerophosphate dehydrogenase (mGPDH) is an enzyme with highly different expression in mammalian tissues. Activity of mGPDH differs up to 100-times, when liver and brown fat mitochondria (BAT) are compared (1). In KCN- or antimycin A-inhibited BAT mitochondria we have found pronounced glycerophosphate (GP)-dependent hydrogen peroxide production that is directly linked to the mGPDH function. We also observed that the rate of the GP-dependent hydrogen peroxide generation could be five-fold increased by one electron acceptor ferricyanide. This activating effect is linked only to GP-dependent and not to succinate- or NADH-dependent peroxide generation. As shown by combined spectrophotometric and oxygen uptake measurements, the rate of ferricyanide-activated hydrogen peroxide generation decreased in parallel to reduction of added ferricyanide. At increasing ferricyanide concentrations both, the rate of hydrogen peroxide generation and ferricyanide/oxygen ratio decreased. This indicates that mGPDH is less protected against electron leak possibly due to the absence of Coenzyme Q-binding protein in mGPDH enzyme complex (2). In case of liver mitochondria, where mGPDH is very low, we found that triiodothyronine activated mGPDH to a level comparable with that of succinate dehydrogenase (Complex II). The hormonally induced increase of mGPDH activity correlated with an increase of GP-dependent ROS production. As a result of hormonal treatment, a 3-fold increase in GP-dependent hydrogen peroxide production by liver mitochondria was detected by polarographic and luminometric measurements. Destruction of the mitochondrial membranes by freezing thawing, connected with the release of endogenous cytochrome *c*, increased the rate of GP-dependent hydrogen peroxide production. Addition of cytochrome *c* to disrupted mitochondria decreased ROS production. Similar inhibitory effect had also Coenzyme Q. We may thus speculate that mGPDH could be a potential risk for the cell metabolism and therefore its expression is highly reduced in most animal tissues. However, hormonal activation of mGPDH biogenesis could be also considered as a useful regulatory device, because cellular ROS production has been shown to selectively and reversibly inhibit the activity of various mitochondrial enzymes (3).

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OUT-OF-CONTEXT ACTIVATION OF MEMORY IN RAT

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We present a series of recent experiments suggesting that rats can activate their memory in a situation that has no physical contextual relationship to the original learning experience. This phenomenon - "Out-of-context activation of memory" (OCAM)- resembles the context-independent recall that is characteristic of human declarative, episodic memory. We found the memory activation as a consequence of a stressful experience. Rats trained on Day 1 in a footshock-reinforced Y-maze left/right discrimination task were given a forced swim on Day 2. When tested on Day 3, they expressed stronger Y-maze memories than the control rats that did not swim. This result was replicated in a food-reinforced T-maze paradigm. If the activation occurred as a consequence of forced swim, then electroconvulsive shock (ECS) given shortly after should impair the reconsolidation of activated memory. We found that ECS following the swim blocked its memory-enhancing effect in contrast to a group that received ECS 5 hrs after the swim. If the Day 1 learning was lateralized to one hemicortex by cortical spreading depression in the opposite hemicortex, the forced-swim induced an interhemispheric transfer of the lateralized Y-maze memory, which was only ever shown to happen when the memory is activated and both hemicortices are intact. Human episodic recall seems to be hippocampus-dependent, and since the Y-maze memory itself does not require an intact hippocampus, we could test if the OCAM itself is hippocampus-dependent. Temporary inactivation of the dorsal hippocampi during the forced swim blocked the swim-induced mnemonic effects in both the intact and lateralized OCAM paradigms. The hippocampus is thus necessary for the activation of a memory which is itself hippocampus-independent. The OCAM phenomenon provides a unique opportunity to study the retrieval of a memory that is not confounded by the environmental activation of the sensory pathways that were activated when the memory was formed.

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MITOCHONDRIAL UNCOUPLING PROTEINS – FACTS AND FANTASIES

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We describe the basic undisputed facts and a modest contribution of our group to the fascinating area of the research on mitochondrial uncoupling proteins. After defining the terms uncoupling, leak, protein-mediated uncoupling, we discuss the prediction that due to their low abundance the novel mitochondrial uncoupling proteins (UCP2 to UCP5) can provide only a mild uncoupling, *i.e.* can decrease the protonmotive force only by several mV. Contrary to this, the highly thermogenic role of UCP1 in brown adipose tissue is not given only by its high content (~5 % of mitochondrial proteins) but also by the low ATP synthase content and high capacity respiratory chain. Fatty acid cycling mechanism as a plausible explanation for the protonophoretic function of all UCPs and some other mitochondrial carriers is described as well as experiments supporting it. The phylogenesis of all UCPs, estimated UCP2 content in several tissues and details of UCP2 activation are described on the basis of our experiments. Functional activation of UCP2 is proposed to decrease reactive oxygen species (ROS) production. Moreover reaction products of lipoperoxidation such as cleaved hydroperoxy-fatty acids can activate UCP2 and promote feed-back down-regulation of mitochondrial ROS production.

EFFECT OF LIPID DIET ON ISCHEMIC TOLERANCE OF CHRONICALLY HYPOXIC RAT HEARTS

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Adaptation of rats to chronic hypoxia increases cardiac tolerance to ischemia/reperfusion injury and modifies the proportion of n-3 and n-6 polyunsaturated fatty acids (PUFA) in myocardial phospholipids (1). The aim of this study was to find out whether diets with different fatty acids (FA) composition could influence the protective effect of chronic hypoxia. Adult male Wistar rats were fed non-fat diet enriched by 10 % of lard (saturated FA, SFA), fish oil (n-3 PUFA, n-3) or corn oil (n-6 PUFA, n-6) for 10 weeks. After 4 weeks on diets, each group was divided into two subgroups which were either exposed for 6 weeks to intermittent high altitude hypoxia of 7000 m in a barochamber for 8 h/day, 5 days/week or kept under normoxic condition for the same period of time. Infarct size (tetrazolium staining) was measured in open-chest animals subjected to 20-min coronary artery occlusion and 3-h reperfusion. Ventricular arrhythmias were analyzed from ECG recordings. Lipids were extracted from non-ischemic hearts, phospholipids were separated by TLC chromatography and FA were analyzed by gas chromatography. In myocardial phospholipids, the n-3 diet increased the proportion of monounsaturated FA and n-3 PUFA and decreased the proportion of n-6 PUFA as compared with SFA group; the n-6 diet had opposite effects. The ratio of SFA to unsaturated FA was not changed by any diet. Diets enriched with PUFA tended to have an antiarrhythmic effect during ischemia; the n-3 diet significantly decreased the score of reperfusion arrhythmias and this effect was even more pronounced in chronically hypoxic hearts. Among normoxic groups, rats fed n-6 diet exhibited smaller infarct size (43.6±3.2 % of the area at risk) as compared to n-3 and SFA groups (56.1±3.9 % and 49.3±2.3 %, respectively). Chronic hypoxia reduced infarct size in n-3 and SFA groups (45.0±1.8 % and 38.2±2.4 %, respectively) but not in n-6 group (42.6±2.5 %). Our results indicate that the extent of ischemic injury can be significantly modified by the diet lipid composition. Infarct size-limiting effects of the diet and chronic hypoxia are not additive.

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BILATERAL AND UNILATERAL EPILEPTIC FOCI: BEHAVIORAL AND ELECTROPHYSIOLOGICAL CORRELATES

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Bilateral symmetric epileptic foci (BSEF) were studied in human patients and on various animal models in last decades. However studies on animal models were focused mainly on electrophysiological findings. Papers dealing with behavioral correlates of BSEF are missing. To study behavioral correlates we have developed new model of BSEF in freely moving laboratory rat. The experiments were performed on adult Wistar rats, which had chronically implanted stainless steel cannulas over the left and right sensorimotor cortex. Implanted cannulas also served as recording electrodes for registration of electrocorticogram (ECoG). Seven days after surgery 5µl of 2mM bicuculine methiodide was applied through the cannulas. Bilateral application led to the development of BSEF (group B, n=12). Behavior and ECoG were monitored before, during and after the application. Results were compared with behavioral and ECoG correlates observed in animals with unilateral application of bicuculine (group U, n=7). ECoG correlates were characterized by the presence of bilateral interictal discharges and ictal afterdischarges in both groups. Analysis of morphology and amplitudes of ECoG graphoelements shows differences between BSEF and bilateral discharges, caused by propagation of epileptic activity from unilateral focus to the contralateral hemisphere The present work for the first time describes the behavioral correlates of BSEF in laboratory rat, which were characterized by jerks of both forelimbs, head and trunk, rearing and wet dog shakes. During interictal periods limb jerks involved both sides of the body in 75 % of animals in group B in contrast with 14.3 % in group U. During the seizures involvement of both sides increased and was present in 100 % of animals in group B and in 57.1 % animals in group U. Unilateral motor phenomena were converted into bilateral ones by elevation, if the limbs did not further support the body. Postural mechanisms exhibit thus significant influence on motor correlates of epileptic foci.

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PROTEASOME INHIBITOR MG132 HAS DIFFERENT EFFECT ON PROTEIN SYNTHESIS IN HEALTHY AND SEPTIC RATS

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Ubiquitin-proteasome system is one of the major sites for protein degradation in mammalian cells. It has recently been revealed, that this system is activated in many disorders accompanied with cachexia, as are cancer, burn, acidosis, and sepsis. Proteasome inhibitor MG132 is known to strongly reduce proteolysis in rat muscles (1), but the effect on protein synthesis is not clear. We investigated direct effect of proteasome inhibitor MG132 on protein synthesis in the muscles of healthy and septic rats. Sepsis was induced in rats by cecal ligation and puncture. Muscles of both septic and healthy rats were dissected and incubated in medium containing 30 µmol/l MG132. Control muscles were incubated in medium without inhibitors. Two types of muscle were used - m. soleus (SOL) and m. extensor digitorum longus (EDL). The rate of protein synthesis was determined during 1h incubation in medium containing L-[1-^{14}C]leucine and calculated as the amount of L-[1-^{14}C]leucine incorporated to proteins. MG132 decreased protein synthesis in the muscles of healthy rats. In the muscles of septic rats, protein synthesis did not change significantly. Considering that protein synthesis is decreased in rats with sepsis (2), and that MG132 in our experiment did not provoke further decrease in protein synthesis, we suppose, that this effect could be beneficial in potential use of proteasome inhibitors in treatment of septic cachexia.

Protein synthesis (nmol leu/g of protein/hour)			
	Muscle	Saline	MG132
Normal rats	SOL (n=8)	1349 ± 74	*1067 ± 115
	EDL (n=8)	699 ± 55	*636 ± 55
Septic rats	SOL (n=8)	1145± 105	1362 ± 150
	EDL (n=8)	525 ± 51	614 ± 75

Mean ± SE, *P<0.05; paired t-test.

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3D COMPUTER RECONSTRUCTION OF LARGE TISSUE VOLUMES BASED ON COMPOSING SERIES OF HIGH-RESOLUTION CONFOCAL IMAGES BY GLUEMRC AND LINKMRC SOFTWARE

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Computer-based visualization of large tissue volumes with high resolution based on composing series of high-resolution confocal images is presented. GlueMRC and LinkMRC programs are introduced, implementing composition of overlapping series of optical sections captured by a confocal microscope, registration and subsequent composition of successive confocal stacks. Both programs are using an interactive approach in combination with automatic algorithms for image registration. Further, the method for obtaining surface renderings of microscopical structure under study is described. For this purpose, structure contours visible in the sections are interactively digitized using a Colon plug-in module running in Ellipse environment. Then the coordinates of the contours are processed by special modules in the graphical programming environment IRIS Explorer and the structure surface is rendered. The method is shown on the 3-D reconstruction of the capillary bed of human placental villi and chick embryonic gut and its vascular bed.

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ERYTHROCYTE NA$^+$/K$^+$-ATPASE RESPONSE TO GREEN LASER LIGHT IRRADIATION AND MC 540-PHOTOSENSITIZATION

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Low-power laser irradiation of the Na$^+$/K$^+$-ATPase - an important enzyme, closely associated with the biological membrane - may help to explain the positive effect of the laser irradiation on the regeneration, already observed in various tissues (1). In our experiments, irradiation of isolated human erythrocyte membranes (protein concentration 1mg/ml) by Nd:YAG laser (30 mW, 532 nm) with fluences 9.5, 19.0, 28.4, 38.0, 47.5 and 63.3 J.cm^{-2} was monitored by measuring the concentration of the liberated inorganic phosphate. Its spectroscopically estimated concentration served for the measure of the enzyme activity. It has been observed a positive effect of the green laser light on the ATPase activity which increased in a radiation energy dependent manner (from 9.5 to 63.3 J.cm^{-2}) with statistical significance $P<0.05$ for the two highest fluences used. In addition, isolated human erythrocyte membranes were incubated with merocyanine 540 (1 µmol/l) for 30 min and then irradiated by 47.5 J.cm^{-2}. While MC 540 in the absence of light did not change significantly the enzyme activity, photodynamic action of light and merocyanine 540 caused a rapid drop of the enzyme activity ($P=0.012$ against non-irradiated control, and $P=0.001$ against membranes with MC 540 in dark). The Na$^+$/K$^+$-ATPase, as a plasma-membrane-bound protein is sensitive to conformational alterations in the membrane, which can cause rearrangements in the active site of the protein molecule. In addition, as the Na$^+$/K$^+$-ATPase molecule is surrounded by an annulus of lipid molecules, the latter can affect the enzyme function due to changes of lipid-protein interactions (2). Thus, the enzyme activity can be altered by the rearrangement of lipid molecules and/or of changed membrane integrity. All these aspects might be liable for the decreased Na$^+$/K$^+$-ATPase activity caused by the combined action of MC 540 and the green laser light.

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FUNCTIONAL EXPRESSION OF MAMMALIAN Kir 2.1 POTASSIUM CHANNEL IN YEAST

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The high degree of homology of essential cellular metabolism shared by yeast *Saccharomyces cerevisiae* and higher eukaryotes offers the possibility to use yeast as an expression and model system for mammalian K^+ channels. Inwardly rectifying potassium (Kir) channels have two main physiological roles: they stabilize the resting membrane potential near the K^+ equilibrium potential and mediate K^+ transport across membrane. Inward rectifier Kir2.1 potassium channels are widely expressed, e.g. in brain, heart and skeletal muscle. The mouse mKir 2.1 potassium channel was functionally expressed in two cation influx and/or efflux defective strains of *Saccharomyces cerevisiae*. B31 strain (*ena1-4Δ::HIS3 nha1Δ::LEU2*) possesses sensitivity to high external cation concentrations, MAB 2d strain (*ena1-4Δ::HIS3 nha1Δ::LEU2 trk1Δ::LEU2 trk2Δ::HIS3*) is sensitive to both low and high external K^+ and is able to grow on 100–600 mM KCl. For heterologous expression in yeast, mKir 2.1 cDNA was cloned into a multi-copy plasmid behind the *CUP1* promoter (expression induced by Cu^{2+} ions) and tagged with GFP at the N-terminus (pYEX-BX-GFP/mKir2.1 provided by J. Ludwig; Multiplex). B31 and MAB 2d cells transformed by the plasmid were grown on media containing different salt concentrations. Functional expression of mKir 2.1 in the B31 strain was found to enhance the phenotype of high potassium sensitivity due to rise of cation influx via this channel (growth inhibited by $[K^+]_{out}$ ~ 200 mM). High selectivity for the larger potassium ion over the smaller sodium ion was observed. Functional expression of mKir 2.1 in MAB 2d strain complemented efficiently the cation influx defect – restoration of growth on media containing low concentrations of K^+ was observed as a result of cation influx via Kir 2.1 channel.

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SERUM CONCENTRATION OF LIPOPROTEIN (a) IN ROMANY CHILDREN

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Lipoprotein (a) - Lp(a) is an important risk factor premature atherosclerosis that is independent of other risk factors of the lipid and lipoprotein metabolism (1). An increased concentration of Lp(a) is undesired also due to its prothrombogenic and coagulating effects. To find out serum concentration of Lp(a) in small children of ethnic Romanies, regarding the risk of premature atherosclerosis. In addition to the concentration of Lp(a), the serum concentrations of apolipoprotein B (apo B), total cholesterol (TCH) and triacylglycerols (TG) was observed in 40 Romany children at the age of 1-3 years of which there were 16 boys and 24 girls. The concentrations of the determined parameters were compared with those found in 37 non-Romany children at the age of 2-4 years (15 boys and 22 girls) in the control group (C). Lp(a) was determined using the immunoturbidimetric method and apo B by the electroimmuno-precipitating method. TCH and TG were determined using the Czech commercial biochemical tests of Lachema company. Romany children statistically increased concentrations of Lp(a) ($p<0,001$) as well as apo B ($p<0,001$) were found. The differences were not significant between the Romany boys and girls. The concentration of TG was statistically significantly increased in Romany children compared with the control group ($p<0,001$). The differences between Romany boys and girls were not significant. In the concentration of TCH no statistically significant differences were found between the groups of Romany and non-Romany children, neither between Romany boys and girls. We would like to point out the fact that the high concentrations of Lp(a), found in the group of small Romany children, in combination with the high concentrations of apo B and TG are dangerous regarding the development of premature atherosclerosis, while Lp(a) could be considered as a very apparent risk parameter (2).

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CHANGES IN ENZYME ACTIVITIES AND GLYCO-CONJUGATE MOIETIES OF INTESTINAL BRUSH BORDERS AFTER COLONIZATION OF GERM-FREE PIGLETS WITH NONPATHOGENIC *E. coli*

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A possible probiotic effect of nonpathogenic *E. coli* strains *O83* and *O86* on the development of digestive functions and carbohydrate structures was studied in originally germ-free and colostrum-deprived 22-day-old piglets. We tested enzyme activities biochemically in isolated brush-border membranes and histochemically in cryostat sections. *E. coli* monoassociation of germ-free piglets speeds up the maturation and biochemical differentiation of enterocytes, the activities of glycohydrolases being most affected. Thus, compared to untreated germ-free piglets we found a reduced activity of lactase and increased activities of sucrase and glucoamylase. Histochemical detection of dipeptidyl peptidase IV showed, besides labeling intestinal brush border, also an increased number of mucosal lymphocytes which might coincide with elevated level of TNF-α in sera of monoassociated piglets. Specific lectins (*Sambucus nigra, Maackia amurensis, Aleuria aurantia, Ulex europaeus*) used in ELISA tests revealed alterations in the expression of sialylated and fucosylated glycoconjugates. Colonization with *E. coli O83* and *O86* led to a) partially reduced α2,6-sialylation and stimulated α2,3-sialylation, predominantly in the ileum, b) an increased expression of both 1,6-fucosylated and 1,2-fucosylated moieties, the highest effect being seen in the duodenum. Thus, *E. coli* strains studied contribute to precocious maturation of specific glycosylation (appearance of fucosylated glycoforms) in the porcine small intestine.

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CHANGES OF BRAIN FUNCTIONS IN NORMAL AND NEURODEFECTIVE MICE EXPOSED TO HIGH-FREQUENCY ELECTROMAGNETIC FIELD DURING THE SECOND POSTNATAL MONTH

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We studied the effect of long-term high-frequency electromagnetic field (HF EMF) exposition 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 of number 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 second month of postnatal life (day 31-60). After the exposition learning and motor ability as well as CNS excitability were examined. Learning ability was tested using the Morris water maze (2) and step down (passive avoidance) method. CNS excitability was tested by the method of audiogenic epilepsy. Spatial learning ability tested in the Morris water maze was poor in both wild type and Lurcher mutant mice of the C3H strain. As compared with controls HF EMF exposition ameliorated spatial learning ability in wild type mice and slightly also in Lurchers. In passive avoidance learning was observed in wild type mice, in Lurchers learning was not so marked. Changes in dependence on HF EMF exposition were unclear. In motor tests wild type mice reached significantly better results than Lurchers. Motor abilities were not changed by HF EMF exposition. In the contrary to exposition during early postnatal development this experiments showed a positive effect of HF EMF exposition on spatial learning (3). Other examined brain functions were not influenced or the effect of HF EMF was only weak.

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VALIDATION OF HEMODYNAMICS PARAMETERS OBTAINED BY NON-INVASIVE VASCULAR DIAGNOSTIC SYSTEM

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Non invasive vascular diagnostic methods to assess artery function are widely used. They are based on detection of either pressure or velocity or volume component of arterial pulse wave (PW). PW is a complex physiological phenomenon spread in circulation in the course of heart systole (1). The hemodynamic parameters depend on e.g. respiration and vary from pulse to pulse. The aim of this study was to assess the stability of most used approaches. System used in this study is based on the detection of volume PW of surface artery. The new in this method is the way PW is taken. The artery volume changes, evoked by the PW, are measured through an adhesive, very thin membrane, which is enclosed, to the palpation spot near the artery. The volume changes, affecting the fluid behind the membrane, transfer themselves into pressure changes and move towards the positive input of a very sensitive differential pressure transducer. The differential pressure transmitter converts the pressure differences to electrical voltage. This system offers high sensitivity to volume changes of artery diameter. The output of transducer is connected to the measuring PC. The computer analyzing system is built in LabView software and allows measurement of arbitrary hemodynamic parameters within the time and frequency domain. The PW evaluated parameters were the following: crest time, interwave distance, elasticity index (2), systolic amplitude, dicrotic time, dicrotic amplitude, and Aix (3). The complex parameter was PW velocity. The whole PW course can be submitted to first, second derivation or FFT. Some parameters correlate. Most parameters vary from pulse to pulse in the range of 10% because of the low circulatory rhythms in blood pressure. Using the first or second derivation of PW course can prevent this variation. The parameters using time intervals have better stability than those using amplitude ones do.

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IS THE EXPRESSION OF CLOCK GENES IN THE SUPRACHIASMATIC NUCLEUS AFFECTED BY PHOTOPERIOD IN RAT PUPS?

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The suprachiasmatic nucleus (SCN) of hypothalamus is site of generation of biological rhythms. At the molecular level biological clocks are based on the rhythmic expression of clock genes that form transcriptional-translational feedback loops. Previous results from our laboratory (1) demonstrated that phase, waveform and amplitude of the rhythmic clock genes expression are affected by the environmental daylength, i.e., photoperiod in adult rats. Because there is a lack of information about the effect of photoperiod on clockwork during ontogenesis we decided to analyze the expression of clock genes (per1, per2, clock, cry1 and bmal1) in the SCN of 3-day and 10-day old rat pups. White Wistar rat dams were kept under artificial short, 8-h light (L) and 16-h dark (D) or long (L16:D8) photoperiod for at least 5 weeks before experiment. On the day of experiment mothers with their pups were released into darkness and pups were sampled in 2 h intervals throughout 24 h cycle. Levels of mRNA were analyzed by *in situ* hybridization. Our results show that photoperiod affects differently the rhythmic clock genes expression and the effect is more expressed later in postnatal development. Hence, although the main counterparts of the feedback loops are already present at the time of birth, the effect of photoperiod develops only gradually during ontogenesis.

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MORPHOLOGY OF REACTIVE ASTROCYTES IN CULTURE

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There is an increasing interest in the response of astrocytes to various physiological and pathological factors. Here we report morphological and some cytochemical features of the astrocytic C6 glioma cells exposed to sublethal doses of Cisplatin, as a new advantageous in vitro model. The cells were exposed to 5 μg Cisplatin/ml and examined 24 to 96 h later. The number of cells progressively decreased due to apoptosis (1) monitored by flow cytofluorometry of Annexin-V and propidium iodide supravital staining at 48h and onwards. Surviving cells showed morphological changes including enlargement of cells and formation of longer processes. EM analysis revealed larger nuclei with more often invaginations of the nuclear envelope and fine and diffusely distributed chromatin. There were multiple and more prominent nucleoli. Mitochondria increased in size and the free ribosomes were substantially more numerous. Cisterns of the rough endoplasmic reticulum were often elongated and occasionally distended with fine, moderately dense precipitates. Bundles of fine actin-like filaments occurred more frequently under the plasma membrane, especially of the cell processes. Heterogeneous secondary lysosomes and lamellated dense bodies occurred in the perinuclear cytoplasm together with clusters of lipid droplets. Autophagic and heterophagic activity was frequently apparent. These findings, together with the earlier reported higher protein content per cell and enhanced expression of the Glial Fibrillary Acidic Protein (2) suggests that the Cisplatin treatment surviving, and/or, the more resistant cells undergo hyperptrohic reaction accompanied by higher turnover of cytoplasmic organelles and astrocyte-specific differentiation. These cells are also vividly cleaning the debris originated from the apoptotic fragments of more sensitive cells by phagocytosis.

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GEOMETRY AND STRUCTURE OF CONDUIT ARTERY OF HYPERTENSIVE NO-DEFICIENT NEWBORN AND ADULT RATS

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The aim of the study was to compare the structure of conduit artery of adult NO-defective hypertensive (NODH) rats (16 weeks of age) and NODH newborn rats (24 days of age), both groups were compared to age matched controls. Blood pressure (BP) was measured noninvasively on tail artery, using the plethysmographic method. The animals were anaesthetized (thiopental 100 mg/kg i.p.), the chest was opened and a cannula localized into the left ventricle. Cardiovascular system was perfused with a glutaraldehyde fixative under the pressure 120 mmHg. Middle part of carotid artery (CA) was excised and processed according to standard electron microscopic procedure. Geometry of CA: wall thickness (WT) and inner diameter (ID) were measured on semithin sections in light microscopy. Cross sectional area of tunica intima and tunica media (CSA) and WT/ID ratio were calculated. Using point counting method volume densities of cells - endothelial (EC), smooth muscle cells (SMC) and extracellular matrix (ECM) in CA were analyzed in electron microscopy. BP was in NODH adult rats 172±1.7 mm Hg vs 103±1.1 mm Hg, ($p<0.01$) in controls, and in newborns NODH rats 150±2.3 mm Hg vs 105±2.1 mm Hg, ($p<0.01$) in controls. In adults an increase of WT was found (40.78±1.33 μm and 25.02±1.76 μm in controls, $p<0.01$), and was confirmed by calculating the CSA (107.93±4.37 $\mu m^2 \times 10^3$ and 64.24±3.07 $\mu m^2 \times 10^3$, $p<0.01$). ID did not differ in experimental and control vessels, however the value of WT/ID ratio was higher (5.08±0.29 x 10^{-2} and 3.16±0.33 x 10^{-2} in controls). Both components of arterial wall were increased: cells 51.6±2.19 $\mu m^2 \times 10^3$ and 32.6±1.06 $\mu m^2 \times 10^3$ in controls ($p<0.01$), and ECM 56.4±3.7 $\mu m^2 \times 10^3$ and 31.7±1.6 $\mu m^2 \times 10^3$ in controls ($p<0.01$). Completely contrasting structure pattern of newborn CA was disclosed. WT was found declined: 22.48±0.66 μm and 27.38±0.63 μm ($p<0.01$) in controls, similarly CSA 38.53±0.99 $\mu m^2 \times 10^3$ and 46.15±1.45 $\mu m^2 \times 10^3$ ($p<0.01$) in controls. The value WT/ID ratio was lower 4.32±0.20 and 5.40±0.11 ($p<0.01$) in controls. Both components of CA wall indicated a decline: cells 16.89±0.66 $\mu m^2 \times 10^3$ and 22.45±1.16 $\mu m^2 \times 10^3$ ($p<0.01$) in controls, and ECM 22.03±0.63 $\mu m^2 \times 10^3$ and 22.22±1.04 $\mu m^2 \times 10^3$ ($p<0.01$) in control artery. In spite of high BP in both age groups in adults an expected CA wall thickening was found with contribution of both cells and ECM. However, in newborns a weakening of CA wall mainly due to decrease cell components was observed.

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URINARY IODINE EXCRETION AND THE STATUS OF IODINE IN BLOOD PLASMA

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The renal excretion of iodine has priority importance in its homeostasis. The urine iodine is generally used for simply evaluation of saturation of animals and human beings with iodine (1). Relations between concentration of iodine in urine and in blood plasma are not more accurately defined in contrary to the other trace elements, especially, by concurrent applying of homeorhesis during lactation (2). This study brings basic information about interrelations between content of iodine in urine and in blood plasma in milking and beef cows during the deficient, sufficient and surplus supplementations with iodine in mineral mixtures. Samples (n=465) for this observation were collected in 1999-2001 from 8 farms in south-west Bohemia where the daily intake of iodine ranged from 1.9 to 17.7 μg per cow according to the level of its supplementation. Iodine was assayed after alkaline digestion by modified method of Sandell-Kolthoff (3). The level of urinary iodine ranged from 22.9 to 500.1 $\mu g \cdot l^{-1}$ and the content of iodine in blood plasma ranged from 27.4 to 878.8 $\mu g \cdot l^{-1}$. Statistically significant relationship (rxy = 0.53) was found between concentration of iodine in urine and blood plasma. The average value 203.1 $ug \cdot l^{-1}$ of urine was accompanied by the average concentration of iodine in blood plasma 154.2 $ug \cdot l^{-1}$, which is exceeding the references about the upper physiological level of iodine in blood plasma (4). This finding challenges to the experimental verifying of efficiency of the homeostatic renal excretion of iodine

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ACTION OF PHYSOSTIGMINE ON THE NICOTINIC ACETYLCHOLINE RECEPTOR

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Physostigmine (eserine) is known as a reversible inhibitor of acetylcholine esterase. Data accumulated indicate that it could also act as a low potency agonist and modulator on different types of nicotinic receptor (1,2). Physostigmine (Phy) could activate even the desensitized receptor (3). This drug apparently binds to different binding sites other than nicotinic agonists and virtual competitive antagonists (1). Currents induced by direct application of Phy can be recorded only in the single channel patch-clamp mode because of the low efficacy of this drug as an agonist. The action of physostigmine on mouse muscle nicotinic receptors was studied by the patch-clamp technique in the COS-7 cell line. We recorded currents in the whole-cell mode 3-7 days after cell transfection by plasmids coding appropriate combination of receptor subunits. Drugs were applied using a rapid microcomputer controlled perfusion system. A complete change of the solution around the cell varied between 30-60 ms which is critical for avoiding rapid desensitization. Cells clamped at -40 mV responded to application of acetylcholine by desensitizing the inward current. No reliable specific whole-cell membrane responses could be induced by Phy application of up to 1 mM. Physostigmine in concentrations of 10^{-6}-10^{-4} M accelerate desensitization of currents induced by acetylcholine and increase the final level of desensitization in concentration dependent manner. This finding is in contrast to the suppression of desensitization caused by Phy and 1-methyl-galanthamine observed in Torpedo receptors (4).

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CONFOCAL STEREOLOGY IN RADIATION BIOLOGY

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This presentation reviews the methodology and format of using confocal microscopy and stereological methods to quantify tissue parameters, e.g. tissue volume, cell number, and microvessel length. Stereological methods and their concepts are shown on an ongoing study of the dose response of the microvessels in proton irradiated hemibrain (1). Geometrical parameters of microvessels and their 3D arrangement in the cortex and white matter, differing in dose tolerance, are compared. Methods for estimating the volume of the brain and brain compartments, total number of endothelial cells in microvessels, length of microvessels, and number of their branchings in the cortex and white matter are presented. It is shown that stereological techniques, based on sound theoretical basis, are powerful and suitable for objective evaluation of the effect of dose response on brain tissues.

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KNOCKOUT MICE: CATECHOLAMINE SYNTHESIZING ENZYMES AND THEIR MODULATION BY IMMOBILIZATION STRESS

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Corticotropin-releasing hormone knockout mice (CRH KO) and c-fos knockout mice (c-fos KO) can serve as interesting models for studying the mechanisms involved in the response of the hypotalamic-pituitary-adrenal axis to stress. In our work we focused on investigation of changes in tyrosine hydroxylase (TH), aromatic L-amino acid decarboxylase (AADC), dopamine-β-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) gene expression and protein level in adrenal medulla of immobilized CRH KO and c-fos KO mice. Levels of TH, AADC, DBH and PNMT mRNA were determined by RT-PCR, the amount of corresponding proteins was determined by Western blot analysis. After single and repeated stress exposure we observed similar increase in TH, AADC and DBH mRNA levels in adrenal medulla of CRH KO and WT mice compared to unstressed controls. Contrary to these enzymes, PNMT mRNA was significantly decreased after single and repeated immobilization stress exposure in adrenal medulla of CRH KO mice compared to WT mice. Moreover, PNMT immunoreactive protein was also decreased after repeated immobilization in CRH KO mice. In c-fos KO mice, single immobilization stress exposure significantly increased adrenomedullary TH, DBH and also PNMT mRNA levels compared to unstressed controls. Our data suggest that CRH deficiency can influence adrenomedullary PNMT mRNA level during stress. On the other hand, c-Fos probably does not play a crucial role in TH, DBH and PNMT gene expression in adrenal medulla under the stress conditions.

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PROTECTIVE EFFECT OF S-ADENOSYLMETHIONINE AGAINST THIOACETAMIDE-INDUCED INJURY OF RAT HEPATOCYTES IN PRIMARY CULTURE

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Thioacetamide (TAA) is a model hepatotoxin activated by biotransformation to toxic metabolites thus leading to centrilobular necrosis. The alteration of mitochondrial membrane was also described. We evaluated the protective effect of S-adenosylmethionine (SAMe) against TAA-induced hepatocyte injury with regard to prevent mitochondria. Hepatocytes were isolated from Wistar rats (230-270 g) by collagenase (SEVAC, Prague) perfusion and cultured in William´s E medium supplemented with antibiotics, fetal bovine serum, insulin, prednisolon and gassed atmosphere. Cells were plated at a density of 2 million cells per 60 mm dish. After cell attachment TAA at 70 mM or TAA together with SAMe at the final concentration of 0.005 and 0.050 mg/ml were added to culture media for 24 hours. Then the medium was replaced and hepatocytes were incubated for 48 hours only with SAMe. Hepatocyte integrity and functional capacity were analysed by lactate dehydrogenase activity (kit, Merck) and urea concentration (kit, Sigma) in cultivation media, concentration of malondialdehyde (MDA) measured using thiobarbituric acid served as a marker of lipid peroxidation, glutathione (GSH) content was measured using HPLC. Mitochondrial membrane potential was evaluated by accumulation of Rhodamine 123 (Sigma). Except MDA production and GSH content SAMe treatment attenuated in dose dependent manner all measured markers namely TAA-induced release of LDH, decrease in urea synthesis, and changes of mitochondrial membrane potential. Our results indicate that SAMe protects hepatocytes against TAA-induced damage rather by stabilization of mitochondrial and cellular membranes than by direct inhibition of lipid peroxidation or prevention of glutathione depletion.

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TISSUE-SPECIFIC DISTRIBUTION OF CORTICOSTERONE METABOLISM IN CHICKEN

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The biological activity of glucocorticoids depends not only on the plasma level of the hormone, number of receptors and the responsiveness of the target cells but also on the local metabolism of glucocorticoids that is able to decrease or increase the local concentration of biologically active steroid. Inactivation of glucocorticoids is associated with oxidation of corticosterone on carbon C_{11} or reduction on C_{20} and depends on the activity of 11β-hydroxysteroid dehydrogenase isoform 2 (11βHSD2) and 20α- or 20β-hydroxysteroid dehydrogenase (20HSD). The increase of local corticosterone concentration depends on the activity of 11HSD isoform 1. However, localization of these steroid enzymes in avian tissues other than intestine is unknown. That's why non-radiometric assay was performed in ileum, kidney and liver. Steroid metabolites of corticosterone and progesterone were identified and quantified by liquid chromatography and mass spectrometry. Moreover, internet databases and alignment programs were used to acquire mRNA sequence of 11HSD2 for Real-Time RT-PCR. Tissue fragments of ileum and kidney converted corticosterone to 20-dihydrocorticosterone and less to 11-dehydro-20-dihydrocorticosterone; liver fragments metabolized corticosterone exclusively to 20-dihydrocorticosterone. If progesterone was used as a substrate instead of corticosterone the tissue reduced progesterone to 20β- and not to 20α-dihydroprogesterone. Sequence of putative 11βHSD2 enzyme was constructed using chicken cDNA library and compared with known mRNA sequences of other species: 53% homology vs. fish and 65% vs. mammals. Its expression was found in mineralocorticoid target tissues: kidney and colon, but not in liver. These observations indicate that corticosterone is inactivated in avian tissues by 11βHSD2 and 20βHSD and that both enzymes are coexpressed in some tissues.

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THE CHANGES OF QRS AMPLITUDE OF SURFACE ELECTROCARDIOGRAM AND CONTRACTILITY OF ISOLATED CARDIOMYOCYTES IN RATS TREATED BY ENALAPRIL AND LACIDIPINE

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Lacidipine (calcium antagonist) and enalapril (angiotensin converting enzyme inhibitor) have been reported to induce regression of LV hypertrophy in experimental models even in doses not reducing high blood pressure (BP). The aim: to analyze the changes of the QRS amplitude of surface electrocardiogram and of the contractility of isolated cardiomyocytes in spontaneously hypertensive rats (SHR) treated by lower doses of enalapril and lacidipine for eight weeks. Four groups of age-matched male experimental animals at the age of 20 weeks were studied: (1) Control Wistar-Kyoto rats (WKY); (2) spontaneously hypertensive rats (SHR), (3) SHR treated with enalapril (10 mg/kg per day) and (4) SHR treated with lacidipine (3 mg/kg/day in two doses). Frank orthogonal electrocardiograms were recorded and the maximum spatial QRS vector magnitude (QRSmax) was calculated. Left ventricular mass (LVM) was measured after rats were sacrificed. The specific potential of myocardium (SP) was calculated as the QRSmax to LVM ratio. Left ventricular myocytes were isolated using the enzymatic digestion of the heart. Contractility, i.e. percentage of cell shortening was analyzed from video sequences of stimulated contractions. (data presented as mean and S.D. *: $p<0.05$ vs WKY, †: $p<0.05$ vs SHR):

	WKY	SHR	Lacidipine	Enalapril
BP [mmHg]	127±6	201±4*	205±13*	206±14*†
LVM/BW [g/kg]	1.93±0.10	2.91±0.13*	2.77±0.13*	2.68±0.16*†
QRSmax [mV]	0.73±0.01	0.5±0.01*	0.45±0.3*	0.48±0.02*
SP [mV/g]	1.08±0.06	0.5±0.03*	0.61±0.04*	0.73±0.04*†
Contractility [%]	13,10±3,27	7,32±3,93*	5,46±3,96*	6,57±4,71*

In conclusion, enalapril effected the LWM/BW ratio and the SP (the relative QRS voltage) of surface electrocardiogram even in the dose which did not decrease the high blood pressure. Neither lacidipine nor enalapril had significant effect on the contractility of isolated cardiomyocytes.

GLUCOSE ABNORMALITIES INDUCED BY OLIGEMIC BRAIN HYPOXIA IN SHR/N-CP LEAN RATS OF KOLETSKY STRAIN AND IN RATS OF WISTAR STRAIN AND EFFECTS OF TERGURIDE TREATMENT

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Many experimental studies have emphasized the role of elevated glucose in worsening of degree of infarction. Elevation glucose may also be associated with worsened brain edema and hemorrhage into infarction (1). Histological evaluations unambiguous documented, that hyperglycemia exaggerated brain damage due to transient ischemia. Ischemic brain injury associated with hyperglycemia increased incidence of seizures, mortality and worse neurological outcome. (2-3) Recent clinical studies have shown benefit of tight glucose control in patients in the neurological intensive care unit. (1). In this paper we investigated abnormalities of glucose metabolism. The effect of Terguride (trans-dihydrolisuride) on glucose metabolism was tested. The experiments were performed in male and female rats of Wistar and Koletsky strain. Glucose intolerance was induced in both strains 4-hour-occlusion of both common carotid arteries followed by 44-hour reperfusion. Brain water content was used as a marker of brain edema. Brain hypoxia induced glucose intolerance in both rat strains. Brain edema after hypoxia was observed only in male of Wistar strain. Basal glycemia was significantly increased by the brain hypoxia in male and female Wistar rats, but not in Koletsky rats. When we evaluated the effect of Terguride treatment of glucose abnormalities on "area under the glucose tolerance curve" (AUC), we found significant decrease of AUC in both sexes of Wistar strain and in female of Koletsky strain. Basal glycemia was significantly decreased only in male of Wistar strain. Our data show that Terguride decreased hyperglycemia in rats with hypoxia-damaged brain.

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INVOLVEMENT OF ADENOSINE RECEPTORS IN NK CELL-MEDIATED CYTOTOXICITY IN DIFFERENT MAMMALIAN SPECIES

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Neuroimmune modulation depends on many molecular structures, where the substantial role plays adenosine receptors in cooperation with dopamine. This is especially exerted when the system undergo stressful conditions or tumor target cell recognition. Our study was designed to assess the effect of adenosine A 1 (CCPA), A 3 (NECA), and A 2A (CPCA) receptor agonists in natural killer (NK) cell-mediated cytotoxicity across several mammalian species (mouse, rat, human and pig). The experimental models of restraint stress or transplanted tumors under dopaminergic impact in mice were followed. An inhibitory action of A 2A agonist at high concentration (10 -5M) were demonstrated, while A 1 and A 3 adenosine agonists don't influence significantly the NK cells effector function. On the other hand, low concentrations of A 2A agonist stimulated the cytotoxicity in healthy unconditioned animals, and contrary evoked inhibition under stress or tumor pressure. Moreover, the final effects of adenosine receptor agonists were dependent on both, the effector and the target cells pretreatment. Analysis of G-protein changes induced by adenosine agonists showed equal participation of Gi and Gs subunits during NK cell signaling as previously demonstrated in nervous system. Thus, the signaling via adenosine receptors is linked to the dopaminergic receptors and functional effects are generally comparable in all studied species.

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THE EFFECT OF CAPTOPRIL ON VASOACTIVE INTESTINAL PEPTIDE METABOLISM IN THE RAT HEART

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Although neutral endopeptidase (NEP) is a major enzyme metabolizing vasoactive intestinal peptide (VIP) in the plasma and tissues, angiotensin I converting enzyme (ACE) has been suggested to contribute to the degradation of VIP in the rat heart (1). Hyperthyroidism is known to be associated with the increased activity of ACE in the plasma and heart and a decreased VIP concentrations in the heart atria of adult rats (2). In the present study, we studied the effect of ACE inhibition by captopril on VIP levels in the plasma, adrenal glands and heart atria of rats treated with thyroxine (T4) 1mg/kg/day for 10 days and their age-matched controls (C). The heart atria and adrenal glands of T4 and C rats were dissected and homogenized in 50 mmol/l Tris-HCl buffer with 1 % NaCl and 60 µmol/l NEP inhibitor thiorphan. The plasma samples and the homogenates of individual atria and adrenal glands were divided into four equal samples; the first one was extracted immediately for determination of VIP concentration (T0 value), the remaining samples were incubated for 30 min at 37 °C without or with 2 and 10 µmol/l captopril, respectively. The reaction was terminated by boiling in 0.1 mol/l HCl. VIP concentrations in the extracts were measured by radioimmunoassay and expressed in ng/g tissue wet weight and pg/ml of plasma. Concentrations of VIP were 6.8±1.2 and 138±7.5 ng/g in the atria and adrenals, respectively, and 83±12 pg/ml in the plasma of C rats.T4 administration had no effect on VIP concentrations in the adrenals and plasma. In contrast, VIP levels were significantly lower in the atria of T4 rats. Incubation of the samples without captopril resulted in VIP levels decreased by 84 % and even 97 % (compared to T0 values) in the atria of C and T4 rats, respectively, whereas the peptide concentrations in the adrenals and plasma were not significantly affected. VIP levels in the samples incubated with captopril in both concentrations did not differ from the T0 values in the C atria. In contrast, VIP concentrations in T4 atria incubated with 2 and 10 µmol/l captopril represented 60 % and 90 % of the respective T0 values. In conclusion, VIP seemed to be metabolized by ACE in the atria, but not in the plasma and adrenals in both C and T4 rats. In addition, increased activity of ACE might contribute to the decrease in VIP levels in the atria of hyperthyroid rats.

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CHANGES OF N-ACETYLGLUCOSAMINIDASE HYALURONIC ACID, AND ALPHA-2 MACROGLOBULIN IN PATIENTS WITH CHRONIC VIRAL HEPATITIS TREATED BY INTERFERON ALPHA

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Liver fibrosis is a common development in chronic liver disease. Determining the stage of liver fibrosis is important in managing patients with chronic viral hepatitis. Liver biopsy is the gold standard for assessing fibrosis, but it is invasive procedure. Biochemical markers for liver fibrosis are an alternative to liver biopsy in patients with chronic hepatitis. Aim of the study was to investigate the effect of therapy with Interferon alpha (IFN-alpha) and ribavirin on potential biochemical markers of fibrosis, such as hyaluronic acid (HA), alpha-2-macroglobulin (AMG) and N-acetylglucosaminidase (NAG) in patients with histologically verified Chronic hepatitis C. Blood samples from patients with Chronic hepatitis C and from healthy controls were assayed for hyaluronic acid (Hyaluronic acid „Chugai", sandwich HA binding protein assay), N-acetyl-glucosaminidase (fluorometric method) and alpha-2-macroglobulin (immunochemical method). In the group of the patients all investigations were performed before and after treatment with (IFN-alpha) and ribavirin (48 weeks)..The serum HA level correlated with the extent of liver fibrosis ($r=0.87$, $p<0.001$). All markers of liver fibrosis were significantly increased in patients with Chronic hepatitis C in comparison to healthy controls (HA: 95.1 $ng.ml^{-1}$ vs. 15.7 $ng.ml^{-1}$, NAG: 18.5 $U.l^{-1}$ vs. 8.7 $U.l^{-1}$, AMG: 2754 $mg.l^{-1}$ vs. 1308 $mg.l^{-1}$).before treatment. After therapy by Interferon alpha and ribavirin (48 weeks) there was significant decrease of the level of HA (57.0 ng.ml. vs. 95.1 $ng.ml^{-1}$). The level of AMG was in patients with Chronic hepatitis C after therapy also decreased (2535 $mg.l^{-1}$ vs. 2754 $mg.l^{-1}$). There was no change in the activity of NAG after therapy in comparison with the activity before beginning of therapy. There was significant positive correlation between serum HA levels and the levels of AMG in patients with Chronic hepatitis C during therapy ($r=0.585$, $P<0.001$). Thus serum HA measurement is a good and clinically useful non-invasive marker of liver fibrosis. It could be therefore used for monitoring of the stage of liver fibrosis and as a measurement of response to antifibrotic therapy. The present study results also suggest that serum AMG levels are related to the therapeutic outcome of IFN-alpha and ribavirine in patients with Chronic hepatitis C.

EXAMINATION OF INTERLEUKIN 10 AND ASSESSMENT OF HEPATIC FIBROSIS IN SUBJECTS WITH CHRONIC VIRAL HEPATITIS TREATED BY INTERFERON

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Cytokines are soluble mediators that control many critical interactions among cells of the immune system. Hepatic fibrosis is a reversible accumulation of extracellular matrix in response to chronic injury. We have examined serum hyaluronic acid (HA) concentration (Hyaluronic acid „Chugai") in a group of patients with histologically verified chronic viral hepatitis B (VHB) and C (VHC) treated 48 weeks with Inteferon alpha (IFN alpha) and healthy blood donors. Serum HA level before the treatment correlated with the extent of liver fibrosis ($r=0.7$, $p<0.001$). We observed statistically significant decrease in HA in all patients (good responders and non-responders as well), after the finishing of the treatment by IFN alpha. All patients with VHB and good responders in the VHC group had significantly higher pre-treatment IL-10 levels, when compared to controls. During the treatment, a constant decrease in IL-10 was observed in VHB good responders subgroup, reaching the significant difference only in month 6. In VHC patients in the good responders subgroup a significant decrease in IL-10 levels was observed in month 1, while an increase was observed in non-responder group. Serum HA measurement is a good and clinically useful non-invasive marker of liver fibrosis. It could be therefore used for monitoring of the stage of fibrosis as a measurement of response to antifibrotic therapy. IL 10 might be useful in the follow up of patients with VHB and VHC treated with IFN alpha.

INHIBITION OF $Ca_v3.1$ CHANNEL BY SILVER IONS

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Silver ions were shown previously to interact with the voltage sensor of L-type calcium channel. We have investigated whether they may affect T-type calcium channels, too. Our experimental object were $Ca_v3.1$ calcium channels stably expressed in HEK 293 cells. The effects of two silver salts, AgCl and $AgNO_3$, on current-voltage (I-V) relationship, steady-state inactivation (SSI) and on the kinetics of channel activation and inactivation were examined. Whole cell currents carried by 2 mM Ca^{2+} were measured using the patch clamp method. The holding potential was –100 mV in all experiments. AgCl solves in water in concentrations up to 15 μM. Therefore the highest AgCl concentration tested in our experiments was 10 μM. At this concentration, AgCl inhibited approximately 24 % of the current amplitude at a membrane potential corresponding to the peak of I-V relationship. The block was voltage dependent and increased linearly with the amplitude of depolarizing pulses. 10 μM of AgCl caused significant hyperpolarizing shift of I-V relationship by -6.6 mV. SSI was not significantly affected. Furthermore, kinetics of the channel activation was significantly accelerated in the presence of AgCl. The inactivation kinetics was not altered. The effect of $AgNO_3$ was examined at concentrations ranging between 0.1 and 300 μM. The inhibition of calcium current amplitude was moderate with an extrapolated IC_{50} of 3.5 mM. The effect of $AgNO_3$ on current amplitude was slightly voltage dependent. Block increased monotonically with increasing depolarization. Neither I-V relationship nor SSI were significantly affected by this the silver salt. Also, $AgNO_3$ had no effect on kinetics of the channel activation or inactivation. In conclusion, Ag^+ is a weak T-type calcium channel blocker. The mechanism of the interaction depends on the form, in which Ag^+ enters the experimental solution.

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THE CIRCADIAN RHYTHM OF PER1 PROTEIN IN SUPRACHIASMATIC NUCLEUS OF RAT PUPS AND ITS MODULATION BY DAYLENGTH

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In mammals the core mechanism underlying circadian rhythmicity is based on the transcription-translational loops within the cells of the suprachiasmatic nuclei (SCN) of the hypothalamus. Per1 gene and PER1 protein are one of the basic elements of this loops. Per1 gene expression as well as its protein production in the SCN of adult rat is rhythmic and depends on the daylength, i.e. the photoperiod (1). The previous study shows that the internal rhythmicity of the SCN can be slightly modulated by the photoperiod already in 10-day old rats (2). The aim of this study was to analyze development of the rhythm in PER1 protein production during ontogenesis and then to determine whether the photoperiod modulates this rhythm in 10-day old rats. Pregnant rats and rats with their pups were maintained under a light-dark cycle (LD) with 12-h light and 12-h dark and under artificial short (LD 8:16) and long (LD 16:8) photoperiod. Rat pups were killed at the postnatal day (P)3 and P10. The daily profiles of PER1 protein were assessed by immunohistochemistry. Our results show that there is a circadian rhythm in the number of PER1-labeled cells in 3-day and 10-day old rat pups. The photoperiod slightly influences this rhythm in 10-day old rats but the modulation does not yet attain that of adult animals.

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CARDIAC PHENYLETHANOLAMINE-N-METHYLTRANSFERASE (PNMT) GENE EXPRESSION AND ITS REGULATION IN RATS EXPOSED TO STRESS

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Recently we have described the existence of adrenaline-synthesizing enzyme PNMT mRNA in the heart of adult rats. The aim of this study was to determine distribution of the PNMT mRNA in the heart and to examine whether the gene expression of this enzyme is affected by immobilization (IMO) stress in a time-dependent manner. PNMT mRNA levels were detected in all seven parts of the heart studied (atria, atrial ganglia, ventricles, septum) with the highest levels in the left atrium and its ganglionic part. Both Southern blot and sequencing verified the specificity of PNMT detected by RT-PCR. Single IMO for 2 hours increased gene expression of PNMT, as determined by both RT-PCR or Real-Time PCR in right and left atria, ventricles and septum. Surprisingly, the ganglionic parts of atria did not respond to stress stimulation. Peak levels of PNMT mRNA were found in the interval of 3 h after the IMO terminated, and also 24 h after the first or sixth IMO. In atria, the effect of IMO was clearly modulated by glucocorticoids, since adrenalectomy prevented the increase in PNMT mRNA levels. In ventricles, adrenalectomy did not affect the IMO-induced increases in mRNA and therefore the PNMT gene expression in cardiac ventricles might be regulated by other factors. Glucocorticoid regulation of PNMT gene expression in heart atria has been confirmed in corticoliberin knock-out mice. Thus, our data have shown that at least two mechanisms exist in the regulation of cardiac PNMT gene expression. The stress-induced increase in atria is dependent on the presence of glucocorticoids, however in ventricles another mechanism is involved.

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MOLECULAR ANALYSIS OF SEX HORMONE-BINDING GLOBULIN GENE IN THE RAT HYPODACTYLOUS MUTATION (*HD*)

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Sex hormone-binding globulin or androgen-binding protein (ABP/SHBG) is an extracellular androgen and estrogen carrier. In the rat, ABP/SHBG is secreted by Sertoli cells of the testis and is thought to regulate androgen bioavailability in the male reproductive tract. During ontogenesis, ABP/SHBG is expressed in many mesoderm-derived tissues, including interdigital mesenchyme of the developing autopodium (hand and foot). *Shbg* is thus a candidate for rat hypodactyly (*Hd*), consisting of two different phenotypes - male infertility resulting from spermatogenesis impairment and digital arch reduction manifesting as decreased number and/or altered morphology of the digits. Moreover, linkage mapping of *Hd* mutation revealed that an DNA polymorphism D10Wox12, intragenic marker for *Shbg*, was nonrecombinant with *Hd*. However, sequencing of the entire coding sequence of *Shbg* failed to identify any variation in hypodactylous animals, distinct from two control strains. RT-PCR analysis of *Shbg* expression in testicular tissue did not reveal any significant difference between infertile hypodactylous rats and fertile SHR controls. We therefore conclude that *Shbg* is, at the gene and mRNA expression level, not altered in testes of infertile *Hd* rats. However, we cannot exclude mutation in regulatory sequences or chromosomal micro-rearrangement, exerting minor quantitative effects upon *Shbg* expression. Analysis of DNA sequences flanking *Shbg* gene to understand regulation of *Shbg* expression in mutant rats will address such possibility.

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ANTICONVULSANT ACTION OF AN ANTAGONIST OF TYPE I OF METABOTROPIC GLUTAMATE RECEPTORS IN IMMATURE RATS

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MPEP (2-methyl-6-(phenylethynyl)-pyridin) is described as a potent, selective antagonist for the type I metabotropic glutamate receptor (subtype 5 - mGluR5) active after systemic administration. The aim of our study was to examine anticonvulsant action of MPEP in immature rats. Two models of epilepsy seizures were used, i.e. cortical epileptic afterdischarges (ADs) and low-dose PTZ (pentetrazol) model of non-convulsive absence seizures. The anticonvulsant action was studied in 12 (P12), 18 (P18), and 25 (P25) day-old Wistar rat pups with implanted cortical electrodes. MPEP (Tocris) was freshly dissolved in isotonic saline in a concentration 5 mg/ml. The control siblings were treated with an equal volume of physiological saline. Individual age and dose groups were formed by 8 rats. In ADs model electrical stimulation of sensorimotor cortex was repeated six times with 20-min intervals. The first stimulation served as a control. Absolute values of intensity ranged from 2.8 to 4.5 mA. MPEP, in doses of 20 mg/kg, 40 mg/kg and 80 mg/kg (P25 only) i.p., was administered 15 min. after the first AD. The EEG was recorded before and during stimulation, during the ADs and 1 min afterwards. The type and duration of ADs were evaluated. Racine's five-point scale was used for the quantification of behavior. In model of absence seizures two age groups P18 and P25 were used. EEG recording started 10 min before MPEP treatment, 15 min after MPEP PTZ was administered. Recording was terminated 30 min after PTZ injection. Animals were treated with MPEP in dose of 40 mg/kg and 80 mg/kg (P25 only) i.p., and with 35 mg/kg of PTZ i.p. The latency of onset of PTZ action, and the length and the number of spike-and-wave episodes were counted. MPEP exhibited an anticonvulsant effect in ADs model (shortening of ADs and decrease in seizure severity) in all age groups studied. This effect was better expressed in younger rats than in the P25 group. None of the parameters of absence seizures was significantly affected by MPEP. An antagonist of mGluR5 MPEP exhibits different anticonvulsant action in two models of epileptic seizures. This result confirms the difference in the mechanisms involved in generation of these seizures.

DEPLETION OF MEMBRANE CHOLESTEROL INHIBITS CHOLINE TRANSPORT IN CHOLINERGIC NG108-15 CELL LINE

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It has been speculated that an increase of amyloid beta production resulting in deterioration of cell function and cell death commonly found during progression of Alzheimer´s disease is linked to disturbances of lipid metabolism, namely to an increase of circulating cholesterol. We have examined in cholinergic cell line NG108-15 (mouse neuroblastoma x rat glioma (1,2)) an influence of acute depletion of membrane cholesterol induced by treatment with methyl-ß-cyclodextrin (MBCD) on some aspects of cell metabolism. Preincubation of cells in the presence of MBCD (30 minutes, 13 mg/ml) decreased cholesterol content by 50-70 % both in control and differentiated cells grown in serum-containing or defined (serum free) medium without any effect on cellular protein content. Depletion of membrane cholesterol has no effect on oxidative activity (2',7'-dichlorodihydrofluorescein oxidation) measured after preincubation with MBCD in control cells and slightly but significantly decreased oxidative load in differentiated cells. In contrast, this depletion of membrane cholesterol induced a large reduction of total uptake of choline. High affinity component of choline uptake was abolished in control cells and inhibited by more than 50 % in differentiated cells. This inhibition of high affinity choline uptake was proportional to the inhibition of total uptake. These data demonstrate that even large depletion of cellular cholesterol in a short-time range has no overt noxious influence on the oxidative activity of NG108-15 cells. However, choline is an essential precursor of membrane phospholipids and a capability of its production in mammalian cells is very limited. It seems apparent that a lasting limitation of choline delivery would have adverse influence on cell growth and functions. Our data thus point to the importance of suitable membrane cholesterol content for the delivery of the essential nutrient choline (3) through two different transport mechanisms.

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THREE ISOELECTRIC POINTS OF THE CREATINE KINASE M-SUBUNIT, PURIFIED FROM MYOFIBRILS

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One of the molecular problems of muscle energetics which remains unclear, is an operation principle of enzyme molecules in energetic reactions. Myofibrillar creatine kinase (EC 2.7.3.2.) (CK) is a homodimeric enzyme, consisting of two M-subunits, with molar mass of 43 kDa. Computed force fields indicate that the substrate-induced energy minimizing principle is not a sufficient condition for the regulation of enzymatic activity (1). Lifetime fluorometry of double labeled CK verified the resolution range of the method for measurements of conformational changes (2). For the further progress of a loading protocol with fluorophores, an identity of the M-subunit was revealed, using mass spectroscopy (MALDI) and sequential 2D SDS electrophoresis methods. MMCK from the rat psoas muscle was eluated from a pure myofibrillar fraction by our procedure, using a low ionic strength medium and subsequently purified by liquid chromatography techniques. The M-subunit was detected by SDS electrophoresis as a single band, corresponding to 43 kDa. However, MALDI detected the band overlaid with the band of actin. Chromatofocusing results and 2D SDS electrophoresis identified three subunits of MMCK (M1, M2, M3) of different isoelectric points within a narrow range of pH, being 7.17, 7.28 and 7.47, respectively.

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DEPOLARIZATION DOES NOT CHANGE AFFINITY OF MUSCARINIC M_2 RECEPTORS EXPRESSED IN CHO CELLS

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It has been proposed that in addition to calcium influx, exocytotic release of acetylcholine (ACh) requires membrane depolarization which results in a sharp decrease of affinity of presynaptic inhibitory muscarinic M_2 receptors relieving their tonic activation by residual ACh (1-3). In our present experiments we have investigated an influence of membrane potential on the affinity of pure population of M_2 receptors expressed CHO cells (4). In kinetic measurements we have found no effect of potassium depolarization (73 mM) on the affinity of equilibrium binding of antagonist [^{3}H]-N-methylscopolamine (^{3}H-NMS). Affinities of muscarinic agonists carbachol and ACh were estimated using displacement experiments. Again, potassium depolarization did not cause any appreciable change of the affinity for both agonists. The lack of effect was not due to a failure of depolarization because the same results were obtained in the presence of potassium ionophore valinomycin. In addition, direct measurement of membrane potential using potential sensitive fluorescent probe DiS-C_3(3) has confirmed depolarization of cells. The discrepancy of our and aforementioned results could indicate that either the extent of depolarization of non-neuronal CHO cells was not sufficient for the effect to become apparent or muscarinic M_2 receptors itself is not the voltage sensor and has to interact with additional protein which is not present in the membrane of CHO cells.

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EFFECT OF PRETREATMENT OF MELATONIN ON IMPAIRED LEARNING AFTER EPILEPTIC SEIZURE ELICITED BY FLUROTHYL

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One epileptic seizure elicited by flurothyl impairs learning in Morris water maze. Duration of this seizure is usually only 3-4 min and the seizures are not recurrent. No dying neurons were observed after this seizure. In our previous work was demonstrated that learning impairment after seizure is prevented by hypobaric hypoxia. Priming effect was observed when the interval between the hypoxia and seizure was 72 hours. One-hour interval was insufficient. In the present study we tested whether scavenger of reactive oxygen species (ROS) melatonin prevents learning impairment in our model of epileptic seizures. Male rats of the Wistar strain were divided in 5 groups. Rats from the first control group were handled and then tested in maze without any other experimental manipulation. Second group was tested in maze after seizure only. Animals in the third group received solution with melatonin (100 mg/kg) and were tested in maze. Both experimental groups were tested after the seizure. In experimental groups the animals were treated by solution with or without the melatonin 1 hour before the seizure. Learning in maze started always 24 h after previous manipulation (seizure or applications). Melatonin increased the threshold for tonic-clonic flurothyl seizures. The pattern of seizures was similar as in control animals. After the seizure the rats pre-treated by melatonin learned better than rats without melatonin. Our results support the possibility that ROS may be important as a ground for some consequences of epileptic seizures. ROS produced by hypoxia could be signal for production of internal scavengers. Increased level of these scavengers may be involved in non-specific priming effects.

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GAMMA-GLUTAMYLTRANSFERASE ACTIVITY IN REACTIVE C6 ASTROCYTES IN CULTURE

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Gamma-glutamyltransferase (GGT) is a major enzyme of gamma-glutamylated peptide metabolism, including glutathione. The function and regulation of GGT activity in astrocytic cells is still insufficiently understood. Here, we report an up-regulation of GGT activity in the astrocytic C6 glial cells induced by reduction of serum supplement and administration of db-cAMP. The change in GGT was accompanied by inhibition of cell proliferation and growth of cells in size. In addition, the cells became of a more mature, and/or, astrocytic appearance. In these cultures, the signs of cellular stress were evidenced by the release of some cells from the culture dish bottoms and their subsequent shrinkage and death in culture medium. Furthermore, higher oxidation of 2´7´dichlorofluorescein diacetate to its fluorescent form indicated a more intense formation of the reactive oxygen radicals in serum low and db-cAMP treated cultures. The study suggests that up-regulation of GGT activity is a part of the glutathione-related adaptation response of the astrocyte-like C6 cells to increased metabolic load or oxidative stress induced by changes in chemical composition of the cellular microenvironment. The GGT response can be a new factor determining some phenotypic properties of reactive astrocytes in the brain in situ as well as the often reported resistance of brain tumor gliomas to chemo- and radiotherapy.

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THRESHOLDS FOR ELICITATION OF CORTICAL AFTERDISCHARGES IN IMMATURE RATS ARE INFLUENCED DIFFERENTLY BY NMDA AND NON-NMDA ANTAGONISTS

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Excitatory amino acids play an important role in generation and maintenance of epileptic seizures and their antagonists were demonstrated to exhibit anticonvulsant action in adult as well as immature laboratory rats. In the present study we compared action of a noncompetitive NMDA antagonist dizocilpine (MK-801) and a competitive AMPA antagonist NBQX using a model of cortical epileptic afterdischarges for quantification of their effects. Wistar rat pups 12, 18 and 25 days old with implanted electrodes were used. Rhythmic electrical stimulation of sensorimotor cortical area was repeated with an increasing current intensity (from 0.2 to 14 mA) and EEG activity and motor phenomena were recorded. Dizocilpine was administered in doses of 0.1 or 0.5 mg/kg, NBQX in doses of 30 or 60 mg/kg, both intraperitoneally 15 min before the first stimulation series. Control siblings were injected with solvens (physiological saline or dimethylsulfoxide). Each age and dose group was formed by 8-10 rats. In comparison with appropriate control groups both drugs exhibited only moderate effect on movements directly elicited by stimulation of sensorimotor cortex. In contrast, threshold currents for epileptic afterdischarges of the spike-and-wave type and accompanying clonic seizures were significantly increased by both drugs. The youngest age group was the most sensitive one, only dizocilpine was efficient in 25-day-old rats. There is the second type of afterdischarge due to a spread of epileptic activity into limbic structures. This type of afterdischarge is best expressed in 25-day-old rats. Threshold for this limbic type was increased by dizocilpine but not by NBQX. Our data confirmed high sensitivity of immature brain to excitatory receptor antagonists. The results speak in favor of involvement of both NMDA and nonNMDA receptors in generation of spike-and-wave type of ADs but of only NMDA system in the transition of epileptic activity into the limbic structures.

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THE INFLUENCE OF NIMODIPINE ON EXCITABILITY OF CEREBRAL CORTEX IN RATS EXPOSED AND NON-EXPOSED TO SHORT-TERM HYPOBARIC HYPOXIA

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Changes in excitability of cortical neurons in young rats exposed to short-term hypobaric hypoxia (1 hour, simulated altitude of 7 000 m, O_2 = 7.2 %) were registered. In all age groups exposed to hypoxia (12, 25 and 35-day-old), prolongation of the first evoked cortical seizure was estimated. With repetition of the stimulation of sensorimotor cortex (5 times with 1-min interval between the end of the seizure and the next stimulation) decrease excitability in older rats (35-day-old) and increase excitability in younger animals (in comparison to control rats) was found. Pretreatment by nimodipine, L-type calcium channel antagonist, (15 min before the exposition to hypoxia in a dose of 10 and 5 mg/kg i.p.) was tested in rats exposed and non-exposed to hypobaric hypoxia. In 12-day-old rats non-exposed to hypoxia, significant shortening of evoked epileptic seizures ($p<0.01$) was registered. In older animals, nimodipine had no effect on excitability of the brain. The increase of cortical excitability with repetition of the stimulation was expressed in 35-day-old rats exposed to hypoxia ($p<0.01$) and the pretreatment by nimodipine did not influenced the duration of evoked seizures in younger rats. Excitability changes after nimodipine pretreatment can by explained by the effect of nimodipine on cerebral microcirculation and by antagonistic effect on α_1 subunit of voltage sensitive channels that mediate long-lasting Ca^{2+} currents in response to depolarization of cortical neurons.

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PROTEIN KINASE C EXPRESSION IN SKELETAL MUSCLE OF HEREDITARY HYPERTRIGLYCERIDEMIC RATS: EFFECT OF DIETARY INDUCED OBESITY

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Pathophysiological mechanisms of insulin resistance in tissues that play an important role in progress of non-insulin dependent diabetes mellitus are not fully explained yet. There is growing body of evidence that protein kinase C (PKC)-dependent signaling pathway might contribute to the development of insulin resistance (1). First of all, PKCθ and PKCε isoforms are involved in the regulation of insulin signal transduction in skeletal muscle and related enzyme activities. During PKC activation its inactive form is translocated from cytosol to membranes where it is bound as an active form. The aim of the study was to determine the protein amount and cellular localization of PKCθ and PKCε in skeletal muscle of insulin resistant hereditary hypertriglyceridemic (HHTg) rats. *Musculus gastrocnemius* from 18 months old non-obese HHTg rats and control male Wistar rats (fed standard diet) and obese HHTg rats (fed with high-sucrose diet for last 7 months before sacrifice) was removed and homogenized. Membrane and cytosolic fractions were prepared by differential centrifugation (105×10^3 g) and relative protein amount of PKC isoforms was determined by Western blotting following immunochemical detection by specific antibodies and quantified by computer densitometry. Insulin sensitivity of skeletal muscle (*Musculus soleus*) was assessed in vitro by incorporation of ^{14}C-U-glucose into glycogen. There was the significantly higher protein content of PKCθ (by 55 %) in membrane fraction and the lower protein content of PKCθ (by 36 %) in cytosolic fraction from skeletal muscle of HHTg rats in comparison with Wistar rats. High-sucrose diet induced obesity in HHTg rats, decreased skeletal muscle insulin sensitivity and promoted moderately PKCθ translocation to membranes. In contrast, the amount of membrane PKCε was downregulated (by 27 %) under high-sucrose diet in HHTg rats. In conclusion, results suggest that the alterations in the expression and cellular translocation of PKCθ, which is the major PKC isoform in skeletal muscle, may be involved in the mechanism of insulin resistance in non-obese and obese HHTg rats.

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FAS ASSOCIATED DEATH DOMAIN (FADD) IN EARLY ODONTOGENESIS

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Mammalian teeth develop from a series of increasingly well-characterized reciprocal interactions between oral epithelium and mesenchyme. These interactions are mediated by exchange of cell signaling proteins and downstream activation of gene transcription. Cell-cell signaling occurring during early tooth development drives the fate of tooth germs by determination of their type (incisor, molar), shape, size and position in the jaw. Apoptotic pathways contribute to this signaling network. Fas mediated apoptosis is considered as a candidate machinery controlling cell elimination during tooth development since its role has been shown in bone formation. Fas belongs to the tumor necrosis factor family and induces apoptosis upon receptor oligomerization. Cellular response to Fas ligand triggered signal is transducted by structurally related receptors containing a conserved “death domain” (DD) - Fas associated death domain (FADD). Overexpression of FADD causes apoptosis. FADD purified goat polyclonal antibody raised against a peptide mapping at the aminoterminus of FADD of mouse origin (x200, Santa Cruz) was used to detect FADD expression in first molar of the field vole (*Microtus agrestis*). Formalin-fixed, paraffin-embedded head of embryos were employed. Simultaneously, apoptotic cells were evaluated using morphological criteria after hematoxylin-eosin staining and TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) assay. FADD positive cells were also correlated with Fas receptor detected immunohistochemically by monoclonal mouse antibody (x200, Santa Cruz). Epithelial and mesenchymal cells from the first molar tooth germs were investigated at the stage of embryonic days 13.5-15.5 when so called primary enamel knot appears and its signaling function is gradually eliminated by apoptosis. FADD was found at all stages under study and strongly co-localized with Fas-receptor molecules, in particular in epithelial cells of the developing tooth germs.

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EFFECT OF ENDOTHELIN-1 ON MOTOR PERFORMANCE IN IMMATURE RATS

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The purpose was to examine the changes in motor ability after unilateral injection of endothelin-1 (ET-1) into immature rat hippocampus. Motor performance was studied using a battery of tests (negative geotaxis, wire mesh ascending, bar holding and rotarod test). Experiments were performed in Wistar 12-day-old (P 12 group) and 25-day-old rats (P 25 group) (the day of birth is defined as P0). ET-1 was infused under the general halothane anesthesia in a dose 20 pmol (0.5 μl) or 40pmol (1 μl) into the left dorsal hippocampus. Control siblings received an equal volume of vehicle instead of ET-1. Individual age and dose groups consisted from 9-10 rats. Starting 24hours after the surgery (until to 56 days), the rats were repeatedly exposed to the motor tests chosen according to level of maturation. The bar holding and rotarod tests were used in both age groups, whereas the negative geotaxis and wire mesh ascending only in P12 group. In P12 animals, 24 hours after administration of ET-1 (20 pmol) significantly worse performance in the wire mesh ascending and bar holding tests was observed. In contrast, P12 animals with a higher dose of ET-1 (40 pmol) were significantly poorer in mentioned tests only 3 days after administration. Interestingly, there were the changes in the bar holding test 6 and 56 days after administration of ET-1 (40 pmol). No differences between controls and experimental rats in other tests used (negative geotaxis and rotarod test) were found. In P25 animals, ET-1 (20 and 40 pmol) caused significant deficit in the bar holding test on the 13th day of testing. In the rotarod test, only 20 pmol dose of ET-1 caused the changes on the 1st and 13th day after insult. The results of the present study suggest that ET-1 hippocampal damage results in the significant changes in motor ability and this effect depends on the dose of ET-1 and age when insult is elicited.

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EFFECT OF N-ACETYLCYSTEINE ON TISSUE METALLO-PROTEINASES ACTIVITY IN RBL-2H3 MAST CELLS EXPOSED TO 24 HOURS OF "*IN VITRO*" HYPOXIA

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Exposure to chronic hypoxia results in hypoxic pulmonary hypertension characterized by structural remodeling of peripheral pulmonary vasculature (1). A release of collagenolytic enzymes - tissue metal1oproteinases (MMPs) from hypoxia-activated mast cells possibly plays an important role in this process (2). We hypothesized that mast cells activation is triggered by reactive oxygen species (ROS). Present study was designed to determine whether antioxidant N-acetylcysteine (NAC) attenuates the effect of hypoxia on MMPs activity in RBL-2H3 mast cells. The RBL-2H3 mast cells line were divided into four groups (50 000 cells per well were seeded): N, N+NAC, H, H+NAC (NAC: l mM N- acetylcysteine). Groups N and N+NAC were cultivated in normoxia (21 % 0_2, 5 % CO_2); groups H and H+NAC were exposed to 24 hours of *"in vitro"* hypoxia (10 % O_2, 5 % CO_2). The cultivation media and cells from 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 visualized by immunohistochemistry. Sets of 100 cells were examined. ANOVA Total MMPs activity in cultivation media was similar in all tested groups. The positive marked cells in each group were: N 8±1 (mean ± S.E.M.); N+NAC 6±1; H 36±7; H+NAC 21±7 ($p<0.003$). Our results showed that NAC decreased MMP-13 formation in RBL-2H3 mast cells exposed to hypoxia suggesting that ROS participate in the enhancement of MMP-13 formation during hypoxia.

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PLACENTAL METABOLISM OF CORTICOSTERONE AT MID- AND LATE GESTATION IN RATS

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The enzyme 11□-hydroxysteroid dehydrogenase (11HSD) catalyzes the interconversion of biologically active glucocorticoid corticosterone and its inactive derivative 11-dehydrocorticosterone. Placental 11HSD has been proposed to regulate the transplacental passage of maternal glucocorticoids to the fetus and the access of glucocorticoids to the placental corticosteroid receptors. We previously showed that the rat placenta expresses two isoforms of 11HSD (1). The isoform 2 (11HSD2) that catalyzes the oxidation of corticosterone to 11-dehydrocorticosterone and the isoform 1 (11HSD1) that operates in opposite direction, i.e. it synthesizes corticosterone via reduction of 11-dehydrocorticosterone. Variations in the activity 11HSD's have been related to fetal growth and development and subsequent development of hypertension in adult life. In the current study we have determined whether 11HSD1 and 11HSD2 change with advancing pregnancy. Quantitative real-time RT-PCR, bioactivity and immunohistochemistry of 11HSD1 and 11HSD2 were used to assess the expression of both isoforms of 11HSD in the placental days 15 and 21 of pregnancy. The transcripts of the isoforms were evident in both mid- and late gestation and their expression increased with gestational age. Reduction of corticosterone to 11-dehydrocorticosterone and oxidation of 11-dehydrocorticosterone to corticosterone were detectable in both stages but in contrast to the developmental patterns of 11HSD1 and 11HSD2 mRNA's the bioactivity did not increase during pregnancy. Developmental pattern of placental 11HSD2 were paralleled in changes by immunohistochemical staining of 11HSD2. These data suggest that the concomitant and marked developmental changes of placental 11HSD1 and 11HSD2 could have an influence on transplacental transfer of maternal glucocorticoids and on the regulation of placental function controlled by corticosteroids.

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CELL ADHESION AND PROLIFERATION ON THE DOPED POLYETHYLENE

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Oxidized cellulose is well known to enhance the wound healing process, including cell growth and repair of tissue functioning. Due to its biodegradability and antimicrobial properties, oxycellulose alone or in combination with other materials is considered to be ideal material for wound dressings (1). We focused on properties of polyethylene (PE) films doped with oxycellulose (*oxy*) and their interaction with cells. PE films with various *oxy*-concentrations (0-20 %) were tested by UV-VIS spectroscopy, X-ray analysis, IR spectroscopy and goniometer measurement. Sample weight changes during boil sterilization (1 hour at 100 °C) and drying (1 hour at 80 °C) were also investigated. Adhesion and proliferation of cells on this material *in vitro* were studied in 3T3 mouse fibroblasts and vascular smooth muscle cells (VSMC) (2, 3). We found that the weight of samples after boiling in water was rising proportionally to *oxy*-concentration. Simultaneously, their weight was partially reduced by *oxy* eliminated in water. The values of sample weight changes did not exceed 0.4 %. Sample weight decreased with increasing *oxy*-concentration during drying (up to 0.015 %), but after four hours they absorbed it again. UV-VIS radiation absorbance rose with increasing concentration of *oxy*. Wetting angle of doped films decreased slightly. Both film surfaces showed different supportiveness for the cell colonization. Cell adhesion was much better on the inner surface of the film. As revealed by scanning electron microscopy, it might be caused by a lower surface roughness. On samples with higher *oxy*-concentration, the cells agglomerated and their spreading area was smaller. X-ray diffraction showed differences between both surfaces and FTIR spectroscopy proved that outer surface contained much more crystalline phase than the inner one. 3T3 cells proliferated best on samples with lower *oxy*-concentrations. Number of adhering VSMC was approximately similar for all *oxy*-concentrations.

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QT DISPERSION ANALYSIS USING NUMERICAL MODEL

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With the respect to our recent findings of increased QT dispersion (QTd) in the group of pregnant women in very late stage of their pregnancy, possible role of changed geometrical relationship was studied, specifically whether the observed changes could be attributed to the pregnancy-related rotation and changed position of the heart. Two numerical models of the electrical field of the heart were used: elementary one represented by the field of time-variable dipole in homogenous volume conductor and finite element model. As the input data, the vectorcardiographic (VCG) signals obtained from pregnant women and control healthy volunteers were taken and the superficial electrocardiograms were reconstructed using the abovementioned models. The original VCG data of control subjects were then transformed in accordance with the changes, expected or found to occur due to pregnancy and the results were compared against the experimental findings obtained in the group of pregnant women. Based on the results of the verification and geometrical manipulations, we can conclude: Estimated QT dispersion is greatly influenced by geometrical relations between the orientation of a terminal vector of repolarization and directions of leads' axes of respective lead system. In cases of horizontal inclination of cardiac electrical axis, as compared to intermediary or more vertical inclinations, the increased voltage of electrocardiograms will be observed in most leads BSPM system. This will contribute to better identification of the end of T wave and thus QTd estimation differences. The change of electrical heart field in terms of rotation alone does not lead to substantial changes in observed QTd, if the dispersion is evaluated from multi-lead system. Conversely, more horizontal position could rather result in better identification of T wave offset, which usually implies lower QTd. The dispersion observed in the group of pregnant women is thus unlikely to be caused principally by the changes in geometry. As likely reasons for observed increased dispersion remain changes in T-loop morphology, specifically increased T wave width that was found in this group. Also, the contribution of nondipolar sources remains unexplored and could play some role. Our results support the hypothesis, that considerable portion of the QT dispersion is a consequence of geometrical relations and as such is subject of inheritably numerous possible estimation errors due to not standardized methodology.

EFFECT OF BISPHENOL A AND BISPHENOL A DIMETHACRYLATE ON STEROID HORMONE PRODUCTION BY PORCINE GRANULOSA CELLS

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Bisphenol A (BPA) and bisphenol A dimethacrylate (BPADi) are chemical compounds used as plasticizers in the manufacture of polycarbonate plastics, and can be leached out of food or medical product covers. Exhibiting weak estrogenic activity, BPA is considered to be an endocrine disruptor. The present study was undertaken to investigate whether BPA and BPADi might induce any changes in steroidogenesis in cultured granulosa cells (GC) isolated from porcine follicles (6-8 mm). After 72 h incubation of porcine GC with tested agents, progesterone (P_4) and estradiol (E_2) levels in cultured media were measure by standard radioimmunoassay. Both tested chemicals significantly suppressed basal as well as FSH and LH-stimulated P_4 production by GC at 10^{-4} mol.l^{-1} concentration. Basal P_4 levels produced by GC were only slightly altered by the action of lower concentrations (10^{-8}-10^{-5} mol.l^{-1}) of the agents. More dramatic effects of the tested compounds were observed on gonadotropin-induced P_4 production by GC. BPA increased FSH-stimulated P_4 levels produced by GC at 10^{-7} and 10^{-6} mol.l^{-1} concentrations, but had no effect on LH-induced P_4 production at these concentrations. BPADi suppressed both FSH- and LH-induced P_4 production by GC in a concentration dependent manner. BPA as well as BPADi (10^{-6}-10^{-4} mol.l^{-1}) decreased FSH-induced E_2 levels secreted by CG to cultured media. The results indicate that the action of BPA and BPADi could interfere with FSH signaling pathway in the stimulation of P_4 production by cultured porcine GC and might induce changes in E_2 synthesis by GC. The impact of BPA on apoptosis in human epidermal carcinoma cells A431 was also investigated. Apoptosis was measured on the level of cell membrane permeability (release of lactate dehydrogenase, LDH). The cells were cultured with BPA (1-100 ng.ml^{-1}) for 16, 24, 48 and 72 h. LDH assay demonstrated that low concentrations exhibit the effect at shorter time of exposure, while longer exposure (24 and 72 h) shift the response to higher concentrations (15 and 25 ng.ml^{-1}, respectively).

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EFFECT OF DISULFIRAM (DS) TREATMENT ON INSULIN IN RAT PANCREAS

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Insulin secreting pancreatic β-cells contain also TRH (1). Although the physiological role of TRH in β-cells remains obscure, its participation in glucoregulation has been hypothesized. In attempt to clarify the role of TRH we blocked posttranslational maturation of TRH molecule by the disulfiram (DS, an inhibitor of peptide alpha-amidation) (2). DS treatment (200 mg/kg/day) *in vivo* during five days resulted in disrupted insulin secretion mechanism. Isolated pancreatic islets from DS rats failed to secrete insulin in response to high level of glucose (16.7 mM) and exhibited abnormally high basal insulin secretion. These defects were accompanied with accumulation of insulin in islets. Both the glucose stimulated insulin secretion and basal secretion from Langerhans islets of DS rats were restored to normal levels after adding of TRH (1 nM) *in vitro*. Our observations demonstrated participation of TRH in the mechanism of glucose induced insulin secretion.

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PROLIFERATION IN THE RAT DENTATE GYRUS IS SUPPRESSED BY WATER MAZE TRAINING AND ENHANCED BY ANTIDEPRESSANT TREATMENT

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Neurogenesis in adulthood in the dentate gyrus of the hippocampus is a generally accepted phenomenon. Proliferation rates decrease during aging and after chronic stress (1). Physical activity or treatment with fluoxetine increases the rate of proliferation (1,2). We investigated the effect of physical and cognitive stimulation in the Morris water maze (MWM) and its potentiation by the antidepressant fluoxetine. Male Wistar rats 3 months old (n=20) were exposed for 15 days to one of the following treatments: daily i.p. injection of fluoxetine (group F), learning in modified acquisition trials in the MWM (group WM), daily injections of fluoxetine and training in the water maze (group FWM), the control group (group C). During day 15, all rats received three i.p. injections of BrdU 8 hours apart and were sacrificed 24 hours after the first BrdU injection. Every sixth section containing the hippocampus (6 sections per animal) was immunostained with anti-BrdU antibody, and the positive cells were counted in the granular cell layer of the dentate gyrus. The mean number of positive cells per each hemisphere in section was calculated for each animal. Fluoxetine treatment increased the rate of proliferation by 26 %. Exposure to the MWM suppressed proliferation in the granular layer by 27 %. The addition of fluoxetine reversed the suppressive effect of water maze exposure on proliferation to a level that was 12 % higher than that of the control group. We conclude that exposure to water maze stimulation suppresses the rate of neurogenesis, presumably due to the stress. Treatment with fluoxetine reversed the effect of stress during the water maze training and increased the rate of proliferation in the granular cell layer of the hippocampus.

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CONFOCAL MICROSCOPY AND STEREOLOGICAL ANALYSIS AS A USEFUL TOOL FOR ASSESSMENT OF THE VOLUMETRIC PROPORTION OF RENAL COMPONENTS IN THE CHICK MESONEPHROS

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Confocal microscopy is special type of microscopy technique that enables capturing of thin optical sections from a thick specimen. Digital images acquired by this technique represent suitable data for quantitative measurement (1,2) and can be used in many fields of cell biology and physiology. Stereological methods are based on evaluation of the structure of three-dimensional (3D) objects performed on 2D sections or 3D subsamples. Different test probes (*e.g.* grid of points, linear or planar probes), nowadays usually generated by a special software, can be applied for estimating different structure parameters. To assess the volumetric proportion of renal components in 10-day-old chick mesonephros we applied the method of spatial grid of points (2). Our previous results showed a high sensitivity of mesonephros to the action of 1,2-dibromoethane (DBE) related probably to the damage to vascular system (3). To verify this assumption we have decided to estimate the volumetric proportion of peritubular vessels and tubules in control and experimental embryos administered intraamniotically on embryonic day 3 through window in the shell (4) with 300 μg of DBE. The preliminary results from stereological analysis suggested significant reduction of peritubular vessels after 300 μg of DBE (round LD50), while the tubular volume was comparable to controls. In conclusion, the method of spatial grid of points enabled assessment of the volumetric proportion of renal components and thus objective evaluation of differences in the developing organ of exposed and control embryos.

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IS PROTEIN KINASE C IMPORTANT FOR CARDIO-PROTECTION CONFERRED BY ADAPTATION TO CHRONIC HYPOXIA IN RATS?

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It has been shown that long-term adaptation of rats to intermittent high altitude (IHA) hypoxia increases the expression of protein kinase C (PKC), mainly its δ isoform, in particulate fractions of ventricular myocardium (1). IHA hypoxia also increases cardiac tolerance to subsequent acute ischemic injury and PKC may be implicated in this process. We examined the potential role of PKC in the mechanism of protection. Adult male Wistar rats were exposed to IHA hypoxia of 7000 m in a barochamber for 8 h/day, 5 days/week; the total number of exposures was 24-32. A control group was kept under normoxic condition for the same period of time. Infarct size (tetrazolium staining) was determined in open-chest animals subjected to 20-min LAD coronary artery occlusion and 3-h reperfusion. Either chelerythrine (1 mg/kg), a non-selective PKC inhibitor, or rottlerin (0.3 mg/kg), a specific PKC δ isoform inhibitor, were administered into the jugular vein as a single bolus 15 min before ischemia. IHA hypoxia decreased the size of myocardial infarction (normalized to the area at risk) to 41.2±3.9 % as compared with 58.2±2.2 % in controls ($P<0.05$). Chelerythrine resulted in a slight but significant reduction of infarction in controls ($P<0.05$) but it did not affect the improvement of ischemic tolerance in hypoxic hearts. Rottlerin attenuated the infarct size-limiting effect of IHA hypoxia ($P<0.05$) but it did not influence infarction in controls. These results suggest that chronic IHA hypoxia-induced cardioprotection in rats is partially mediated by PKC δ; however, the role of other isoforms is obscure.

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THE DETERMINATION OF ANTIBODIES AGAINST CARCINOMA MAMMAE AND LDH VIRUS BY MODIFIED ELISA METHOD

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The present work continues in the testing of fractions obtained by means of high-pressure gel chromatography (HPGC) from the human malignant breast tumor and the blood of inbred C3H/H2K strain mice infected by a mice LDH virus (LDV) (1) as antigens in the ELISA method modified by us (2,3). The procedure is suitable for early diagnosing and monitoring antibodies in a malignant breast tumor simultaneously with senological examinations which include mammography and clinical examinations (4). We determined a titer of total antibodies in blood of 178 women patients with a various degree of a non-malignant disease of the breast and in 355 samples of blood in women patients with a malignant breast tumor. The examination was extended by us onto determination of a titer of common specific antibodies and titer of IgG and IgM class antibodies in all samples tested. The titer of antibodies was determined against the specific antigen prepared from the malignant breast tumor and against the non-specific antigen prepared from the mice LDV. Based on the knowledge regarding a protective influence of sexual hormones on the immunological state of the organism, which decreases with an increasing age, the set of women with a non-malignant breast disease was divided in two groups, with the criterion being the threshold of 35 years of age. We compared obtained results with a group of 74 blood samples taken from Blood Donor Center, examined by antigen prepared from human malignant breast tumor and to that obtained from blood of C3H/H2K strain inbred mice infected with the mice LDV. We conclude, that the strict selection of "healthy" blood donors must be provided without rather viral infections for better quality of control group.

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INTERACTION OF VASCULAR SMOOTH MUSCLE CELLS WITH OXYCELLULOSE-BASED COMPOSITE MATERIALS PERSPECTIVE FOR TISSUE ENGINEERING

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One of the emerging problems in transplantation medicine is shortage of autologous or appropriate heterologous donor tissue. Therefore, the search for new synthetic materials for construction of bio-artificial tissues and organ replacement is always rational. In the present study, we evaluated the adhesion, subsequent growth and potential immunoattractiveness of rat aortic smooth muscle cells (RASMC) in cultures on a composite material consisting of polyethylene enriched with 1 % to 20 % of oxycellulose, prepared in the Research Institute for Organic Syntheses, Pardubice. Oxycellulose has been widely used in health care as a bioresorbable compound with haemostatic, wound healing, immunostimulatory, reparative and bacteriostatic effects (1). We found that when embedded in polyethylene in order to improve its mechanical properties, it allowed adhesion of RASMC. However, the number of initially adhered cells decreased with higher oxycellulose concentrations. On the materials with 15 % and 20 % of oxycellulose, it was 39 % and 32 % of the control value on the pure polyethylene. On day 3 after seeding, the differences in cell numbers on samples with different oxycellulose concentrations almost disappeared, but were still significantly lower than on the control sample. The cell spreading area was usually smaller (about 40-80 % of the control value) on the oxycellulose-enriched polyethylene. The cells were often rounded and clustered in aggregates. The lower cell spreading was probably due to an increase of the material surface roughness at higher oxycellulose concentrations. The potential immunogenicity of the cell-material construct was evaluated by concentration of immunoglobulin adhesion molecules. Expression of ICAM-1 and VCAM-1 was comparable with the control sample or even lower. Nevertheless, these results indicate that the polyethylene-oxycellulose composite material could be considered as biocompatible and applicable for future use in tissue engineering, however, improvement of mechanical properties of this material would be advantageous.

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BAROREFLEX SENSITIVITY IN CHILDREN AND ADOLESCENTS AFTER ANTITUMOUR THERAPY WITH RESPECT TO FATTY ACID METABOLISM

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The successful therapy by new antitumor drugs increased number of patients with side effects after this treatment (cardio- and neurotoxicity). Anthracyclines are suggested to cause cardiomyopathy because of interference with fatty acid metabolism and they also interact with the autonomous nervous system (ANS). The study aimed to evaluating relationship between plasma lipids: cholesterol, HDL, LDL, triglycerides (TG) and body characteristics (height, weight, body mass index-BMI) and circulatory parameters: pulse intervals (PI), systolic and diastolic blood pressures (SBP, DBP) and their variability, and baroreflex sensitivity (BRS) in healthy subjects and patients treated previously for malignant tumors. In our department 303 children and adolescents (11-21 years) were examined: 206 healthy controls (C) and 97 young patients after cardiotoxic therapy (T). SBP and DBP and PI were recorded continuously for 5 min. BRS was determined by a spectral method. Circulatory parameters variability was estimated as standard deviations: PIsd, SBPsd and DBPsd. Laboratory results of plasma lipids were used. Group T was divided into two subgroups: 84 subjects with cholesterol <5mmol/l (T_L) and 13 subjects (13 % of group T) with cholesterol >5 mmol/l (T_H). These subgroups were also tested for age of 11-15 and 16-21 years. Group T patients had higher BMI ($p<0.01$), prolongation of PI ($p<0.01$), increased PIsd ($p<0.05$), decreased SBP ($p<0.001$), DBP ($p<0.001$) and DBPsd ($p<0.05$), and lower BRS ($p<0.01$) in comparison with group C. We found the shorter PI in group T_H than T_L ($p<0.05$). Comparing PI of C vs. T_L or T_H, it was markedly prolonged in T_L ($p<0.01$). In age group 16-21 years T_H had higher BMI ($p<0.01$), lower PI ($p<0.01$), SBP ($p<0.01$) and SBPsd ($p<0.05$) than C, and T_L had lower SBP ($p<0.01$), lower BRS ($p<0.01$) and higher PIsd ($p<0.01$) than C. Concluding, longer mean PI, lower BRS and decreased SBP and DBP in patients after antitumor therapy are signs of increased parasympathetic and decreased sympathetic tonic activity. Increased variability of PI could be explained by higher fluctuation of parasympathetic tone. Even BMI increases in T_H subjects; their blood pressure is persistently lowered. The development of ANS tone seems to be missing in the older population of subjects after cardiotoxic therapy.

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EFFECT OF RESTRAINT STRESS AND AMPHETAMINE ON IN SITU DETECTED cAMP-PDE ACTIVITY IN MYOCARDIUM OF LEWIS AND SPRAGUE-DAWLEY RATS

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Cyclic AMP levels in the heart are associated with its synthesis by adenylate cyclase and its degradation by phosphodiesterase (PDE). We demonstrate the ultrastructural in situ cAMP-PDE distribution and activity in the myocardium of Sprague-Dawley (S-D) and Lewis (LE) rats after their exposure to restraint stress or an acute dose of amphetamine. S-D and Lewis LE rats, the latter known to have a deficient hypothalamo-pituitary-adrenal axis activity, were used in order to disclose the possible significance of rat strain on PDE activity. Animals were divided into 3 groups: controls, rats treated with an acute dose of amphetamine (8 mg/kg, i.p., for 60 min) and rats under restraint stress for 60 min. Control hearts of both strains revealed PDE activity on the sarcolemma of cardiomyocytes and plasmalemma of endothelial cells of microvessels. In LE rats we observed an additional enzyme reaction in junctional sarcoplasmic reticulum. In addition, cardiomyocytes of LE rats revealed a higher PDE activity when compared to S-D rats. Restraint stress decreased PDE activity in cardiomyocytes of LE rats while amphetamine markedly inhibited enzyme activity in cardiomyocytes of S-D rats. Endothelial PDE was more resistant to stress. Differences in myocardial PDE localization and activity in cardiomyocytes of LE and S-D rats might indicate different degradation of cAMP and heart function in genetic various strains. The results also support different myocardial vulnerability to pathophysiological conditions and the heart contractile responses of the particular rat strains to the cAMP-PDE inhibitors.

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QUANTITATIVE HISTOCHEMICAL CHANGES IN CYTOCHROME OXIDASE IN THE PIRIFORM CORTEX AFTER STATUS EPILEPTICUS IN ADULT RATS

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The piriform cortex is activated during an early phase of experimental temporal seizures as described by MRI studies (2). It indicates that the early activity of the piriform cortex should be accompanied by increased ATP production. Furthermore, degenerating neurons demonstrated by FluoroJade B staining are visible early after status epilepticus as well as after longer intervals. Cytochrome oxidase as the terminal enzyme in the electron transport chain in mitochondria catalyses the transfer of electrons from its reduced substrate to molecular oxygen to form water. An important aspect of this reaction is the generation of ATP via the coupled process of oxidative phosphorylation. Cytochrome oxidase activity in the brain may be used as an endogenous metabolic marker for neurons because glial contribution is minimal. A total of 21 adult male Wistar rats formed experimental and control group. SE was induced by a single dose of pilocarpine (40 mg/kg) in LiCl-pretreated animals. Animals were transcardially perfused with 0.5 % glutaraldehyde and 10 % sucrose in 0.1 M PBS (pH 7.6) one week or three months after the SE. Cryocut 20 μm thick coronal sections were mounted onto gelatin coated glass slides. Series of nine sections were made. Every second and third sections were used for cytochrome oxidase histochemistry. Staining was performed according to Bilger and Nehlig (1). Analysis of optical density was performed with image analysis software under stereomicroscope. After subtraction of the background the optical density of the piriform cortex was measured. Because of the nonhomogeneity of the piriform cortex optical density was measured separately in its anterior and posterior part. Posterior part was identified by an absence of the laterally olfactory tract. Optical density of the anterior part of the piriform cortex remained nearly unchanged at both 1 week (99 % of control) and 3 months (96 % of control) poststatus intervals. Posterior part of the piriform cortex showed a decrease of optical density in both groups: to 81 % and 83 % of control values one week and 3 months after SE, respectively. Our results demonstrated that damage pf the piriform cortex is not homogenous and thus that their parts are differently involved in epileptic activity.

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BIORESORBABLE POROUS SCAFFOLDS FOR TISSUE ENGINEERING

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The aim of tissue engineering is to construct biological tissues *in vitro* or regenerate tissues *in vivo*, preferably by using autologous cells. Thus, tissue engineering requires biomaterials for scaffolding the cells for their differentiation, proliferation and creation of extracellular matrix. The most important features of ideal scaffolds seem to be: very good biocompatibility, bioresorbability, adequate degradation kinetics, three-dimensional (3D) open porous microstructure and thoroughly controlled surface properties (physical and chemical). Among all resorbable biomaterials, copolymers and terpolymers of L-lactide, glycolide and ε-caprolactone appear to be the most attractive for tissue engineering applications. Such materials are regarded as biocompatible, however their synthesis is usually carried out with highly toxic tin compounds as initiators. Therefore, to improve the biocompatibility it was recently shown, that it is possible to carry out the synthesis process with the use of, so called bio-elements, e.g. calcium, iron, zinc and zirconium compounds. In this study it is reported a method of preparation of porous 3-D scaffolds from two copolymers of glycolide and L-lactide (PGLA) and glycolide and ε-caprolactone (PGCap) and one terpolymer of glycolide, L-lactide and ε-caprolactone (PGLCap), synthesised with the use of zirconium acetylacetonate. The scaffolds, produced by solvent casting – particulate leaching, were characterised by X-ray photoelectron spectroscopy, scanning electron microscopy and infrared spectroscopy (FTIR). Porosity of the scaffolds was also measured. The scaffolds were submitted to degradation in pH 7.2 phosphate-buffered saline (PBS) at 37 ^{o}C for 22 weeks. The degradation was monitored by mass change, viscosity, gel permeation chromatography as a function of incubation time. Selected materials were studied with fibroblasts and osteoblasts *in vitro* and implanted into experimental animals. Obtained results show that it is possible to produce a wide variety of biocompatible scaffolds, possessing high porosity (about 90 %vol), different size of pores (600, 200 and 40 μm, respectively) and different degradation kinetics. Degradation time decreased according to the sequence: PGLA – PGCap and PGLCap indicating that the polymer composition is a key factor in the degradation of porous scaffolds.

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THE EFFECT OF SERUM GONADOTROPIN ON CATECHOLAMINE LEVELS IN THE HYPOTHALAMUS OF EWES

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Hypothalamic catecholamines participate in the regulation of gonadotropin hormone secretion in the estrous cycle of animals (1,2). In the current science there is a lot of information about the function of monoamines related to regulated reproduction. The effect of hormonal preparations used for inducing superovulation in farm animals on catecholaminergic system of hypothalamus and hypophysis is not fully understood. The effect of serum gonadotropin (2000 IU SG) superovulation hormonal preparation were investigated on catecholamine levels (norepinephrine, dopamine and epinephrine) in the hypothalamus of ewes with synchronized estrus in period by radioenzymatic methods. Changes in catecholamine concentrations were determined in the corpus mamillare, eminentia medialis and area preoptica of ewes. The study was performed with 28 ewes of the Slovak merino breed during the estrous period. The estrus was synchronized by Agelin vaginal tampons. The administration of 2000 IU SG resulted in significant decrease ($p<0.05$) concentration of epinephrine as compared to control group with synchronized estrus in the area preoptica. Hormonal stimulation with SG decreased the levels of hypothalamic dopamine in the areas studied and these differences were significant in the eminentia mediana ($p<0.001$) and corpus mamillare ($p<0.01$). These changes are supposed to be connected with an increase estrogens alternation after administration of PMSG by means of a feedback mechanism.

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BIOLOGICAL HALF-LIVES OF BROMIDE AND SODIUM IN THE RAT ARE RELATED AND DEPEND ON THE PHYSIOLOGICAL STATE

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Bromide has often been used as inert indicator for the closely related chloride in connection with physiological kinetic investigations. The biological half-life of bromide can be decreased by administering surplus halide (e.g. chloride) ions. On the other hand, the already long half-life of bromide may be increased considerably by a salt-deficient diet [1]. These findings were interpreted in the literature as a marked dependence of the biological half-life of bromide on chloride concentration in the diet. However, keeping in mind the differences between the metabolism of sodium and chloride, we hypothesized that the biological half-life of bromide depends on the magnitude of sodium intake rather than on the intake of chloride [2]. Here, we demonstrated the parallel course of the excretion rates of sodium and bromide ions in adult male rats administered simultaneously with ^{24}Na-sodium chloride and ^{82}Br-bromide. These excretion rates were inversely proportional to the magnitude of sodium intake in the animals. In addition, we investigated the biological half-life of bromide, as substituting for sodium or chloride, in lactating and non-lactating female rats as well as in young rats of varying ages (2, 4, 6, and 10 weeks of age). The radioactivity retained in mothers and in whole litters was measured *in vivo* at appropriate time intervals (up to 240 h) after the application of ^{82}Br-bromide to the mothers. The time course of the changes in the ^{82}Br radioactivity of the young was calculated as the difference between the rate of ^{82}Br intake in mother's milk and the ^{82}Br excretion through the kidneys into the urine. Non-weaned young rats (12 d) had the longest half-life (269 h) and lactating dams the shortest (44 h). The determined values demonstrated that non-weaned young apparently conserve sodium, while lactating dams, due to their large food intake, waste sodium.

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EFFECTS OF EXCESSIVE BROMIDE ON THE METABOLISM OF IODINE IN THE RAT

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There is no evidence in mammals of bromide accumulation in any particular organ that might indicate a specific physiological function of this ion. On the other hand, considering the chemical similarity of bromine to iodine, goitrogenic effects of bromide cannot be excluded. Indeed, we have shown recently that excessive bromide in the adult male and female rats affected in several ways their metabolism of iodine (1). An enhanced bromide intake markedly reduced accumulation of iodide in the thyroid (2) as well as in the skin (3). Bromide also increased the excretion of iodide by the kidneys, changed the time-course of iodine elimination from the body and in this way significantly shortened the biological half-life of iodine in the rat (4). In the present paper, we examined some of the possible reasons for the observed adverse effects of a high bromide intake in lactating rat dams on their performance in the course of nursing period and on the prosperity of their young. In the dams, there were two distinct consequences undoubtedly caused by high bromide intake: stagnation in the extent of diet and water consumption in the course of lactation period, and a drop in the production rate of mother's milk. Very high intake of bromide in the mothers (about 220 mg Br per day per dam) caused a marked decrease in the body weight increments in their pups. Only about one-half of these sucklings survived and their general condition was very poor. Moreover, excess bromide in the mothers significantly depressed the extent of iodine transfer from the dams through mother's milk to the sucklings. It was also proved that bromide similarly to iodide readily penetrated into the rat milk and via mother's milk was transferred in a large extent into the body of suckling young.

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STRESS: DO THEY AFFORD ROLE OF INDUCTION AND NUCLEAR TRANSLOCATION OF MAJOR HEAT SHOCK PROTEINS IN THE RAT BRAIN FOLLOWING HEAT, PROTEOTOXIC AND EXCITOTOXIC NEURONAL PROTECTION?

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Heat shock proteins (HSP) - a major subgroup of stress proteins - are characterized as tools of cellular defense against heat stress, ischemia, trauma and toxicity. Originally considered only as protection against the stress-induced protein denaturation, most of them is now identified as molecular chaperones. Recently, they were recognized to be specifically involved in processes of cell death and survival. However, most brain neurons were reported not to induce HSP70 at all (heat stress) or to induce it later than glial cells and vessels (ischemia and trauma). The protective effect of large amounts of constitutive chaperones HSC70 and HSP90 in neuronal cytoplasm remains to be established, especially as their nuclear transfer seems to be dampened. In order to elucidate the relationship between HSP and neuron protection *in vivo*, we compared fate of induced and constitutive chaperones in neurons and glial cells following hyperthermia (HS), proteotoxic (PTS) and excitotoxic stress (ETS) in the rat. HSC70/HSP70 expression was evaluated following 30 min of hyperthermia (HS) or intraventricular injection of arsenite (PTS) in anesthetized rats (urethane, 1.5 g/kg). ETS was induced by kainic acid (10 mg/kg, i.p.). HSP were detected in vibratome or antigen-retrieved paraffin sections with an ABC technique. In HS and PTS, HSP70 was induced in glial cells (mostly oligodendrocytes), vessels and a few discrete neuronal groups, but not in astrocytes. The induced HSP70 translocated immediately to glial nuclei and relocated to cytoplasm between 8-24 h. HSC70 remained localized mainly in neuronal cytoplasm, unaffected by HS and PTS. However, we did not see neuronal cell death in HS and PTS, while there was pronounced microglial activation and cell death in PTS. In contrast to HS and PTS, ETS treatment induced HSP70 mainly in cortical and hippocampal neurons. Earliest detection was seen by 6 hours postinjection in only few neurons and it culminated at 24 hours in many CA 1, 3, and hilar neurons. Despite that, many hippocampal neurons containing HSC70 and expressing HSP70 died at 48 hours. However, HS rescued most of these neurons if HS preceded ETS induction by 24 h. If HSP70 is involved in this heat-induced neuronal rescue, then it may indicate the major role for glial HSP70.

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CHANGES IN OXYGEN KINETICS OF CYTOCHROME *C* OXIDASE IN LEIGH SYNDROME CAUSED BY *SURF1* MUTATIONS

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The oxygen partial pressure (pO_2) drops from 20 kPa in the inspired air to a level of less than 1 kPa in some tissues. In these physiologically hypoxic conditions a sufficient rate of cellular respiration is maintained due to the remarkable oxygen affinity of cytochrome *c* oxidase (COX) (1). Here we present the results of a first study aiming to determine, how the cells with genetic defect of COX react with oxygen at low pO_2. We analyzed the COX oxygen affinity in a neurological disorder Leigh syndrome caused by mutations in the *SURF1* gene – the most frequent COX deficiency in infancy. These mutations prevent synthesis of the Surf1 protein necessary for the assembly of COX and result in severe COX deficiency (2) associated with changes in enzyme structure and both electron- and proton-transport properties (3). The oxygen kinetics was evaluated in cultured fibroblasts harboring *SURF1* mutations using high-resolution respirometry and expressed as p_{50} (pO_2 at half-maximal respiration rate). The measurements were performed in two experimental settings – respiration of intact coupled cells with endogenous substrates, and unrestricted oxidation of exogenous succinate in digitonin-permeabilized cells after FCCP uncoupling. In intact cells, the p_{50} in patient fibroblasts was 2.3-fold elevated in comparison with control fibroblasts (0.0392 to 0.0886 kPa). Under the conditions of maximal respiratory rate, the increase was 3.6-fold (0.0323 to 0.1148 kPa). We hypothesize that the depressed oxygen affinity may lead to limitations of respiratory rate in tissue hypoxia resulting in impaired energy provision, and thus contribute to severe pathophysiological state of patients suffering from Leigh syndrome.

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INSULIN RESISTANCE IN NON-OBESE PATIENTS WITH EARLY AND ESTABLISHED PRIMARY HYPERTENSION

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Primary hypertension (PH) and insulin resistance (IR) are multifactorial diseases and the penetration of genotype critically depends on similar non-genetic risk factors (e.g. life style, nutrition, physical activity, smoking, environment, age). It is well-established fact that IR may contribute to development of PH in obese subjects. However the presence of IR in patients with PH as well as its possible contribution to the development of PH is not clear in non-obese ones. In the present study we evaluated glucose homeostasis in non-obese patients with early primary hypertension (HT1) and in patients with established hypertension (HT2) in comparison to healthy control subjects. Plasma glucose, insulin and C-peptide concentration were measured in plasma after intravenous glucose load. We did not find any significant difference in fasting glucose concentrations in both HT1 and HT2 as well as in insulin and C-peptide response to intravenous glucose load in HT2 patients compared to controls. Fasting plasma insulin and C-peptide were significantly ($p<0.01$) higher in HT1 and HT2 groups when compared to controls. Response of insulin and C-peptide were significantly higher in HT1 compared to controls ($p<0.01$). Insulin resistance index IR HOMA was higher ($p<0.05$) in both HT1 and HT2 patients when compared to healthy controls. The presence of IR in non-obese hypertensive patients may suggest the development of metabolic syndrome. More attention should be paid to detection of IR in clinical practice.

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PROBIOTICS AND THEIR EFFECTS ON THE SELECTED METABOLIC PARAMETERS IN SMALL CHILDREN

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Probiotics are bioactive substances or functional edibles containing microorganisms in sufficient amount, which after their implantation or colonization change the host microflora. This fact enables them to manifest their health beneficial effects. During last decade in the field of probiotics there has been a considerable scientific progress in selection and characterization of specific probiotic cultures and their effects on health. The aim of the study is to obtain new knowledge on the mechanism and effect on the molecular and cellular levels. We have been investigating whether the selected probiotics will influence the metabolism of small children, namely in the composition of proteins, lipids, immunological profile and antioxidants. As probiotic organisms are used, above all but not solely, the gram positive bacteria, belonging to two genera Lactobacillus and Bifidobacterium. Ten children at the age of 1-3 years were administered milk with probiotic cultures enriched by *Streptococcus termophilus* and *Lactobacillus bifidus*. The control group consisted of ten children at the same age, who were in the same institution with identical social and boarding regimen, but they got milk without probiotics. The results were compared at the beginning of administration, at the end of the third month of application, and a month after discontinuing probiotics. A significant decrease in the plasma MDA ($p<0.01$) was found due to the effect of probiotics. The values of TAS exhibited statistical significance ($p<0.001$) at comparison between the individual months of probiotics application. The values of catalase significantly decreased ($p<0.001$) at comparison between months 1 and 4. Beneficial, but insignificant effect was found in the concentrations of IgG, M, A, and PAF. In the children nourished with milk containing probiotics, an increase in the serum concentration of HDL and a decrease in the serum concentration of LDL cholesterol and thrombocytes were recorded. On the basis of our results we consider the application of probiotics as an important component of healthy nutrition and we expect that probiotics can be consumed not only by adults but also by children as a mean for prevention of certain diseases and modulation of the host immunity.

SELECTIVITY OF NEURONAL DEATH IN CONDITIONS OF FUNCTIONAL OVERLOAD

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Death of selected populations of hippocampal neurons can be both the result of seizure activity and of the possible direct opening of Ca^{2+} channels. To understand better the mechanism of neuronal vulnerability, effect of electrical stimulation and that of the administration of excitatory and neurotoxic drugs on the incidence of dying neurons in different hippocampal fields was studied. Animals were allowed to survive 2 days after the experimental elicitation of seizures by drugs or electrical stimulation. Perfusions fixed brains were processed for DNA staining (Hoechst) in combination with Fluoro-Jade to differentiate surviving and dying cells. Material was evaluated and photographed under OLYMPUS Provis fluorescence microscope. High number of Fluoro-Jade positive cells (dying cells) with the typical distribution within hippocampal region was seen after the kainate administration. Only few positive cells were identified after the NMDA administration. All other experimental interventions did not have any detectable effect. Some chemically induced seizures have the tendency to repeat. Regularly it happens after the kainate administration, no repetition was observed after the electrically and Flurotyl induced seizures. Morphological signs of neuronal loss were detected only after those stimuli, which were mediated by the glutamate receptors. The principal role of balance between the activation of NMDA and that of non-NMDA receptors induced by the individual agonists, should be therefore considered. If the balance is preserved, neurons appear to be more resistant.

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CORRELATION BETWEEN CALCIUM SIGNALS AND RELEASE-DEPENDENT INACTIVATION OF CALCIUM CURRENT IN RAT VENTRICULAR MYOCYTES

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The L-type calcium channel current (I_{Ca}) in rat ventricular myocytes has a fast component of inactivation dependent on calcium release from the sarcoplasmic reticulum. Here we studied the relationship between activation of calcium release by brief depolarizing prepulses and the consequent release-dependent inactivation (RDI) of calcium current. Calcium currents isolated from other membrane currents were measured in isolated rat ventricular myocytes using the whole-cell patch clamp technique under conditions supporting full phosphorylation of calcium channels. Calcium release was measured as spatially resolved calcium transients using confocal microscopy and the calcium indicator fluo-4. Prepulses from a holding potential of –50 mV to positive voltages, lasting 2-12 ms, induced calcium release. At the same time, they induced inactivation of the test peak I_{Ca} and decreased the extent of calcium release during the test pulse to 0 mV that commenced 10 ms after the prepulse. The extent of prepulse-induced calcium release and of I_{Ca} inactivation increased with the amplitude and duration of the prepulse. Calcium release evoked by the prepulse began shortly after the end of the prepulse, while calcium release evoked by the test pulse started around the peak of test I_{Ca}. The prepulse-evoked decrease of calcium release during the test pulse was manifested as a decrease in the number of activated release sites and as an increase in the temporal dispersion of release. We conclude that direct measurements of spatially resolved calcium release confirm our previous observations that the relative potencies of Ca^{2+} ions to trigger calcium release and I_{Ca} inactivation depend on the previous history of calcium influx (1). Additionally, when calcium current was partially inactivated, the decreased extent of calcium release was accompanied by a decrease in calcium release synchronization (2).

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PRENATAL METHAMPHETAMINE EXPOSURE ALTERS POSTNATAL DEVELOPMENT OF RAT PUPS

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There are studies showing that drug abuse during pregnancy may have a long-term effect on progeny of drug-abusing mothers. Methamphetamine (MA) is one of the most common illicit drugs throughout the world. The purpose of the present study was to assess the effect of prenatal MA exposure on postnatal development of rat pups before the time of separation from their mothers. Female rats were injected with MA (5 mg/kg daily) for the duration of their pregnancy. Pups were then tested throughout the lactation period. They were weighed daily and the ano-genital distance was measured on postnatal day (PD) 1. Development of postural motor reaction was tested by righting reflex on surface between PD 1-12, and righting reflex in mid-air after PD 12 until successfully accomplished. On PD 23 sensori-motor coordination was examined using the rotarod test. Additionally, the markers of physical maturation, such as eye opening, testes descent in males and vaginal opening in females were also recorded. We demonstrated that pups exposed prenatally to MA are slower in righting reflex on surface and that their physical and functional development is retarded. Additionally, the sensori-motor coordination was impaired in prenatally MA-exposed pups when testing in the rotarod test. However, there were no changes induced by prenatal MA expose in weight gain or in sexual maturation. There were no sex-dependent alterations in any measures. Thus, the present study demonstrates that prenatal MA exposure impairs functional development of rat pups during the first three weeks after birth.

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ALLOSTERIC MODULATION OF MUSCARINIC ACETYLCHOLINE RECEPTORS: A MATHEMATICAL FORMALIZATION

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Muscarinic receptors belong to a large family of G protein-coupled receptors (1). Allosteric sites on muscarinic acetylcholine receptors represent novel drug targets. 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 rate of association and rate of dissociation. There are seeming paradoxes: an increasing concentration of the positive modulator slows gradually more even the association rate for the competitive antagonist N-methylscopolamine at M2 subtype of muscarinic receptor. Similarly, negative modulator slows the dissociation rate in a concentration-dependent fashion. To overcome the problem we hypothesized that a compulsory order of ligand binding proceeded (4). The cyclic kinetic scheme is used to describe the ternary complex model (TCM). Up to now, there is lack of studies, which would analyze the suggested model with respect to the qualitative behavior. The formalism of linear algebra proved useful for comparative analyses of these two reaction systems. The number of independent reactions equals 3 in the four-state TCM. Using Laplace transformations on the system of differential equations we found out simple solutions for the convenient initial conditions. We can visualize the kinetic behavior of the cyclic vs. opened four-state models. Using proof by contradiction, we demonstrate a physical inconsistency of the model with cyclic reaction scheme when applied to allosteric interactions at muscarinic receptors (cf. 5).

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TACRINE AND MUSCARINIC RECEPTORS: UNUSUAL MECHANISM OF INTERACTION

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It has been observed repeatedly (1, 2, 3) that the inhibitor of cholinesterases tacrine (1,2,3,4-tetrahydroacridin-9-amine) inhibits the binding of orthosteric muscarinic ligands to muscarinic receptors with unusually steep binding curves (high hill slopes). Definite explanation of this phenomenon is not available. A model has been proposed according to which tacrine binds to the orthosteric site and to the allosteric site simultaneously with homotropic positive cooperativity (4). Another hypothesis suggests that tacrine binds to two spatially separated allosteric sites on muscarinic receptor with positive cooperativity or its binding to the common allosteric site modulates receptor-receptor inter-actions (2). We have discovered recently that structurally related compounds 7-methoxytacrine, acridin-9-amine, and proflavine (acridine-3,6-amine) yield characteristically steep binding curves while quinacrine (6-chloro-9-([4-diethylamino]-1-methylbutyl) amino-2-methoxyacridine) behaves like a com-petitor and displaces the orthosteric ligand [^{3}h]*n*-methylscopolamine yielding the standard curves with hill slope close to 1. We hypothesize that the bulky substitution at position 9 on basic acridine skeleton sterically hinders the binding quinacrine into orthosteric and allosteric binding sites simultaneously. However, the most important finding of our study is that tacrine and the com-pounds under test self-associate in solutions stronger than 10^{-5} m. The variation in molar absorptivity with the concentration of these compounds were measured in the UV-VIS spectra. Deviations from Lambert-Beer's law with increasing concentration were detected. We provide the mathematical expression for corrected concentration of monomer in solution.

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THE LUSITROPIC PROPERTIES DIFFER IN THE NEWBORN AND ADULT RABBIT VENTRICULAR MYOCARDIUM

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Relaxation is a part by which cardiac muscle actively returns to its precontractile state. The trace of relaxation and inter-beat diastolic properties are termed "lusitropic functions". Although lusitropic pattern of relaxing adult myocardium have been studied and analyzed; no comprehensive parametric description of isometric lusitropy in newborn myocardium was investigated. Therefore the purpose of present study was to determine the frequency-dependent changes of lusitropy in immature and adult ventricular myocardium. Using programmable stimulator the newborn and adult right ventricle papillary muscles were excite by different rates at steady state. To evaluate age-related differences in the isometric lusitropy, next record were used: a) relaxation maximal speed of isometric force decline (dF/dt_r, or –F), b) maximal rate of isometric force increasing during contraction (dF/dt_c, or +F), c) ratio between maximal rates of contraction and relaxation (+F/-F), d) peak force of contraction (MG), e) time required for the fall relaxation force to half of its peak value (R/2), f) ratio between maximal rate of isometric force decline and MG (-F/MG), g) +F/MG. As frequency decreased (cycle length, CL, protract,), adult –F decline, and newborn –F show CL-independent course. The intervention which cause decreasing of –F is termed negative lusitropic effect. While newborn ratio +F/-F is CL-independent, the adult ones rapidly decline during initial short CL and then steeply increased. Newborn MG is CL-independent; adult MG start at short CL from an extensively high force and within prolongation of CL it decline under newborn value. No difference was found between newborn and adult R/2. Lengthening of CL, in both, newborn and adult papillary muscles, causes decrease of -F/MG (negative relaxant effect). The values R/2 in both age group indicated that rate of SR Ca^{2+} uptake is similar in newborn and adult rabbit. Much pronounced negative slope of adult –F as a function of CL-prolongation and CL-independent newborn –F support the assumption that the amount of SR in newborn is significantly less than in adult. The decreasing values of –F/MG during lengthening of CL and symmetrical shift-down of newborn –F/MG curve suggests that this parameter can be a useful index of lusitropy in isolated cardiac muscle. The accordance between age-related lusitropic behavior and increasing of SERCA2a during early postnatal development tend to realistic hypothesis that, the cause of lusitropic ontogeny is an amplification of SERCA2a function.

HORMONAL COUNTERREGULATORY RESPONSE DURING HYPOGLYCEMIA IN YOUNG MEN WITH EARLY HYPERTENSION

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Neuroendocrine system may play a role in development of essential hypertension. Goldstein (1) found only in about 40 % of 78 comparative studies statistically significant higher baseline catecholamine levels in hypertensive (HT) compared to normotensive (NT) subjects. In dynamic test, Amerena and Julius (2) reported increased sympathetic response during physical exercise in HT subjects. However, in the literature there is a substantial lack of data on complex neuroendocrine function in hypertension. To evaluate baseline hormonal concentrations and neuroendocrine response during hypoglycemia in HT subjects. Insulin tolerance test (ITT, 0.1 IU/kg, Actrapid HM, i.v.) was performed in young non-obese men with untreated early hypertension and in matched NT controls. Counterregulatory and volume-regulating hormones were determined in venous plasma taken before and 30, 45 and 60 minutes after insulin administration. The mean blood pressure and heart rate tended to be higher in HT subjects than in NT subjects, however these differences were not significant. No significant differences between HT and NT subjects were observed in baseline values of estimated variables. The fall of glycemia during ITT was similar in HT and NT subjects. During ITT decreased levels of epinephrine ($p<0.05$), growth hormone ($p<0.05$) and prolactin ($p<0.01$) were observed in HT subjects compared to NT subjects. No significant differences were found in levels of C-peptide, norepinephrine, cortisol, aldosterone and plasma renin activity during ITT between HT and NT subjects. Decreased epinephrine response during hypoglycemia in HT subjects did not influence its counterregulatory effect. It may be speculated that the sensitivity of adrenal receptors is changed in hypertensive patients. Decreased growth hormone and prolactin response to hypoglycemia in HT subjects suggests an impairment of ascendent pathways of glucose counterregulation.

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ONTOGENETIC DEVELOPMENT OF THE CYTOKINE´S PROFILE OF THE PIG´S EXPERIMENTAL MODEL

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The pig´s ontogenetic model for the specific type of the placentation of this animal kind has already had long-time tradition in evolutionary immunology. The development of the whole organism is sensitively controlled through signal molecules – cytokines. We observed interleukins as the first group of cytokines, from which we chose IL-1β and IL-8. IL-1 β is produced by macrophages and it increases the expression of other cytokines. IL-8 is able to attract cells (especially neutrophils) to the place of inflammation and it influences the process of hemopoesis. Interferons are the second group which we studied. IFN-γ is the elective representative of this group and it facilitates defense antiviral mechanisms and it activates macrophages and Tc-lymphocytes. We used 2 litters of piglets from the birth to the 15th weeks of their life. Blood samples were obtained at regular intervals by paramedial puncture in the caudal part of the regio coli ventralis. Levels of cytokines were determined by the ELISA method the sensitivity of which was increased by using the biotin-avidin system. Concentrations of individual cytokines were estimated from absorbations that were measured on ELISA-reader at 450 nm. Levels of observed cytokines rose in average: IL-1 between 1. and 7. days 8.01 pg/ml; 7. and 18. days 665.79 pg/ml; 18. and 28. days 1198.97 pg/ml; 28. and 31. days 1070.42 pg/ml and between 31. and 66. days 381.45 pg/ml. IL-8 between 1. and 7. days 245.49 pg/ml; 7. and 18. days 580.38 pg/ml; 18. and 28. days 658.07 pg/ml; 28. and 31. days 532,29 pg/ml and between 31. and 66. days 82,78 pg/ml. IFN-γ between 1. and 7. days 25.91 pg/ml; 7. and 18. days 73.49 pg/ml; 18. and 28. days 84.35 pg/ml; 28. and 31. days 58.06 pg/ml and between 31. and 66. days 63.25 pg/ml. The measurement was also practiced with using the referential filter (630 nm). Concentrations were however almost identical, therefore we did not mention them. The sharp limit between cytokines and hormones could not be determined because some cytokines have the similar character as hormones and vice versa. For this reason we extended our research and we also started to observe level's changes of cortisol. Its level was detected by the HPLC (Waters) technique and absorbation was consecutively detected at 254 nm. Our results show the continual level increase of all observed matters during the postnatal period. It is connected with the progressive colonization of the organism by microbe phloem to which the organism is forced to react by the complicated cascade of specific and nonspecific immune reactions.

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CHRONIC N-ACETYLCYSTEINE ADMINISTRATION PREVENTS HYPERTENSION DEVELOPMENT IN L-NAME-TREATED RATS

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Oxidative stress is known to play an important role in the pathogenesis of various forms of experimental hypertension. The aim of this study was to evaluate the production of reactive oxygen species as well as their role in the induction and/or maintenance of high blood pressure in rats with NO-deficit hypertension elicited by chronic L-NAME treatment. Adult Wistar rats treated with L-NAME (60 mg/kg/day/4 weeks) were compared with rats treated simultaneously with L-NAME and either N-acetylcysteine (1.5 g/kg/day/4 weeks) or Tempol (0.7 g/kg/day/4 weeks) or with untreated control. The similar experiments were done on SHR and WKY (as control) rats. Basal blood pressure, superoxide concentration (estimated by lucigenin chemiluminescence) in the aorta rings, NO synthase activity and the levels of conjugated dienes in the heart and kidney were measured at the end of the experiment. Chronic NO synthase inhibition by L-NAME treatment increased blood pressure of rats, enhanced superoxide concentrations and elevated the levels of conjugated dienes in the heart and kidney. These changes were prevented by a simultaneous chronic administration of N-acetylcysteine which also restored lowered NO synthase activity. A simultaneous chronic administration of Tempol caused only a borderline blood pressure reduction in L-NAME treated rats, but it increased their NO synthase activity, lowered conjugated dienes and tended to normalize superoxide production. The acute administration of Tempol caused a significant blood pressure in L-NAME hypertensive rats. The effects of L-NAME and N-acetylcysteine in WKY rats were similar to those observed in Wistar. In contrast, chronic N-acetylcysteine treatment of adult SHR had only marginal blood pressure effects, although it caused a significant increase of lowered NO synthase activity and decrease of conjugated dienes in the heart and kidney. It seems evident that reactive oxygen species are important for both the induction and the maintenance of hypertension elicited by chronic L-NAME treatment.

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EFFECTS OF N-TERT-BUTYL-ALFA-PHENYLNITRONE (PBN) ON RECOVERY OF IMMATURE RATS DURING ONE WEEK AFTER STATUS EPILEPTICUS

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Status epilepticus (SE) leads to long lasting consequences, such as neuronal loss and functional impairment, both in adult and in immature rats. Free radicals play an important role in brain damage induced by various insults, including epilepsy in adults. N-tert-butyl-alfa-phenylnitrone (PBN), one of the free radical scavengers, has been recently tested for neuroprotective effect in various models, however, no data are available for immature animals with epileptic seizures. The aim of the present study was to examine the effect of PBN on recovery following SE induced in immature rats. SE was induced in 12- (P12) and 25-day-old (P25) Wistar rats using lithium-pilocarpine model. PBN (2 x 100 mg/kg i.p.) was injected: (1) First dose of PBN 30 min prior to pilocarpine, and the second dose immediately after beginning of SE, (2) PBN was given immediately and one hour after beginning of SE. Paraldehyde was administrated after 2 h (90 min at P25) of continuous SE to prevent transition into generalized tonic-clonic seizures. Control animals received corresponding volume of saline. Latency of the first motor manifestations, as well as severity of seizures, were recorded. Weight gains were evaluated during one week following SE. Motor functions were tested 3 and 6 days after SE with behavioral tests, including bar holding and rotarod test. PBN-pretreated animals developed SE after longer latency in both age groups ($p<0.05$). Severity of seizures was not changed in younger group, whereas in P25 pups PBN treatment resulted in a significant decrease of wild running and an absence of generalized tonic-clonic seizures. P25 treated animals showed significantly higher weight gains, as compared with untreated rats, and they also performed better in the bar holding and rotarod test. No effect was found in behavioral changes in P12 group. Positive effect of PBN on recovery process following SE in immature animals is age-specific.

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EFFECT OF B-LYMPHOCYTE SUPPRESSION ON THE DEVELOPMENT OF ADJUVANT ARTHRITIS AND ENDOCRINE RESPONSES IN MALE LONG EVANS RATS

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To evaluate the effect of the immunosuppressive fraction of boar seminal vesicle fluid (ISF) that is known to selectively inhibit the production of IgG by B-lymphocytes on the development of adjuvant arthritis in male and female Long Evans rats. Adjuvant arthritis (AA) was induced by single subcutaneous injection of complete Freund's adjuvant at the base of the tail. ISF was administered to arthritic rats: A - by i.p. injections in 3 day intervals during the whole time course of the disease (22 days), B – during the preclinical phase of the disease (day 1-10), C – during the clinical phase of the disease (day 10-20), D – into the joint of the left hind paw during the whole time of the disease. A - the disease was attenuated as evaluated by the hind paw swelling, body and thymus weights. The decreased levels of leptin which was found in arthritic rats and corresponded with reduced body weight, recovered due to the treatment as well. B – ISF treatment remained without any effect. C – hind paw swelling was partially attenuated, as were recovered body and thymus weights, but the leptin levels remained suppressed. D – local administration of ISF did not ameliorate the severity of the disease. It can be concluded that ISF can attenuate AA which is considered as T-lymphocyte mediated disease. It demonstrates also the involvement of B-lymphocytes in this process. ISF is effective only when given systematically, probably via the suppression of IgG. During the disease it exerts recuperative rather than preventive effects.

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HYPOLIPIDEMIC EFFECT OF RESPIRATORY UNCOUPLING IN WHITE ADIPOSE TISSUE

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Hypolipidemic effect of certain drugs such as fibrates or thiazolidinediones is associated with the induction of uncoupling protein (UCP) gene expression in depots of white fat. This suggests that respiratory uncoupling in white adipocytes could represent a mechanism how these compounds lower plasma triacylglycerol (TG) levels. To analyze this, we evaluated systemic lipid metabolism in the transgenic mice (aP2-*Ucp1* mice) with ectopic expression of various levels of UCP1 in white adipose tissue. Three-month-old, C57BL/6J male mice with different levels of the aP2-*Ucp1* transgene (i.e. hemizygous and homozygous animals) together with their non-transgenic littermates were kept on a standard chow diet or fed a high-fat diet for approximately 3 months. At the end of experiment plasma samples were obtained for the analysis of TG and free fatty acid levels and the composition of plasma lipoproteins. The activity of lipoprotein lipase was assessed to estimate the capacity of adipose tissue for the removal of fatty acids from the circulation, while hepatic TG secretion was estimated by intravenous injection of 10 % Tyloxapol (Triton WR-1339). Clearance of exogenous lipids was evaluated by intragastric gavage of sesame oil and subsequent analysis of plasma TG levels during a 4-hour period after the gavage. Transgenic mice, especially the homozygous animals, fed a low-fat diet had reduced levels of total plasma TG. Plasma free fatty acids followed a similar trend. A rise in plasma TG associated with a high-fat feeding was diminished in hemizygous and even reversed in homozygous transgenic mice. The concentration of very-low-density lipoprotein (VLDL) particles in plasma of transgenic mice was relatively low in spite of minor changes in hepatic TG secretion induced by the transgene. However, the activity of lipoprotein lipase in epididymal fat of transgenic mice was enhanced, particularly on a high-fat diet, and postprandial clearance of plasma TG was augmented by a high-fat diet in the transgenic but not in control mice. Thus, respiratory uncoupling in white fat may lower plasma lipids by enhancing their *in situ* clearance and catabolism.

A COMPARISON OF A NEW REAL-TIME PCR METHOD FOR MONITORING Hsp70 PROTEIN FAMILY EXPRESSION IN HepG2 CELLS WITH A COMMERCIAL Hsp-70 (SEARCH-LC) KIT

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We focused on expression of Hsp70 protein family in the human hepatocyte HepG2 cell line and attempted to elaborate a method for its monitoring at the mRNA level using LightCycler™ instrument (Roche). In this contribution, we compare the new method with results obtained using a commercial Human Hsp-70 (Search-LC) kit. Cell lysates were prepared and purification of mRNA was done by either the magnetic separation method (Dynal) or using a High Pure RNA isolation kit (Roche). Corresponding cDNA was synthesized first. Amplicon production with our optimized primer pair selected for specific amplification of individual Hsp70 targets was compared to that achieved using the above Hsp-70 commercial kit. Amplification reactions were successfully effected in SYBR®Green detection format. As a result, the measurements have led to mutually congruent data. Nevertheless, we feel that the knowledge of the specific sequences in optimized primer sets can be of key importance in future specialized studies. The present method is currently in progress in monitoring of Hsp70 family mRNA levels in signaling cascade studies in HepG2 cells in this laboratory.

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IP PROSTANOID RECEPTOR IS NOT PRESENT IN DETERGENT-INSENSITIVE MEMBRANE DOMAINS

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Various detergent (Triton X-100) concentrations (0.1-1 %) were used for preparation of detergent-insensitive membrane domains (DIMs) in order to critically analyze the presence of IP prostanoid receptor in these membrane structures. Distribution of Flag-epitope tagged version of IP prostanoid receptor - $G_s\alpha$ fusion protein (FhIPR-$G_s\alpha$) in HEK293 cells was compared with that of the cognate $G_s\alpha$ protein and the prototypical membrane domain markers, caveolin and flotillin-2. At an optimum TX-100 concentration (0.1-0.25 %), more than 90 % of caveolin was present in DIMs. Under these conditions, however, $G_s\alpha$ represented about 20 % of its total cellular amount and there was no detectable FhIPR-$G_s\alpha$. Whereas other studied G protein subunits, $G_{11}\alpha$ and $G\beta\gamma$, showed similar behavior to that of $G_s\alpha$, adenylylcyclase IV was undetectable in DIMs. The levels of DIM-associated molecules were reduced by using increased TX-100 concentrations (0.5-1 %), which may suggest that the structure of DIMs is at least partially disrupted by high detergent concentrations.

DEVELOPMENT AND OPTIMIZATION OF A REAL-TIME PCR METHOD FOR MONITORING Hsp70 PROTEIN FAMILY EXPRESSION IN HepG2 CELLS

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Hsp proteins have been implicated in various cellular pathological events. We focused on expression of the Hsp70 protein family in the human hepatocyte HepG2 cell line and attempted to elaborate a method for its monitoring at the mRNA level using LightCycler™ instrument (Roche). Cell lysates were prepared and purification of mRNA was done by either the magnetic separation method (Dynal) or using a High Pure RNA isolation kit (Roche). Corresponding cDNA was synthesized first. Six primer pairs for the Hsp70 family were examined. Among them, both primers designed in this laboratory and primers adopted from literary data were examined. Amplification reactions were effected in SYBR®Green detection format. Purity and length of PCR products as well as those of the source mRNA were checked electrophoretically. As a result, an optimized primer pair giving a product of adequate degree of purity and expected chain length was selected. The method will be utilized for monitoring Hsp70 family mRNA levels in signaling cascade studies in HepG2 cells that are currently performed in this laboratory.

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SYNERGISTIC EFFECTS OF ALUMINIUM AND FLUORIDE ON THE PATHOPHYSIOLOGICAL HALLMARKS OF ALZHEIMER´S DEMENTIA

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The hypothesis that the accumulation of aluminium in the brain is the cause of Alzheimer´s dementia (AD) has been postulated and discussed very often. Recent fundamental research of the pathogenesis of AD brings evidence that this disease is connected with the alterations in neurotransmission, abnormal polymerization of cytoskeletal protein τ, alterations in mechanisms of calcium homeostasis, changes in β-amyloid precursor protein and β-amyloid production, and apolipoprotein E accumulation (1). The mechanisms of how aluminium could evoke the multiple pathophysiological changes in AD are not known. The intensive laboratory research of mechanisms of signal transduction brings numerous experimental data, which could change our understanding and interpretation of aluminium action on the cell level. Reflecting many laboratory studies we suggest that some of pathological changes are not raised by aluminium alone, but by the synergistic action of aluminium and fluoride (2). Aluminofluoride complex (AlFx) acts as a new high affinity analogue of phosphate and nonspecific activator of G proteins. The cholinergic hypothesis suggests that the reduction of cholinergic neurotransmission could explain the most important cognitive deficit in AD. Cholinesterases are todays the targeting point for most used group of AD drugs. We studied the effect of 20 μM $AlCl_3$ and 0. 01-1 mmol L^{-1} NaF on the AChE activity in human red blood cells (RBC). While 20 μM $AlCl_3$ increases the AChE activity to 158 %, fluoride with 20 μM $AlCl_3$ inhibits the AChE activity. We measured the level of cytosolic Ca^{2+} in platelets isolated from the blood of AD patients in comparison with the groups of healthy young and age-matched controls and found the significantly decreased values in platelets of patients with AD (3). AlFx decreased the cytosolic Ca^{2+} level in young and age-matched controls. AlFx evoked the significant changes in cytoskeletal proteins of RBC and fibroblasts.

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PLASMA LEVELS OF BNP-32 DID NOT CHANGED 60 MIN AFTER PULMONARY EMBOLISM IN EXPERIMENTAL RATS

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Pulmonary embolism (PE) is a "medical evergreen" with not decreasing incidence. PE complicates primary diseases (hypertrophic cardiomyopathy, atrial fibrilation, flebothrombosis...) or long-lasting major surgical procedures, catheterizations or finally false i.v. application of suspensions during i.m. administration. Natriuretic peptides (NP) belongs to the family of hormones with auto-, para- and endocrine effect. Cardiac NP (ANP, BNP) restrain effect of sympatho-adrenal and renin-angiotensin-aldosterone system. ANP, Nt-ANP, BNP and Nt-BNP are recently considered to be important independent markers of cardiac diseases. Some recent publications showed correlation between ANP and size of PE. Relationship of BNP with PE remains unclear. Aim of our study was to investigate plasmatic levels of rat BNP-32 after 60 minutes of induction of PE. Thirty-nine (male, outbreed, 450-500 g) Wistar rats were randomized into two groups, PE and K. In the group PE (n=23) pulmonary embolism was induced using intra venous application of suspension 2 ml of microspheres (Embosphere 40-120 μm, 8 ml original suspension *ad* 100 ml of isotonic sodium chloride solution) at time 0 during 12 minutes. Sham intervention in the group K was based on infusion of pure isotonic sodium chloride solution using the same way of administration. Sixty minutes after start of induction of PE arterial blood was sampled in both group (a. carotis) for analysis of BNP. For the sample analysis, We used EIA rat BNP-32 set (Phoenix Peptide Inc.) was used. Whole experimental protocol was performed under general anesthesia (ether + droperidol + xylazine + ketamine). Mean plasma BNP level in group PE 1.37±0.30 ng/l, did not significantly differ from control group K 1.14±0.37 ng/l (p=0.21). Our results showed that there is no significant increase of plasma BNP level 60 minutes after pure/non-thrombotic pulmonary embolism. Previously described elevated plasma BNP levels seems not to be caused by acute pulmonary embolism.

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CHANGES IN PROTEIN EXPRESSION OF NR2A/NR2B SUBUNITS OF NMDA RECEPTOR IN ADULT RAT BRAIN: CONSEQUENCES OF NEONATAL BRAIN DAMAGE

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Some aspects of schizophrenia are associated with a dysfunction of ionotropic glutamate receptors of N-methyl-D-aspartate (NMDA) type. As developmental processes in the brain can be adversely affected by perinatal infections (1), our animal model is set up on the viral hypothesis (2). Recent findings have demonstrated that virus-infected macrophages/microglia increase production of quinolinic acid (QUIN), an excitotoxic metabolite of tryptophan, with an ability to activate NMDA-R. Resulting neurodegeneration and abnormal synaptic plasticity have specifically been looking at the role of NMDA-R subunits in schizophrenia. Therefore, this study was focused on the protein expression of NR2B subunit in right and left hippocampi of 50-day-old (post-pubertal) rat males influenced by bilateral infusion of 0.25 μmol QUIN/0.25 μL phosphate-buffered saline/ventricle on PND 12. Detailed Western blot analysis revealed quantitatively different protein expression of NR2B and NR2A subunits in synaptosomes isolated from the right and left hippocampus in young adults. The NR2B subunit expression was higher in the right hippocampus of naive rats (30±6.7 %, $p<0.02$; paired t-test used). In controls, neonatal anesthesia and "sham" operation altered the expression of NR2B (but not NR2A) subunit. However, in QUIN-treated rats the protein expression of NR2A subunit was decreased both in the right (33±8 %, $p<0.02$) and left (30±9 %, $p<0.02$) hippocampus. Moreover, also the expression of NR2B subunit exhibited a more pronounced decrease [in the left hippocampus 37±4.5 % ($p<0.005$) and in the right hippocampus 29±2.3 % ($p<0.001$)] in comparison with corresponding controls. We conclude that long-term dysregulation of NR2B and NR2A subunits may be involved in NMDA receptor dysfunction, hyper-locomotion and in deficit of prepulse inhibition. Although the NR2B/2A subunits may be involved in certain aspects of schizophrenia further investigation of the role of these NMDA receptor subunits is needed.

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EXPRESSION OF CLOCK GENES IN THE RAT SUPRACHIASMATIC NUCLEUS DURING ONTOGENESIS

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The rat suprachiasmatic nucleus (SCN) is a central pacemaker responsible for the generation of circadian rhythms. Its mechanism consists of several interlocked transcriptional and translational feedback loops of clock genes and their products and develops already prenatally. The aim of our study was to discover the development of the molecular pacemaker during ontogenesis. Gravid females and mothers with their pups were released into constant darkness and decapitated in 2-3 h intervals. Daily mRNA profiles of Per1, Per2, Cry1, Bmal1 and Clock genes in the SCN of 19 day old fetuses and 3 and 10 day old newborn rats were examined by *in situ* hybridization with radioactive riboprobes. In addition, daily profiles of PER1, PER2 and CRY1 proteins in the SCN of 19 day old fetuses were assessed by immunocytochemistry. No significant rhythms of expression of any studied gene were found in the SCN of 19 day old fetuses. Per1, Cry1 and Clock mRNA levels were constitutively low, whereas Bmal1 mRNA level was high and Per2 mRNA level medium. Furthermore, no PER1, PER2 or CRY1 immunopositive cells were found in the SCN of 19 day old rats. However, mRNA rhythms of all examined genes except Clock were clearly expressed in the SCN of 3 day old pups and became even more prominent in 10 day old rats. In conclusion, our data imply gradual development of the molecular pacemaker in the rat suprachiasmatic nucleus.

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LIFESTYLE DECREASES RISK FACTORS OF CIVILIZATION DISEASES: COMPARISON OF VEGETARIANS AND NONVEGETARIANS

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The lifestyle is capable to decrease the risk factors (RF) of civilization diseases without drugs. Our previous work showed, that one-week-lasting reconditioning stay containing low-fat-low-energy vegetarian diet, physical activity, without stress, smoking, alcohol (lifestyle New Start) decreased some risk factors of civilization diseases (body weight, BMI, blood pressure, serum cholesterol and blood glucose) in 50-years old volunteers (1). In the present work we a) confirmed it in the higher number of persons (N=625, 147 men, 478 women, mean age 49.0±14 years). The body weight decreased from 70.8 to 70.1 kg, BMI from 24.8 to 24.6 kg/m^2, systolic blood pressure from 129.6 to 123.2 mmHg, diastolic blood pressure from 80.1 to 77.2 mmHg, serum cholesterol from 5.05 to 4.41 mmol/l, blood glucose from 4.62 to 3.90 mmol/l ($p<0.0001$). b) We compared these parameters in lacto-ovo-vegetarians, (L-O-V, N=185, 65 men, 130 women, mean age 47±11.2 years, duration of diet 5 years) and in Seventh Day Adventists (SDA, N=130, 44 men, 86 women, mean age 46±10.8 years), and in controls, participants in reconditioning stays. The measured parameters decreased less during one-week, lasting stay in L-O-V and SDA than in control population. Moreover, the initial values of some parameters before the stay were lower in L-O-V and ASD than in controls: body weight 61.9, 70.2, 72.1 kg ($p<0.05$); BMI 22.1, 24.1, 25.4 kg/m^2 ($p<0.05$); systolic blood pressure 118.1, 127.1, 134.2 mmHg ($p<0.05$); diastolic blood pressure 70.8, 75.8, 81.5 mmHg ($p<0.001$); serum cholesterol 4.91, 4.03, 4.94 mmol/l ($p<0.01$); blood glucose 3.99, 3.41, 4.48 mmol/l ($p<0.001$). The results showed that a) short lasting reconditioning stay is capable to decrease the RF of civilization diseases, b) low level of RF parameters in L-O-V and SDA group is due to their keeping of more conditions of lifestyle including diet (nonsmokers, exercise, temperance).

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THE IMPACT OF DIABETES ON THE SENSORY INNERVATION IN THE RAT HEART

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Diabetic cardiomyopathy affects both the contractile cardiomyocytes and the afferent sensory and efferent motor innervation of the heart (1). Recent results led to hypothesis that both the signaling systems of fast-acting classical neurotransmitters as well as long-acting neuromodulators of the peptide-class are critically disturbed in diabetes, thereby contributing to pathogenic progression (2). The aim of this study was to test the components of sensory innervation, acting via calcitonin-gene related peptide (CGRP), in the time course of developing diabetes mellitus (4-16 week) in the well-established model of streptozotocin-induced experimental diabetes in the rat. CGRP was quantified at peptide level by radioimmunoassay and localized at cellular level by immunohistochemistry, its respective receptors were quantified at mRNA level by real-time RT-PCR. Cardiac neuropathy did not lead to any changes in CGRP concentrations in both atria in week 4 and 8 after the onset of the disease, but the peptide levels significantly increased in week 16 when compared to the respective control values. In the ventricles, however, the significant increase in CGRP concentrations occurred even in the 4th week being followed by further elevation until week 16 when the peptide levels reached ~200 % of the control values. Changes in CGRP levels in the time course of diabetes were accompanied by down-regulation of CGRP receptors since a decrease in relative expression of mRNA encoding for this receptor was observed. The decline was more prominent in the right heart compartments then in the left ones. In conclusion, the elevation of CGRP levels in the diabetic heart may play a protective role, as the peptide is likely to exert the trophic effect upon cardiomyocytes and enhance myocardial blood flow.

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EFFECTS OF STIMULUS INTENSITY AND ISOMETRIC MUSCLE CONTRACTION ON AMPLITUDE CHANGES OF CORTICAL 16–30 HZ OSCILLATIONS INDUCED BY REPETITIVE CUTANEOUS STIMULATION

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Effects of cutaneous stimulation on cortical 16-30 Hz oscillations were analyzed in two experiments. In Experiment 1, painful and innocuous stimuli (0.2 ms pulse duration, ~2 s inter-stimulus interval) were applied to the right index finger in 10 right-handed subjects. EEG was recorded from 88 scalp sites, and analyzed using event-related desynchronization method. Amplitude of 16-30 Hz oscillations over the contralateral and ipsilateral sensorimotor cortex showed initial decrease peaking 0.2-0.3 s after stimulus onset and subsequent increase over the contralateral sensorimotor cortex at 0.6-0.8 s. The 16-30 Hz power increase was significantly stronger during painful than during innocuous stimulation. In Experiment 2, effects of isometric muscle contraction of ipsilateral hand muscles amplitude changes of 16-30 Hz after painful stimulation were studied in 10 right-handed subjects. Both the post-stimulus decrease and subsequent increase of 16-30 Hz oscillations were significantly smaller during contraction than during relaxation of ipsilateral hand muscles. Results suggest that synchronization of cortical 16-30 Hz oscillations differentiate painful and innocuous stimuli, and that stimulus-induced amplitude changes of 16-30 Hz rhythms are attenuated by concurrent contraction of ipsilateral hand muscles.

MORPHOLOGICAL CHANGES IN THE REPRODUCTIVE APPARATUS OF EWES AFTER HORMONAL STIMULATION

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Qualitative and quantitative histological changes in the reproductive apparatus of Slovak merino sheep were studied after synchronization and hormonal stimulation. Observations were carried out in 40 sheep in the period of physiological anoestrus. Synchronization was ensured with Agelin (chlorsuperlutin 20 mg) for 10 days. And by hormonal stimulation with 750, 1000 and 1500 IU PMSG (Serum gonadotrophin Ivanovice na Hane). The animals were killed 120 h after the removal of intravaginal sponges on average. Samples from individual parts of the reproductive apparatus were processed by common histological methods for examination under a light microscope and by the method of Murakami et all. (2) for examination under a scanning electron microscope Stereoscan Cambridge 2A and Jeol. We observed that the influence of PMSG in the anoestric period resulted in a significant increase in the average weight of ovaries. With the increasing dose of PMSG increased also the number of newly formed corpus luteum, i.e. the highest number was observed in sheep stimulated with 1500 IU PMSG. The rinsing of the gonadal apparatus of sheep of the latter group provided the highest number of ovula per one sheep. The hormonal stimulation resulted in similar significant increase in the weight of uterine cervices and uteri. The number of glands, height of their epithelium and the height of the cervical surface epithelium also increased significantly following the administration of hormones. The administration of hormonal preparations used to induce superovulation in sheep increased the contact surface, caused multiplication of cilia of spherical protrusions, increase in the number of glands and marked desquamation in the reproductive apparatus in sheep (1).

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AGONIST-STIMULATED HIGH-AFFINITY GTPASE IN YOUNG AND ADULT RAT BRAIN CORTEX

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The present study analysed GTPase activity in cerebrocortical membranes prepared from young (12-day-old) and adult (90-day-old) rats. High-affinity GTPase activity was significantly higher (by 45 %) in adult than in young rats and the $GABA_B$ receptor agonist baclofen effectively increased GTPase activity (by about 56 %) in both types of samples. Similar results were obtained by concomitant GTPγS binding experiments. The number and affinity of $GABA_B$ receptors (assayed by the specific antagonist [^{3}H]CGP54626A) in carefully washed cerebrocortical membranes from young (12-day-old) and adult (90-day-old) rats was not different. Agonist-stimulated GTPase activity was further markedly enhanced by the addition of RGS1 but not by RGS16. RGS1 per se slightly increased GTPase in adult rats, but neither RGS1 nor RGS16 influenced GTPase activity in preparations from young animals. These findings indicate a rising high-affinity GTPase activity in the developing rat cortex and suggest a role of RGS1 in modulation of this enzyme activity.

OVEREXPRESSION OF P-GLYCOPROTEIN (P-GP) IN L1210/VCR MURINE LEUKEMIC CELLS IS ACCOMPANIED WITH ATP- AND UDP-SUGARS LEVELS DEPRESSION

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Multidrug resistance of murine leukemic cell line L1210/VCR (obtained by adaptation of parental drug sensitive L1210 cells to vincristine) is associated with over-expression of mdr1 gene product P-glycoprotein (1) - the ATP-dependent drug efflux pump (for review see 2). ^{31}P-NMR spectra of L1210 and L1210/VCR cells (the latter in the presence of vincristine) revealed besides the decrease of ATP level a considerable lower level of UDP-sugars in L1210/VCR cells. The difference in the level of ATP between sensitive L1210 and resistant L1210/VCR cells obtained from ^{31}P-NMR was verified by enzyme estimation procedure using luciferin/luciferase reaction. The difference in UDP-sugars content between sensitive and resistant cells was also monitored by estimation of UDP-glucose (major UDP-sugar) content using NAD/UDP-glucose dehydrogenase reaction. We established the amount of UDP-glucose on 2.72±0.15 nmol/mg of proteins in sensitive cells, while amounts of UDP-glucose in resistant cells and resistant cells cultivated prior estimation in vincristine were less than 0.6 nmol/mg of proteins, i.e., the minimal value that can be estimated according to our experimental condition. The level of ATP in resistant cells was about 35 % lower as in sensitive cells. Cultivation of resistant cells in medium containing 0.2 mg/l vincristine caused additional decrease of ATP level to about 50 %.

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SIMULTANEOUS USE OF INDEPENDENT REFERENCE SYSTEMS IN NAVIGATION: SPATIAL BEHAVIOR OF RATS INTERACTING WITH A BIOLOGICALLY SIGNIFICANT MOVING OBJECT

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In a stable environment the animal navigates according to visual, acoustic, odor, self-motion generated or other perceivable cues, anchored to one coordinate system, so called spatial frame. But when any substantial part of the environment moves with respect to stable surroundings, the animal's world dissociates into two spatial frames. In nature, it is not only important to pay attention to landmarks but also to predators, preys, etc. We presume that animals are capable to develop a spatial behavior, in which the animals like predator, prey or conspecific form a reference point of a new spatial frame. For that purpose, we trained 5 pairs of rats in a rat model of antipredator behavior where the "prey" rat learns to maintain a safe distance from the freely moving "predator" rat, approach of/to whom is punished by a mild footshock. The predator can be replaced by a programmable robot which makes the task easier because of its predictable path. In another, appetitively motivated task, ten pairs of rats were rewarded whenever they subsequently fulfilled two requirements: 1) increased their mutual distance to 40cm or more, and then 2) decreased their distance to 10cm or less. Good performance is thus dependent on mutual coordination of locomotion of both animals or, at least, one animal must not interfere with the behavior of the other animal that leads to pellet triggering. These tasks are intended to serve as a model for electrophysiological examination of brain structures involved in the above mentioned spatial processes. All experiments were in accord with the directive of the European Communities Council No. 86/609/EEC.

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ENERGY STATE OF SKELETAL MUSCLE AFTER EXPOSURE TO LOW FREQUENCY WAVE MAGNETIC FIELD

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Electromagnetic fields (EMF) are one of the most common environmental factors that influence living systems. With the progressive development of emerging technologies the systems that generate EMF are rapidly increasing and possibly influencing humans and animals. The research of biomedical effects of EMF is therefore also in progress as there are many examples documenting either positive or negative influence of EMF on the health. In this study we tested potential influence of the low frequency EMF on the energy metabolism of the skeletal muscle. Homogenous wave magnetic field (MF) was produced by the solenoid (f = 50 Hz, the induction B = 10 mT). The rats were exposed inside the solenoid for the defined time. Two different exposures were selected: 1) repeated 1 hour exposure, 2 times a week for 3 months (with the control group kept under same conditions but without any exposure to artificial MF), and 2) acute 1.5 hour exposure (and the appropriate control group). Energy metabolites (ATP, creatine phosphate, creatine, lactate, pyruvate, inorganic phosphate and proteins were analyzed by enzymatic and spectrophotometric methods in *musculus gracilis anticus* to evaluate potential effect of two different exposures to MF on the energy state of muscle. Muscles from the animals with repeated exposures (and corresponding controls) were fixed under deep anesthesia with a flat tongs cooled in liquid nitrogen, several hours after the last exposure to MF. Muscles from the animals with acute exposure were removed immediately (also in deep anesthesia induced after the end of exposure) by the same technique. The muscles after fixation were stored in -80 °C until analysis. Evaluation of results (comparison of exposed animals with their appropriate controls) by ANOVA or non-parametric Kruskal-Wallis test did not show any significant change in tissue concentrations of all analyzed metabolites, including creatine-charge (which is a suitable criterion of the energy state of skeletal muscle). Conclusion of our study is that neither repeated exposures or the acute exposure of rats to the homogenous wave (50 Hz) magnetic field (10 mT) had any important influence on the energy state of skeletal muscle.

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HIGH-FAT DIET MODULATES ROSIGLITAZONE ACTION ON METABOLIC AND GENE EXPRESSION PROFILES IN A RAT MODEL OF METABOLIC SYNDROME

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Polydactylous inbred rat strain (PD/Cub) is a recently established genetic model of metabolic syndrome. Adult male PD/Cub rats (n=14) were fed high-fat high-cholesterol diet (HFD) for 4 weeks with (n=7) or without 14-day rosiglitazone (RSG, 0.4mg/100g body weight) administration. Oral glucose tolerance test (OGTT) was performed, serum triglyceride (TG), free fatty acid (FFA) and cholesterol (CH) levels were measured using commercially available kits. TG and CH content of liver and muscle tissues was determined. Lipogenesis, glycogenesis and glucose oxidation in tissues were assessed *in vitro* by incorporation of ^{14}C-U glucose into total lipids of adipose tissue, glycogen and CO_2 in soleus muscle, respectively. Total RNA was isolated from the epididymal adipose tissue and the expression profile of >15,000 transcripts (annotated genes and ESTs) was assessed using the Affymetrix RAE230A array. There were no differences in the total body weight and weights of heart, liver and kidneys between the two groups. The RSG-treated group showed higher adiposity index (1.92±0.15 vs. 1.40±0.05). The levels of fasting glucose, TG and CH were not significantly different between the RSG-treated and control groups. We did not observe any differences in the levels of FFA, both fasting and in the 60th minute of OGTT, but the drop during the OGTT test was significantly higher in the control group (0.32±0.08 vs 0.57±0.07 mmol/l). The incremental area under the curve of OGTT was significantly smaller in RSG-treated rats in comparison to the control group (187.0±9.3 vs. 313.4±17.1 mmol/l/2h). There were no differences in TG or CH content of hepatic and muscle tissues. RSG administration resulted in a significant increase of the insulin-stimulated lipogenesis and decrease in glucose oxidation in muscle, while the basal levels of both parameters did not differ between the control and the experimental groups. The expression profiling revealed distinct clusters of genes relatively up- and down-regulated in the RSG-treated-group compared to the control group. We report a case of diet-modulated pharmacogenetic interaction in genetic model of metabolic syndrome. These observations are in contrast with the previously reported metabolic profile of RSG-treated PD/Cub fed high-sucrose diet and suggest HFD as a modulator of pharmacogenetic interaction of RSG and PD/Cub genetic makeup.

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REPEATED METHAMPHETAMINE ADMINISTRATION DURING PREGNANCY AND LACTATION ATTENUATES MATERNAL BEHAVIOR IN RATS

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It has been demonstrated that repeated opiate exposure during pregnancy impairs maternal behavior of rats. However, it is not known whether methamphetamine (MA), a drug whose usage has increased lately, has the same effect on maternal behavior. The present study tested the hypothesis that repeated administration of MA (5mg/kg daily) prior, during and after pregnancy alters maternal behavior. Dams (absolute control, saline- and MA-treated) were observed with their pups in two experiments. In Experiment 1, twelve types of activities and three types of nursing positions of mothers were recorded ten times during each 50-minute session for the 21-day lactation period. A decrease in nursing and active maternal behavior was found in MA-treated mothers relative to control rats. All mothers, regardless of the treatment, displayed significantly less maternal behavior and more non-maternal activities as postpartum time progressed. In Experiment 2, mothers were also tested for pup retrieval from postnatal days 1 through 12. MA-treated mothers were slower in retrieving the first pup, in returning the first pup to the nest and in returning all pups to the nest. Thus, the present study suggests that MA administration prior, during and after pregnancy impairs maternal behavior and increases non-maternal activities of mothers.

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NEUROGENESIS AND GLIOGENESIS IN BRAIN INJURY AND LEARNING

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Glia, especially astrocytes, have been known for years to be capable of cell division during mammalian adulthood. Astrogliosis and glial scar formation are well known brain repair processes. A group of astrocytes placed in restricted layers, the subventricular zone of the lateral ventricle and the subgranular layer in the hippocampal dentate gyrus, have been identified in the adult mammalian brain as neuronal stem cells, which not only give rise to new glia but also serve as a generator of new neurons (1). It is already known that antidepressant treatment increases the number of newly generated cells, both astrocytes and neurons (2). The fate of newborn cells around a photochemical lesion differs after fluoxetine and beamwalking pretreatment: in controls 32 % of the total number of BrdU-positive cells had differentiated into NeuN-positive neurons or GFAP-positive glia, 26 % into neurons and 6 % into glia. In animals subjected to beam-walking pretreatment, 40 % of newborn cells had differentiated, 30 % into neurons and 10 % into glia; in animals with fluoxetine pretreatment 44 % of newborn cells had differentiated, 22 % into neurons and 22 % into glia; and following both pretreatments 36 % of cells had differentiated, 22 % into neurons and 14 % into glia. The proliferation of cells in the subgranular layer is enhanced during learning (3), injury or antidepressant administration and suppressed during stress. A compensatory effect of fluoxetine pretreatment on the decrease in proliferation induced by the stress of swimming has been observed. Currently, there are serious efforts being made to utilize cell therapy in the treatment after brain or spinal cord injury and to image the fate of implanted stem cells (4). A knowledge of neuronal fate determinants could help our understanding of brain repair mechanisms and to construct cell-based therapies for brain disorders.

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THYROID HORMONES AND EXPRESSION OF CONNEXIN-43 IN OLD RAT HEARTS. A PILOT STUDY

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Cardiac myocytes are coupled electrically as well as metabolically by connexin channels that form junctions between the cytoplasm of adjacent cardiomyocytes. Distribution and function of gap junction channels are important determinants of the electrical properties of the myocardium. Changes in thyroid status markedly influence cardiac contractile and electrical activity. L-thyroxine modifies cardiac actions by regulation of expression of several proteins involved in excitation and contraction processes. We have previously shown that thyroid hormones can modulate not only the contractile function but also connexin expression in cultured neonatal rat cardiomyocytes. This study was aimed to examine whether L-thyroxine can affect cell-to-cell communication by modulating the expression and/or phosphorylation of the major intercellular channel protein, connexin-43 (Cx43), in old rat hearts. L-thyroxine was applied orally in doses 100 mg/100g/day during two weeks to female and male Wistar rats aged 18 months. Thereafter, heart samples from control and hyperthyroid rats were processed for SDS-PAGE electrophoresis and Western blot analysis using primary mouse anti-connexin-43 monoclonal antibody and secondary antimouse peroxidase labeled antibody. Our preliminary results indicated that the expression of connexin-43 was not significantly influenced by thyroid hormones in old Wistar rats. Hyperthyroid treatment, however, led to decreased content of phosphorylated isoform of Cx43 and this decrease was more evident in male than in female rats.

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SOFTWARE FOR SKIN CONDUCTANCE RESPONSE

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Software tools for skin conductance response have been developed in Matlab 5.3 and user interface has been developed using GUI (Graphic User Interface) tools. Input of the software tool for skin conductance response processing is the measured signal of skin conductance response. Signals can be filtrated in different ways that are set in the program, namely weighted averaging, wavelet de-noising and digital (Butterworth) filter. Output data are the following parameters (1): arithmetical average of signal, modus of signal, median of signal, maximum of signal, minimum of signal, variance of signal, standard deviation of signal, average deviation of signal, interquartile range, α-percentiles, Fourier transform coefficients, wavelet transform coefficients, basic level of signal, time point of SCR onset, SC value at onset, latency, maximal SCR amplitude, time point of maximal SCR amplitude, rise time, time of recovery on 50 % of amplitude value, time of recovery on 37% of amplitude value, number of humps, integral estimation, frequency of nonspecific SCRs, sum of nonspecific SCRs amplitudes. User guide is programmed in Matlab as well and it enables easier setting of program parameters, processing more signals and more transparent display of results. The advantage of the developed software tool for skin conductance response processing is extraction of both basic and special parameters, typical for skin conductance signals. These parameters are used as input values for classification of persons (2) using artificial intelligence methods (3, 4).

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THE ROLE OF CALCIUM BINDING PROTEINS IN SENSITIZATION OF STT NEURONS

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Sensitization of spinothalamic tract (STT) neurons may play an important role in chronic pain states. Increased calcium influx and/or its release from internal stores are considered to be one of the key mechanisms in the sensitization process. The physiological effect of the Ca^{++} is dependent on activation of Ca dependent secondary messengers and is thus limited by the amplitude of the concentration change and the distance of diffusion from the source of the Ca^{++} (e.g. by the size of the Ca micro-domain). Calcium binding proteins (CBP) represent one of the key factors in the calcium buffering properties of the cells and have thus high impact on the size of the Ca microdomain size after Ca^{++} influx. In this study the role of CBP in sensitization of STT neurons after peripheral inflammation was examined. STT neurons were retrogradely labeled by fluorescent dextrans injected in the thalamus of control and arthritic rats. The animals were injected with a mixture of kaolin and carrageenan into the knee joint for induction of experimental arthritis. Presence of calcium binding proteins (calretinin-CR, parvalbumin-PA, calbindin-CA) in STT neurons was assessed immuno-histochemically in fixed spinal cord slices from lumbar segments L_{3-5}. Our experiments showed that only a small fraction of STT neurons in the rat contain Calbindin and Parvalbumin and about 40 % of them contain Calretinin under control conditions. There was a significant increase in number of STT neurons expressing Calbindin and Parvalbumin on the side of the arthritis compared to STT neurons on the contralateral side and in control animals. However, both the area and the intensity of immunohistochemical staining for Parvalbumin were significantly decreased in the superficial dorsal horn area on the side of the arthritis when compared to the contralateral side. Our results suggest that peripheral inflammatory processes can induce increased expression of calcium binding proteins in STT neurons. This increase of calcium buffering proteins can change calcium homeostasis in these cells and affect calcium dependent processes such as synaptic plasticity - neuronal sensitization.

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BEHAVIOR OF THREE RAT STRAINS IN THE MORRIS WATER MAZE

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The Morris Water Maze is a commonly used device to investigate spatial learning and memory in laboratory rodents. During the last 20 years it has become one of the most frequently used laboratory tools in behavioral neuroscience (1). In our experiments, the main aim was to test the effect of rat strain on the behavior of rats with different activity of hypothalamo-pituitary-adrenal (HPA) axis; these experiments are an extension of our behavioral studies investigating stressor specific changes and the interaction of stressors and amphetamine (AMPH) (2,3). We used three male rat strains, namely Wistar (Velaz, Prague), Sprague-Dawley and Lewis rats (Charles River Laboratories, Germany); the latter are known to have a deficient HPA axis activity and reveal several biochemical and behavioral differences from other strains. We used a typical water maze with an automatic registration of rat movement. Rats were trained to find an invisible escape platform from 4 cardinal points. The rat performance was expressed as mean latency and distance to reach the platform, and several other parameters were calculated. More advanced parameters (path length efficacy, latency, heading accuracy, bearing accuracy, tortuosity, thigmotaxis, segment and rings used) were analyzed by using computer program TRAM. Wistar rats learned the task during 4-5 days. Both average escape latency and distance were different in other two rat strains; Lewis rats revealed slower and Sprague-Dawley rats faster learning than Wistar rats. The extension of learning up to 18 days showed that Lewis rats never reached performance comparable to Sprague-Dawley rats. Our results provide another support for idea of strain differences in learning ability of rats and represent basis for our further experiments testing the interaction of various stressors with psychostimulants, like amphetamine.

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HYPOVENTILATION AND AMPLITUDE CHANGES OF ECG IN THE DEPENDENCE ON THE LIGHT/DARK CYCLE IN FEMALE WISTAR RATS

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Hypoventilation (HV) as one of disorders of normal ventilation decreased the electrical stability of the heart and increased the vulnerability to the ventricular arrhythmias. Therefore the aim of this study was to investigate the effect of 20 minute HV on amplitudes of ECG in the dependence on the alternation of the light and the dark cycle in female Wistar rats. The experiments were performed in the anesthetized (ketamine-100 mg/kg i.m. + xylazinum-15 mg/kg i.m, Léčiva, Praha) female rats adapted to the light-dark regime 12:12 hours. The rats were subjected to the following condition of ventilation: 1) intact group without artificial ventilation, 2) group (after tracheothomy and thoracothomy with 5 min artificial ventilation (parameters of ventilation: 40 breath/min., 1 ml/100 ml g of body weight), 3) group after 20 minute of HV (parameters of ventilation: 20 breath/min., 0.5 ml/100 g of body weight. The ECG parameters were evaluated from the 2nd limb lead of ECG. The significance of differences were evaluated by Student´s t-test. On the strength of comparison of ECG amplitudes in the light and the dark phase, the following significant changes were found, in the intact group, with the higher P wave amplitude (0.06 mv vs. 0.032 mV ($p<0.01$), and the lower R wave amplitude (0.41 mV vs. 0.56 mV ($p<0.01$) in the dark phase. In the 2. group, the lower T wave amplitude (0.02 mV vs. 0.021 mV ($p<0.5$) in the dark phase and in the 3. group, the higher T wave amplitude (0.19 mV vs. 0.12 mV ($p<0.05$) only after 15 min HV in the dark phase. It is concluded that prolonged hypoventilation in the light and the dark phase of the rat regime day has non-unequivocal influence to changes of ECG amplitudes.

EXERCISE CARDIOPULMONARY DATA MEASURING SYSTEM AND SOFTWARE FOR STATISTICAL EVALUATION OF RESULTS

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Exercise testing offers the investigator the possibility of simultaneously studying the cellular, cardiovascular and ventilatory systems responses under conditions of precisely controlled stress. Exercise testing with appropriate gas exchange measurements can also serve to grade the adequacy of cardiorespiratory function. This is of significant practical impact because of the increased number of therapeutic options now available for conditions that cause exercise limitation. Moreover, an individual patient may have mixed defects (e.g., cardiac and respiratory), and consequently, it is often necessary to determine the relative contribution of each to the patient's symptoms. Exercise testing can also provide vital information regarding the limits of systemic function before surgery or other therapy. Application of these systems is possible in work medicine, sport medicine and rehabilitation. The KARD is a system for exercise testing which is used for exercise testing in laboratory (1). For measuring data evaluating, the program KONSIL was developed. All measured data and personal data of the patient are stored in Microsoft database (*.MDB). The program can display and print many types of protocols and graphs (2). The curve can be filtering by least-squares data smoothing or median. For statistical analysis, the program KONS_VYB was developed. This program was developed for statistical analysis of stress test results for group of subject. The programs and systems for automatic stress testing have been developed in co-operation with doctors for more then 18 years and are used in several function laboratories in the Czech Republic.

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BLOCKAGE OF SERCA2 IN NORMAL AND DIABETIC ALBINO RAT HEART, THE EFFECT OF INSULIN

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Insulin is known to influence calcium cycling in cardiomyocytes – it affects L-type of calcium current, Na^+-Ca^{2+}-exchanger and Ca^{2+}-ATP-ase of sarcoplasmic reticulum (SERCA2). The aim of our work was to study the effect of insulin on cardiac contraction-relaxation cycle in control and diabetic albino rats under a blockage of SERCA2. The activity of SERCA2 was inhibited with cyclopiazonic acid (CPA), which is a selective blocker of this pump. Insulin-dependent diabetes was induced with streptozotocin (STZ) dissolved in a citrate buffer (65 mg/kg of body weight). Control animals were injected with citrate buffer only. Experiments were performed on the right papillary muscles 16 weeks after streptozotocin administration. Muscle preparations were stimulated with frequency from 0.1 to 10 Hz in the control Tyrode solution, in the Tyrode solution containing CPA (3 μM/l) and in the Tyrode solution containing CPA (3 μM/l) and insulin (80 IU/l). The following parameters of contraction were recorded by means of a mechanoelectrical transducer: maximum force of contraction (MG), time to peak of contraction (TTP), half-time of relaxation (R/2), time to 90 % relaxation (R_{90}) and time from 50 % relaxation to 90 % relaxation (R_{50-90}). CPA reduced MG in both experimental groups; this effect of CPA was potentiated by insulin. CPA and combination CPA+ insulin did not affect TTP and R/2 in both control and diabetic rats, but insulin itself shortened TTP and R/2 in the control group. R_{90} was significantly longer with CPA in normal and STZ rats. The subsequent application of insulin in the control group returned R_{90} to control values in contrast to the diabetic group, where insulin did not influence R_{90} in the medium with CPA. The similar effect was observed in R_{50-90}, where insulin reversed the prolongation of R_{50-90} induced by CPA in controls but not in diabetic rats. We conclude that CPA had the negative inotropic effect in the myocardium of control and diabetic rats and it decelerated mainly the second half of relaxation. In the control group but not in the STZ one, insulin inhibited the effect of CPA on duration of relaxation. These results suggest that insulin stimulates the activity of SERCA2 in normal rats but it does not affect sarcoplasmic Ca^{2+}-ATP-ase in the heart of diabetic rats.

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EFFECT OF THE LD CYCLE ON THE PRECONDITIONING BY HYPOVENTILATION IN WISTAR RATS

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The aim of study was to evaluate the effect of the light - dark (LD) cycle on the electrical stability of the heart during hypoventilation/reoxygenation after preconditioning by hypoventilation. The ventricular arrhythmia threshold (VAT) was measured in anesthetized female Wistar rats (ketamine/xylazine 100mg/ 15mg/kg, i.m., open chest experiments). The preconditioning (PC) was induced by one (1PC), two (2PC) or three (3PC) cycles of 5 minute hypoventilation followed by 5 min. reoxygenation. The effect of the light period (control group - without preconditioning n=12, 1PC n=8, 2PC n=10 and 3PC n=8) was followed after adaptation of animals to LD cycle of 12h : 12h, with the dark part of day from 18.00 to 06.00 hour. The effect of the dark period (control group - without preconditioning n=19, 1PC n=9, 2PC n=15 and 3PC n=11) was followed after inverse setting of LD cycle, with the dark period from 06.00 to 18.00 hour. The VAT from period of 20 min. hypoventilation and 20 min. reoxygenation were compared to initial VAT values measured after tracheotomy, thoracotomy and 5 min. stabilization under normal artificial ventilation. The parameters of hypoventilation - V_T = 0.5 ml/100g, 20 breaths/ min. and normal ventilation and reoxygenation - V_T = 1ml/100g, 40 breaths/ min. The experiments were performed during the whole year and the results were averaged independently of seasons. In the light part of day, 20 min. hypoventilation significantly decreased the VAT in the control and 1PC groups ($p<0.05$) and non-significantly in 2PC vs. the initial values. Reoxygenation reversed the VAT values to the initial level only in the control group. In 3PC, the VAT was increased from 2.32±0.57 mA to 4.41±1.24 mA during hypoventilation ($p<0.001$) and to 5.71±0.95 mA during reoxygenation ($p<0.001$). In the dark part of day, no statistically significant differences were found between initial thresholds and VATs from hypoventilation and reoxygenation. It is concluded that the cardioprotection against the hypoventilation/reoxygenation - induced decrease of VAT proved to be effective only after three cycles of preconditioning by hypoventilation and only in the light part of day. The rat myocardium is probably more sensitive to the systemic hypoxia induced by hypoventilation in the light part of day than in the dark one.

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REGULATION OF THE EXPRESSED $Ca_v3.1$ CHANNEL BY DITHIOTHREITOL

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Oxidation state of sulfhydryl group may influence behavior of proteins containing cysteine residues in functionally important regions. Dithiothreitol (DTT) is sulfhydryl-reducing agent known to dissolve disulfide bonds formed between two cysteines. It was demonstrated, that DTT affects the function of high voltage activated calcium channels, which contain several cysteine residues within their pore region and several other regions. Effect of DTT on low-voltage activated calcium channels was not described yet. Ca_v 3.1 calcium channel belongs to the family of low-voltage activated calcium channels. In our study we investigate effect of DTT on currents through Ca_v 3.1 calcium channel permanently expressed in HEK 293 cells using whole-cell configuration of patch-clamp method. Holding potential in all experiments was –100 mV. The effect of DTT was evaluated after incubation of HEK 293 cells in bath solution containing 2 mM 1.4-DTT for 20 min. Current-voltage relationship (I–V), steady-state inactivation (SSI) and current amplitude were compared with and without DTT treatment. Both I-V and SSI were significantly shifted in hyperpolarizing direction after DTT treatment (Table).

	activation $V_{0.5}$ (mV)	dV	inactivation $V_{0.5}$ (mV)	dV
Control	-44.9±0.4	3.8±0.1	-67.5±0.3	3.9±0.1
DTT	-48.5±1.2	3.8±0.2	-71.8±1.5	4.2±0.1

When DTT was directly applied into experimental chamber, DTT decreased gradually current amplitude measured at the depolarization to –30 mV (peak of I-V). We concluded, that DTT treatment affects significantly function of the expressed Ca_v 3.1 calcium channel. However, these experiments do not allow to distinguish between direct effects on channel protein and indirect effects mediated by other cellular proteins.

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BEHAVIORAL EFFECT OF SUBUNIT SELECTIVE ANTAGONIST OF NMDA RECEPTOR IN ANIMAL MODEL OF SCHIZOPHRENIA

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Congenital or neonatal viral infections remains compelling, but not yet proved hypothesis of schizophrenia. Even though we cannot induce schizophrenia in an animal we can still use animals to test a proposed role of retroviruses in the etiology of this psychosis. Virus-infected macrophages/macroglia overproduce quinolinic acid (QUIN) that selectively interacts with *N*-methyl-D-aspartate receptor (NMDA-R) heteromers containing NR2B subunit. As protein expression of this receptor subunit prevails in neonatal rat hippocampus, intraventricular (i.c.v.) infusion of QUIN (0.25 μmol/0.25 μL saline/lateral ventricle) on postnatal day (PND) 12 led to behavioral abnormalities in early adulthood (1). The aim of this study was to determine whether neonatal administration of Ro-25-6981 (Sigma/RBI, Prague), a high affinity antagonist of NMDA-R containing the NR2B subunit, protects against the QUIN-induced behavioral deficits. Therefore, Ro 25-6981 was injected (i.p.) in a dose of 10 mg/kg 0.5 h before and 22 h after the i.c.v. infusion of QUIN. Thirty two day after neonatal brain lesions, the behavior of each animal in groups of naive (intact), sham-operated (control) and QUIN-treated rats was assessed under three testing paradigms: (a) spontaneous locomotor activity was registered by Ethovision software (Noldus, The Netherlands), and 2-3 days later, (b) acoustic startle responses (ASR) to 120-dB acoustic stimuli and (c) prepulse inhibition (PPI) of ASR were measured using a startle chamber (SR-Lab System, San Diego Instruments, USA). We established that a decrease in the hippocampal protein expression of NR2B subunit led to locomotor hyperactivities in both QUIN-lesioned and QUIN-lesioned/Ro-25-6981-treated rats comparing with their respective controls. In contrast, ASR in QUIN-lesioned and QUIN-lesioned/Ro-25-6981-treated young adults was non-significantly increased whereas the disruption of PPI was prevented by the neonatal treatment with Ro-25-6981 in QUIN-lesioned rats only. We conclude that NR2B subunit may be involved in certain aspects of schizophrenia-like behavior but further investigation of the functional role of this NMDA receptor subunit is needed.

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THE CHANGES OF MUSCARINIC, BETA-ADRENERGIC AND D_2-LIKE RECEPTORS IN ACETYLCHOLINESTERASE KNOCKOUT MICE

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Acetylcholinesterase (EC 3.1.1.8, AcChE) deficiency (AcChe -/-) leads to the hyperstimulation of muscarinic receptors (MR) resulting in down-regulation. Li *et al.* (1) and Decossas *et al.* (2) have reported a decreased number of MR in different brain regions. In this research we have focused on the properties of MR, beta-adrenoceptors (BAR) and D_2-like dopaminergic receptors (D2R) in AcChE -/- animals in brain cortex, cerebellum, lungs and hearts using radioligand binding studies with ^{3}H-QNB (for MR), ^{3}H-CGP12177 (for BAR) and ^{3}H-spiperon (for D2R). There was a substantial decrease in lung MR (57 % of control) which was accompanied by a decrease in BAR (63 % of control). In brain cortex, the MR were diminished to 45 % of control, the BAR were not changed and D2R were increased to 148 % of control. As with the other tissues, there was a substantial decrease of MR in cerebellar tissue (46 % of control). This decrease in MR was accompanied by decrease in BAR (66 % of control) and in D2R (53 % of control). The heart tissue completely differed in receptor changes in comparison to the tissues tested: the MR in the heart were more than doubled (206 % of control). The BAR were not changed. Our results have shown that not only are MR affected (down-regulated) by the elimination of acetylcholinesterase, but other receptor systems (BAR, D2R) are influenced as well. The only exception was the cardiac tissue in which the up-regulation of MR occurred. These findings raise the question about the nature of the up-regulated MR in the heart.

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FUNCTIONAL AND STRUCTURAL CHANGES IN THORACIC AORTA OF 4-WEEK-OLD HYPERTENSIVE RATS

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In adult animals hypertension is accompanied by endothelial dysfunction characterized by important functional and structural changes in vessel wall (1). The aim of this study was to evaluate functional and structural changes in young rat thoracic aorta during early stage of hypertension. Three experimental groups of rats (all 4 weeks of age) were used: 1) control normotensive Wistar rats, 2) hereditary hypertriglyceridemic rats (hHTG), and 3) nitric oxide (NO)-deficient hypertensive rats (offsprings fed by dams administered L-NAME after delivery for a period of 4 weeks). Aortic rings from all animal groups were mounted in organ baths, and changes in tension were monitored. For morphological evaluation the animals were perfused with a glutaraldehyde fixation and thoracic aorta was excised and processed for electron microscopy. Geometry of thoracic aorta was measured using light microscopy. Systolic blood pressure in hHTG rats was slightly (by 15 %, $P<0.05$) and in NO-deficient hypertensive rats was markedly (by 48 %, $P<0.001$) higher than that of age-matched control rats (95±4 mmHg). The heart weight/body weight ratio in hHTG rats was 5.39±0.09 vs. 4.36±0.25 in controls ($P<0.01$), indicating hypertrophy of the heart, but in NO-deficient rats was 4.30±0.17 only. Concentration-response curves to acetylcholine in phenylephrine-precontracted aortic rings from hHTG and NO-deficient hypertensive rats were not significantly different from that of control normotensive controls. Maximal responses of thoracic aorta to noradrenaline were significantly reduced in both groups of hypertensive rats. The values of wall thickness and cross-sectional area of thoracic aorta in hHTG and NO-deficient hypertensive rats were significantly decreased in comparison to control group. No difference was observed in wall thickness/inner diameter ratio between hHTG rats and age-matched controls, but in NO deficient hypertensive rats this ratio was decreased. The results suggest that alteration in structure of arterial wall proceeds the occurrence of endothelial dysfunction present in adults.

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RHYTHM SUSCEPTIBILITY OF L-THYROXINE-TREATED RATS TO LOW K^+-INDUCED VENTRICULAR FIBRILLATION AND THEIR ABILITY TO RESTORE SINUS RHYTHM

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We examined the contribution of L-thyroxine (T4) administration to incidence of low K^+-induced ventricular fibrillation (VF) as well as to ability of the heart to recover sinus rhythm and mechanical function. The experiments were performed on isolated heart preparation using the heart of 4 and 20 month-old female Wistar rats without and with feeding with T4 50 μg/100g/day during two weeks). Perfusion of the heart with oxygenated Krebs-Henseleit solution at a constant pressure and temperature was followed by perfusion with K^+-deficient one until occurrence of VF (<10 min). 2 min of sustained VF was followed by perfusion with normal solution for 10 min during which sinus rhythm was restored. ECG, left ventricular pressure and coronary flow were continuously monitored. The results showed that compared to untreated rats, the onset of low K^+-induced ventricular premature beats was delayed and their number was decreased in both T4-treated groups. Nevertheless, VF occurred earlier in young T4-treated versus untreated rats (6.78±0.28 *vs* 9.59±0.55 min, $P<0.05$) while the difference was not a significant in old one (6.31±0.69 *vs* 7.18±0.67 min). Furthermore, sinus rhythm appeared earlier in old T4-treated rats compared to untreated (7.28±0.72 *vs* 8.84±0.81, $P<0.05$ min) while almost in the same time in young rat hearts regardless of the treatment. In conclusion, our results indicate that administration of pharmacological dosage of T4 can increases a risk of low K-induced VF in young but not in old animals, in which even facilitates restoration of sinus rhythm. Moreover, post-fibrillation mechanical function was enhanced in both young and old T4-treated hearts.

CHANGES OF CORTICAL INTERHEMISPHERIC EVOKED POTENTIALS AFTER STATUS EPILEPTICUS IN DEVELOPING RATS

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Convulsive status epilepticus (SE) led to an acute degeneration of neurons in many brain structures, among other also in the motor area of the cerebral cortex. To know if this morphological damage has functional consequences cortical interhemispheric evoked responses were studied at different intervals after SE. SE was elicited by pilocarpine (40 mg/kg i.p.) 24 hours after LiCl pretreatment in 12- and 25-day-old rats. Paraldehyde (0.3 ml/kg i.p.) was used to interrupt SE after two hours of continuous seizures. Younger animals were returned to their mothers, older rats needed an intensive care during two days after SE. Control siblings were treated in the same way only saline was administered instead of pilocarpine. Cortical stimulation and registration electrodes were implanted 3, 6, 9, 13 or 26 days after SE and after at least one-hour rest the animals were taken into experiment. Constant-current output stimulator generated biphasic pulses of 1-ms duration. Intensity was increased stepwise from 0.2 to 4.0 mA, eight responses were always averaged. An input-output curve was constructed using an amplitude of the first positive and negative waves as a measure of output. After the end of experiment brains of all rats were histologically checked. No difference in the response was found in short intervals after SE induced in 12-day-old rats. Significantly higher responses were found only at the longest interval, i.e. 26 days after SE. Degenerating neurons as demonstrated by FluoroJade B method 3 days after SE were not numerous in this age group. Their localization and shape indicated that nearly all of them might be interneurons. In contrast, there was a marked tendency to higher responses in rat pups seized at the age of 25 days. Statistically significant differences were demonstrated with lowest intensities of stimulation practically at all intervals after SE. There were many FluoroJadeB positive neurons including also pyramidal cells 3 days after SE in this group. Higher amplitude of monosynaptic cortical responses may indicate an increased excitability of the sensorimotor cortex. Different time course of changes in the two age groups indicate age-specific time course of functions of the motor cortex.

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ALPHA-2-MACROGLOBULIN AS BIOCHEMICAL MARKER OF LIVER FIBROGENESIS

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Liver biopsy is the gold standard for assessing fibrosis. The use of biochemical parameters as fibrosis markers could substantially reduce the number of biopsies performed for the management of chronic hepatic viral infections. Recently was reported good correlation of alpha-2-macroglobulin (AMG) to the activity of liver fibrosis. AMG is secreted by tissue macrophages and fibroblasts and functions in the environment of extracellular matrix macromolecules. Aim of the study: to investigate the levels of AMG, potential fibrogenesis marker, in correlation to histological staging in patients with chronic viral hepatitis and to compare the levels of AMG with the levels of hyaluronic acid which is accepted as marker of liver fibrogenesis. Blood samples from 64 patients with chronic hepatitis (23 hepatitis B and 41 hepatitis C) and 20 blood samples from healthy controls were assayed for AMG. Fibrosis in liver biopsy specimens were staged on scale of 0-4: 0 - no fibrosis, 1 - portal fibrosis without septa, 2 - few septa, 3 - numerous septa without cirrhosis, 4 - cirrhosis. AMG was estimated immunochemically and hyaluronic acid with enzyme-immunoassay. The levels of AMG were elevated in patients with chronic hepatitis and liver cirrhosis. After dividing of patients according to the results of histological grading of fibrosis, there was not difference between group 0 and healthy controls. Patients with fibrosis 1-3 had significantly higher levels of AMG than controls. There was significantly higher levels of AMG in group 3 in comparison to group 1-2. The group of patients with liver cirrhosis had higher level of AMG than patients with chronic hepatitis (2723 mg/l vs. 1954 mg/l). There was significant correlation between levels of hyaluronic acid and AMG ($r=0.43$, $P<0.001$). According to the results of our study we can concluded, that the estimation of AMG significantly correlated with the presence and activity of liver fibrosis and could be helpful in the diagnosis and monitoring of liver fibrosis.

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EFFECT OF ESSENTIAL PHOSPHOLIPIDS ON PLASMA CERULOPLASMIN AND ITS ENZYMATIC ACTIVITY

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Copper is essential trace element. In the blood plasma copper is transported in the form of ceruloplasmin. Ceruloplasmin is not only simple transport protein but it is multifunctional protein with various other physiological functions. At least a part of these functions is connected with ceruloplasmin´s enzymatic activity. Recent studies in humans suggest that the specific enzymatic activity of ceruloplasmin is a more sensitive indicator of copper status than either serum copper and ceruloplasmin or erythrocyte superoxide dismutase. Aim of the study: to estimate the effect of administration of essential phospholipids on the metabolism of copper and ceruloplasmin in patients with liver steatosis. An open clinical trial was performed in patients suffering from liver steatosis. Two capsules of Essentiale forte (Rhone-Poulenc Rorer) were administered 3 times daily for 3 months. The serum level of copper was determined by atomic absorption spectrophotometry (Varian AA-475). The amount of ceruloplasmin protein was estimated immunochemically and the enzymatic activity of ceruloplasmin was estimated as polyphenoloxidase activity. The copper level in patients with liver steatosis was moderately decreased in comparison to healthy controls (15.45 vs. 17.44 $\mu mol.l^{-1}$). The therapy with essential phospholipids had no significant effect on copper level (15.45 vs. 15.43 $\mu mol.l^{-1}$). The difference in ceruloplasmin levels between patients with liver steatosis and healthy controls was also not significant (348 vs 343 $mg.l^{-1}$), but the specific activity of ceruloplasmin in patients was significantly decreased in comparison to controls (0.57 vs. 0.82). The specific activity of ceruloplasmin was after therapy moderately increased in comparison to level before therapy. Thus the decreased specific activity of ceruloplasmin in spite of normal levels of copper and ceruloplasmin in patients with liver steatosis suggested some problems in copper metabolism in these patients. The results of our study showed positive effect of therapy with essential phospholipids on copper metabolism in patients with liver steatosis.

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STRUCTURE OF CORONARY ARTERY OF SHR IN PREHYPERTENSIVE AND EARLY HYPERTENSIVE PERIOD OF ONTOGENIC DEVELOPMENT

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The aim of the study was to evaluate geometry and structure of the septal branch of the left descending coronary artery (RS) of 3 week (3w) and 9 week old (9w) control Wistar and Spontaneously hypertensive rats (SHR). Sixteen controls and sixteen SHR of both ages were used. Blood pressure (BP) was measured non-invasively on the tail artery using plethysmographic method. After sacrificing the rats were perfused with a glutaraldehyde fixative at a constant pressure of 90 mm Hg (3w) and 120 mm Hg (9w) for 10 min via a cannula placed in the left ventricle. After fixation the upper part of the RS was excised and processed according to standard electron microscopy procedure. Wall thickness (WT) and inner diameter (ID) were measured on semithin sections and cross sectional area (CSA) and WT/ID ratio were calculated. Volume densities of endothelial cells (EC), smooth muscle cells (SMC), and extracellular matrix (ECM) were determined by the point counting method in electron microscopy. From the volume densities the areas of EC, SMC, and ECM were counted. No difference in BP between 3w old Wistar rats (83±1.9 mm Hg) and SHR (84±1.4 mm Hg) was observed. The difference in this respect was found between groups of 9w old rats (106±1.1 mm Hg vs. 154±1.4 mm Hg in SHR, $p<0.01$). In prehypertensive period (3w old rats): Both WT (9.60±0.25 μm) and arterial wall mass (CSA) (5790±244 μm²) in controls did not differ from WT (9.67±0.48 μm) and CSA (5194±401 μm²) of SHR. Decreased ID (182±6.0 μm vs. 159±6.2 μm, $p<0.05$) and increased WT/ID ratio (0.054±0.0017 vs. 0.062±0.0033, $p<0.05$) in SHR indicated remodeling of arterial wall (inward). No changes in areas of EC, SMC, and ECM were found between controls and SHR. In hypertensive period of SHR (9w old rats) increase of WT (10.6±0.88 μm vs. 13.83±0.59 μm, $p<0.01$), CSA (7579±619 μm² vs. 9845±385 μm², $p<0.01$), WT/ID ratio (5.06±0.64 vs. 6.62±0.40, $p<0.05$) was found. No difference was observed between groups in ID (220±11 μm vs. 212±5 μm in SHR). Areas of SMC (4964±313 μm² vs. 7220±282 μm², $p<0.01$) and ECM (1424±90 μm² vs. 1795±70 μm², $p<0.01$) were increased in arterial wall of SHR. No difference was found in areas of EC between controls (879±32 μm²) and SHR (825±32 μm²). In conclusion, remodeling of arterial wall (inward) was already observed in prehypertensive period of SHR. Hypertrophy of arterial wall was found in hypertensive period of ontogenic development of the SHR.

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THYROID HORMONES REGULATE DIFFERENTLY MUSCLE MHC ISOFORMS AND THEIR mRNA LEVELS

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We studied expression of myosin heavy chains (MHC) isoforms and their mRNAs in the slow soleus (SOL) and fast extensor digitorum longus (EDL) muscles of adult euthyroid, hypothyroid and hyperthyroid female inbred Lewis rats. The hyperthyroid state was induced by Na 3,3',5-triiodo-L-thyronine (T3), while methimazole was employed for inducing hypothyroidism. The fiber type composition was determined according to the mATPase activity and immunocytochemical determination of MHC isoforms using the C.A.S.T. Grid system (Olympus, Denmark) based on 2-D stereological methods. The MHC isoform composition was determined by the SDS-PAGE and the mRNA level for each MHC isoform by RT-PCR. In euthyroid rats, SOL muscle contained ~97 % of slow type 1 fibers as determined by histo- and immunocytochemistry and 96 % MHC1 isoform determined by SDS-PAGE. EDL muscle contained 5.7 % of slow type 1 fibers, 24.0 % of fast 2A, 37.2 % of fast 2X/D and 42.9 % of fast 2B fibers. As shown by SDS-PAGE, EDL muscle contained 4.9 % MHC1, 14.8 % 2a, 33.5 % 2x/d and 47.2 % 2b MHC isoforms. In hypothyroid rats, the SOL muscle contained almost exclusively slow fibres, while the EDL contained 7 % of slow MHC1 and only 37 % of the fastest 2b MHC isoform. Conversely, in hyperthyroid rats, SOL muscle contained only 93.5 % of slow type 1 fibers and 81.3 % of MHC1 isoform, whereas in fast EDL the percentage of slow fibres decreased by about one half compared to euthyroid rats. Thyroid status thus significantly affected muscle phenotype. On the other hand, expression of mRNAs was less affected by the thyroid treatment and the levels of mRNAs for individual MHC isoforms were not substantially influenced by altered thyroid status. We conclude that thyroid hormone regulation of MHC isoform expression acts differently at translational and transcriptional level.

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A NEW TYPE OF OPTICAL SENSOR FOR THE DETECTION OF TONGUE MOVEMENTS DURING DRINKING IN RATS

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The simultaneous recording of both microstructure of licking (lapping) and of electrical brain activity seems to be a desirable method in some drinking experiments. The licking/lapping sensor has to fulfill some necessary conditions then. Since the animal's body must not be a part of the electrical circuit, electrical sensors should be excluded. An access restriction ensuring that only tongue protrusion will be detected must be minimized. We have constructed a new type of optical lapping sensor, which fulfils both above requirements. The corpus of the apparatus is built up from a plastic Petri dish (diameter 100 mm). Drinking opening (diameter 8.7 mm) is drilled in the cover and the dish is filled with water. An optical gate controls the access to the opening. When a rat wants to get a drop of water the tongue movement interrupts the beam in the gate. Each tongue protrusion is considered to be one lick. Light emitting diode and receiving diode are coaxial and are located in the upper surface of the dish cover; water surface is 5 mm under this level. The photoemissive cell of the optical gate sends the infrared beam (wave length 950 nm) and the receiving diode provides the static signal 14 mV against the 7 mV background. When the beam is interrupted by the tongue the voltage goes down. This analog signal is fetched into the comparator with the threshold level 9.5 mV. The comparator output supplies optical coupler for the computer input. The microstructure of the lapping can be recorded without any artifacts. The configuration of the apparatus ensures a good access to water with a minimal restriction. It was validated by measuring of the lapping frequency. Mean value 6.8 Hz corresponded well with the frequency of the lapping from an open pool of water without any restriction (6.9 Hz) (1).

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TOTAL PERIPHERAL RESISTANCE IN DIFFERENT CLINICAL CONDITIONS – AN UNDERAPPRECIATED AREA IN UNDERSTANDING PHYSIOLOGY AND PATHO-PHYSIOLOGY OF CIRCULATORY SYSTEM

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In principle, changes in total peripheral resistance (TPR) can affect the two other basic hemodynamic quantities such as cardiac output and arterial blood pressure. The present conference contribution pays attention to a variety of chronic clinical abnormalities characterized by either much less than or much greater than normal long-term TPR that causes significant changes in cardiac output and/or regional blood flow but has no effect on the systemic arterial pressure. Different authors explain these phenomena differently, and sometimes in a contradictory manner, in contemporary medical textbooks. Thus, existing theoretical (patho)physiological models are examined in the contribution to put forward a consistent approach to this area.

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BIOLOGICAL EFFECTS OF ELECTROMAGNETIC FIELDS

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A brief survey of some contemporary approaches concerning effects of low or high frequency electromagnetic field (EMF) is depicted. Firsts group of tests included studies with low frequency EMF. Attention was paid to the transport of potentially neurotoxic metal: manganese into the rat's brain as affected by simultaneous exposure to EMF. Several inorganic manganese substances, applied by means of intratracheal instillation were used for tests. Exposure to low frequency EMF was realized within solenoid chamber with parameters of 50 Hz and induction B=10mT. The exposure period was 1 hour. There were 2 exposures per week for 3 months. Significant results, manifesting positive effect of EMF on the increase of brain manganese content in case of soluble manganese sulphate were ascertained. Tests with manganese oxide have shown its penetration into the brain within subchronic exposures, but additive effect of EMF was not verified. The second approach was devoted to the study of energy metabolism of rat skeletal muscle under the exposure to low frequency EMF of the same parameters as above mentioned during acute or subchronic test regimen. Following energy metabolism markers were observed: ATP, creatine phosphate, creatine, lactate, pyruvate, inorganic phosphate and proteins by enzymatic and spectroscopic methods in *musculus gracilis anticus* to evaluate the potential effect of two different exposures to EMF on the energy state of the muscle. Evaluation of results (comparison of exposed animals with their appropriate controls) by ANOVA or non-parametric Kruskal-Wallis test did not show any significant difference in tissue concentrations of all analyzed metabolites. The third approach is responding to the massive usage of mobile phones and its main task is the observation of the physiological and potentially morphological consequences of the exposure of rats to the simulated radiation from the mobile phone within acute and later on subchronic exposure regimen. The transport of metal - manganese - as a marker across the blood-brain barrier under the EMF exposure is being followed. Acute tests have enabled to standardize exposure conditions of electromagnetic field radiation.

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INFLUENCE OF MEMBRANE LIPID CONTENT ON ION TRANSPORT IN ERYTHROCYTES OF DAHL RATS WITH SALT HYPERTENSION

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It is known that salt hypertension of Dahl rats is accompanied by pronounced hypertriglyceridemia and hypercholesterolemia. We were interested how lipid metabolism could take part in salt hypertension. Dahl salt-sensitive rats on high-salt diet had increased levels of plasma triglycerides (1.47±0.09 *vs* 0.26±0.03, $p<0.001$) and total plasma cholesterol (3.09±0.12 *vs* 2.19±0.10, $p<0.001$) compared to salt-resistant rats. Furthermore, salt-sensitive rats differ from salt-resistant ones by total membrane phospholipid content (4.29±0.03 *vs* 4.06±0.04, $p<0.001$), but their membrane cholesterol did not change significantly (3.48±0.11 *vs* 3.39±0.05, NS). Erythrocyte membrane content of cholesterol and phospholipids correlated positively with Na^+ concentration in the erythrocytes ($r=0.486$ and $r=0.683$, $p<0.001$), with the activity of Na^+-K^+ pump ($r=0.566$, $p<0.001$ and $r=0.343$, $p<0.05$) and Na^+-K^+-$2Cl^-$ cotransport ($r=0.443$, $p<0.01$ and $r=0.602$, $p<0.001$) and also with Rb^+ leak ($r=0.419$, $p<0.01$ and $r=0.357$, $p<0.05$). Membrane phosphatidylserines + phosphatidylinositols were significantly associated with Na^+ concentration in the erythrocytes ($r=0.458$, $p<0.01$), with activity of Na^+-K^+ pump ($r=0.383$, $p<0.05$), Na^+-K^+-$2Cl^-$ cotransport ($r=0.491$, $p<0.01$) and with Rb^+ leak ($r=0.589$, $p<0.001$). Membrane content of sphingomyelins was related to the activity of Na^+-K^+-$2Cl^-$ cotransport ($r=0.538$, $p<0.001$). We can conclude that observed relationships between ion transport and the membrane content of cholesterol and/or sphingomyelins, which influence membrane fluidity, indicated how alterations of lipid metabolism might participate in the pathogenesis of salt hypertension.

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THE EFFECT OF HIGH-FREQUENCY ELECTROMAGNETIC FIELD ON SOME BRAIN CHARACTERISTICS IN HEALTHY AND NEURODEFECTIVE MICE

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The high-frequency electromagnetic field (HF EMF) is a factor the effect of which, especially on the brain function, is broadly discussed. The reason is its presence in many devices od daily use, mainly mobile phones. Despite some controversial results of until now performed studies some recent evidences of neuronal damage were found in certain brain structures of rats acutely exposed to HF EMF (1). Also, changes of working memory and evoked potentials were found in human as a consequence of mobile phone use (2,3). In this work we observed the effect of acute and chronic exposition to HF EMF (870 MHz) on brain function in normal and neurodefective – Lurcher mutant mice (+/Lc). The mutants suffer from olivocerebellar degeneration caused by mutation of the δ2 glutamate receptor gene, their healthy littermates are wild-type mice (+/+). In our experiments we used animals derived from two different strains (C3H, C57B1/7). In HF EMF exposed animals (+/Lc, +/+ of both strains) and unexposed controls the spatial learning was tested using Morris water maze. There were almost no significant differences in spatial learning between HF EMF irradiated animals and controls. The CNS excitability tested using audiogenic epilepsy method showed significant differences in chronically HF EMF exposed +/Lc of both strains, when irradiated animals exhibited significantly lower mean reaction than unexposed controls. The evaluation of CNS inhibition after i.p. injection of Pentobarbital (50 and 80 mg/kg body weight) showed that HF EMF exposed animals exhibited longer time of sleep but lower percentage of mortality in comparison with unexposed controls, but the differences were only on the margin of significance. The histochemical detection of NADPH-d activity and immunohistochemical examination of c-Fos positivity in the hippocampal dentate gyrus of some experimental animals showed differences that proved the effect of the exposition on parameters observed. The findings complete the recent results that exposition to HF EMF can influence the brain.

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LATERALISED GATING OF PAIN EVOKED POTENTIALS DURING ISOMETRIC MUSCLE CONTRACTION

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Influence of muscle contraction on pain-related evoked potentials, and whether muscle contraction and painful stimulation are presented on ipsilateral or contralateral hands have not been addressed as yet. To elucidate these points, subjects received painful electrical stimuli (0.2 ms pulses, ~2 s interstimulus interval, amplitude 20 % above pain threshold) on the 3rd finger of the right hand. These stimuli were presented during periods of relaxation of hand muscles, or during periods of isometric muscle contraction (pressing rubber tube with thumb and index finger) of the right or left hand. High resolution EEG (111 closely spaced scalp electrodes, 1024 Hz sampling rate) was acquired in ten right-handed men (average age 22±2 years). The sources of evoked potentials were analyzed using BESA 5.0 software. The source model involved source dipoles in the primary somatosensory and somatomotor areas (SI and MI), contralateral secondary somatosensory cortex (SIIc), ipsilateral SII (SIIi), anterior cingulate gyrus (GC) and in precuneus. Differences of source waveforms for painful stimulation alone, and painful stimulation plus right-hand muscle contraction or left-hand muscle contraction were tested using bootstrap method at a 95 % confidence level. We found significant decrease of peak amplitude in SI/MI during contraction of the right-hand muscles compared to painful stimulation alone. Source activities in SIIc and SIIi were smaller during contraction of the left-hand muscles compared to painful stimulation alone. In cingulate cortex, earlier peak (~160 ms) was attenuated by contraction of the right-hand muscles and the later peak (~240ms) by contraction of the left-hand muscles. Results suggest that pain-related evoked potentials are attenuated during isometric muscle contraction in a similar manner as are non-painful stimuli. Contraction of muscles of the dominant hand shows largest changes in the SI/MI and in early component of the cingulate cortex activity. In contrast to dominant hand, non-dominant hand muscle contraction attenuates bilateral SII cortices and the late component of the cingulate cortex activity. These data strongly suggest lateralized gating of pain-related evoked potentials during isometric muscle contraction.

OXIDIZING REAGENT COPPER-O-PHENANTHROLINE IS AN OPEN CHANNEL BLOCKER OF THE VANILLOID RECEPTOR TRPV1

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The TRPV1 channel plays an important role in generating nociceptive signals in mammalian primary sensory neurons. It consists of 838 amino acids with 6 transmembrane segments (TM1-TM6), a pore-forming loop between TM5 and TM6 and N- and C- terminals located intracellularly (1). It is a homotetramer and forms a nonselective cationic channel that can be opened by capsaicin, weak acids and noxious heat. There are 18 cysteines (Cys), three of which are located on the extracellular side of the receptor in and around the region of the pore-forming loop. Redox state of cysteines plays an important role in regulating activity of this channel (2). Here we report that the TRPV1 channel activated by noxious heat (> 43 °C), capsaicin (1 μM) or extracellular pH 5 in transfected HEK293T cells or cultured rat DRG neurons is blocked in the open state by an oxidizing agent Cu-o-phenanthroline complex (Cu:Phe). The effects of Cu:Phe are concentration dependent (IC_{50} = 5.2 : 20.8 μM) and fully reversible. Cu:Phe applied immediately before exposure to acidic solution, capsaicin or noxious heat is without effect. Substitutions of the extracellular Cys residues (616, 621, 634) by glycine individually or together do not alter the blocking effects of Cu:Phe suggesting that disulfide cross-linking does not represent the underlying mechanism. It is suggested that the complex Cu:Phe, a bulky, positively charged molecule, represents a very effective and reversible open channel blocker of TRPV1.

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PRINCIPLES OF ADRENERGIC AND CHOLINERGIC REGULATION OF QUANTA RELEASE DISPERSION AT FROG ENDPLATE

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Endplate currents are the result of a simultaneous secretion of many neurotransmitter quanta in response to a single nerve spike. The higher degree of synchrony can potentiate the synaptic transmission even when other parameters of postsynaptic responses are unchanged and *vice versa*. Some compounds, noradrenaline in particular, can substantially shorten the release time of evoked quantal release when present in the muscle bath. This results in an increase of the amplitude of reconstructed multi-quantal EPCs. Thus, noradrenaline facilitates synaptic transmission by making the release of quanta more synchronous (1). Acetylcholine (ACh) acts in a reverse manner than noradrenaline, i.e. it increases the dispersion of the synaptic latencies at frog neuromuscular junction (2). These results, contrary to adrenergic influence, led to a substantial decrease of the amplitude of reconstructed multi-quantal EPCs. From the physiological point of view, changed asynchrony by noradrenaline and ACh released either quantally or non-quantally would have a significant physiological impact on cholinergic synapse safety factor during tetanic stimulation and stress conditions, or in the presence of anticholinesterases (3,4).

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IDIOTHETIC NAVIGATION OF RATS IN POOLS OF SIMPLE GEOMETRY

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Research into allothetic and idiothetic navigation of laboratory rats did not yet lead to definite assessment of the importance of hippocampus for these modes of spatial cognition. The purpose of the present study is to develop a task based on navigation to a single intramaze location that can be implemented with the same success from any point at the circumference of the pool either by allothesis or by idiothesis. Convenient test location is the center of the circular pool which can be reached by a rat swimming from start perpendicularly to the wall for a distance equal to the radius of the pool (idiothesis) or by a rat swimming from the start to the most remote point of the pool and stopping at a locus equidistant from all parts of the wall (allothesis). Contribution of the above navigation modes to the animal's ability to search the goal in the center of the pool was examined in adult male Long-Evans rats. Well trained rats were able to locate the pool center in 1-min probe trials (in absence of the escape platform) not only when swimming in adequately illuminated room but also in total darkness, when they could only use idiothesis. The marginal improvement of probe trials in light against probe trials in darkness indicates that allothesis is not critical for performance of this navigation task. The importance of idiothesis was still more apparent in rats trained in a small pool (90 cm in diameter) and given the probe trial test in a large pool (190 cm in diameter) who searched the absent escape platform at the position indicated by idiothesis (45 cm from the start) and not in the center of the large pool indicated by allothesis. When rats alternating escape to the platform in center of a small and of a large pool were given a probe trial in the large pool in darkness, they concentrated their search to two locations corresponding to the above positions. Also navigation to the center of equilateral triangle, square, rhomb or oblong can also be achieved by idiothesis, but require start from specific departure points and stress importance of proximal allothetic cues. All experiments complied with the Czech law and with the directive of the European Communities Council No. 86/609/EEC on protection of laboratory animals.

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EARLY POSTNATAL INTERVENTION TO GLUTAMATERGIC SYSTEMS CHANGES THE NOCICEPTION OF ADULT RATS

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Dysregulation of glutamatergic system by neonatal brain infusion of quinolinic acid (QUIN) and/or *N*-acetyl-L-aspartyl-L-glutamate (NAAG) is implicated in animal modeling of schizophrenia (1). As schizophrenic patients really exhibited decreased pain sensitivity (2), we have hypothesized that the neonatal NMDA receptor dysfunction induced by QUIN (or NAAG) might modify the nociceptive transmission. Thus, we measured thermal nociceptive sensitivity in plantar and the tail-flick tests, using young adult rat males (PND 50) with neonatal brain injury (PND 12). The QUIN- and/or NAAG-injured rats were housed separately, but together with their sham-operated and naive siblings (7-10 rats/cage). In comparison with naive controls, QUIN-injured rats exhibited decreased withdrawal latencies of the tail flick and plantar test whereas NAAG-injured animals increased the latencies marginally. Nociceptive responses of the sham-operated rats paralleled their littermates neonatally treated with QUIN and/or NAAG. The responses were decreased and/or increased, respectively. No significant changes in nociception were observed in naive (control) animals, irrespective of their housing. Our results support the hypothesis that beside expected pathophysiological mechanisms, the nociceptive sensitivity may be influenced by environmental factors with dominant effects of social variables. Similar observations were also made in rats with the model of neuropathic pain, which were selected for high (HA) and low (LA) tendency to autotomy. Autotomy was less frequent in the HA rats if they were housed together with naive animals of the LA group and *vice versa* (3). We can conclude that control (sham-operated) rats share similar pattern of nociceptive behavior as their cagemates with the neonatally induced dysfunction of the brain glutamatergic system. In this respect naive (intact) rats were more resistant. The sham operation may have a nonspecific long-term impact on nociceptive behavior, which able to mimic the behavior of other animals.

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GEOMETRICAL MODELING OF THE ULTRASTRUCTURE OF MUSCLE CELLS

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Function of muscle cells closely relates to their ultrastructure, i.e., to the size, number, shape, and three-dimensional organization of their organelles, which altogether contribute to the characterization of the cell phenotype. These attributes are defined from electron-microscopical images of ultrathin sections of fixed muscle samples and quantified by means of stereological methods. Recent progress in computer sciences makes possible to integrate the knowledge on structure of muscle cells into geometrical models, which would be appropriate for visualization of complex 3D features, for testing hypotheses and for validation of measurements. In this study, we describe techniques of geometrical modeling of organelles and construction of typical parts of the muscle cells. Our approach is based on the theory of implicit surfaces (1) generated by skeletons, blending operations and shape transformations. A volumetric model was generated by interpolation of parallel modeling planes perpendicular to the longitudinal muscle cell axis. A parallel modeling plane is defined by a planar continuous graph that divides the plane into areas limited by closed polygons. In each plane the number, shape and topology of cell organelles (myofibrils, mitochondria, t-tubules, sarcolemma) is defined by means of functionally represented polygons (2). The final volumetric representation makes possible to calculate the volume and surface densities of organelles in the model and to visualize the model. The presented geometrical modeling software was written for PC in C++ under MS Windows 98 environment. It allows interactive modeling, user-friendly visualization, and generation of thin sections across the model at an arbitrary angle and position. Images of the sections are useful for testing feasibility of stereological studies and for optimization of quantification techniques.

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MODELING OF THE REACTION-DIFFUSION SYTEM OF THE DYADIC JUNCTION IN VENTRICULAR MYOCYTES.

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The spatio-temporal profiles of calcium and other cytosolic constituents of the cardiac dyad are not amenable to direct experimental studies. Previous reaction-diffusion models of this system were simplified to less than 3 spatial dimensions by either assuming cylindrical symmetry or by neglecting the height of the dyad. We have attempted to create a physico-chemical model of the dyadic junction capable of high spatio-temporal resolution that takes into account all three spatial dimensions and the kinetics of chemical reactions. The goal of the simulations with this model was to assess the spatio-temporal profiles of Ca^{2+} and Mg^{2+} ions, the two important modulators of the ryanodine receptor (RyR), during Ca^{2+} influx via a calcium spark. The model was implemented using the VLUGR3 solver (1, 2). The reaction-diffusion system in the model of the dyadic gap included Ca^{2+}, Mg^{2+}, ATP and calcium binding sites on the sarcolemma and was coupled to a cytosol model that additionally included the sarcoplasmic reticulum calcium pump, calmodulin and troponin. The calcium release site was implemented as a cluster of 49 RyRs, each carrying 0.36 pA calcium current (a total of 17.64 pA). The effect of RyR single-channel amplitude and the number and density of RyRs on the 4D (xyzt) profiles of Ca^{2+}, Mg^{2+}, ATP, CaATP and MgATP was examined under these conditions. The calcium concentrations of ~1 mM reached at the central part of the release site, as well as the peak cytosolic calcium concentration of ~ 1 μM reached within 10 ms of calcium release flux suggest that the simulated total calcium flux is of appropriate size. The concentration of free Mg^{2+} in the dyadic gap may increase up to twice during calcium release. Although substantial, this increase of Mg^{2+} should not affect RyR kinetics appreciably, because it happens at the background of a saturating concentration of Ca^{2+}. A substantial difference was found between the spatial distribution of calcium and magnesium concentration gradients.

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COMPARISON OF FLUORESCENT INDICATORS FOR MEASUREMENT OF LOCAL CALCIUM SIGNALS

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Fluorescent calcium indicators are extensively used for detection of changes in the cytosolic concentration of calcium, an intracellular signaling messenger. Maximization of fluorescence in response to a given increase in the local calcium concentration is vital for measurements of local calcium signals with high spatio-temporal resolution. The measured fluorescence signal is dependent on the indicator's absorptivity, its maximum fluorescence ratio (F_{min}/F_{max}), as well as on the rates of calcium binding and dissociation to/from the indicators. For measuring calcium transients, the high-affinity indicators fluo-3 and fluo-4 are most often used. Fluo-4 was claimed to produce better results than fluo-3 (1). For measurements of local calcium fluxes, fluo-3 as well as Oregon Green (OG) BAPTA-5N have been used previously. We have compared them to Calcium Green-5N, which has similar properties to OG BAPTA-5N (2), and Rhod-5N, which has a much higher fluorescence increase upon Ca^{2+} binding. We have measured the basal and peak fluorescence and their standard errors during local calcium signals measured in the absence of EGTA (calcium sparks) and in the presence of 4 mM EGTA/2mM Ca^{2+} (calcium spikes), as well as the time-to-peak and duration of the calcium signals. Our results suggest that the difference in the performance of fluo-3 and fluo-4 for measurements of local calcium transients - calcium sparks - is lower than expected from their published properties. For measurement of local calcium fluxes – calcium spikes, Oregon Green BAPTA 5-N is preferable to fluo-3 or fluo-4 due to its faster kinetics of Ca^{2+} dissociation. The two remaining low-affinity indicators, Calcium Green BAPTA 5-N and Rhod-5N, do not provide sufficiently high fluorescent signals to enable measurement of Ca spikes. This was surprising especially for Calcium Green-5N, whose F_{min}/F_{max} as well as calcium affinity are very similar to those of Oregon Green BAPTA-5N (2). The low calcium affinity and relatively low calcium binding rate of Rhod-5N, as well as low excitation power of the He-Ne laser at 543 nm might be responsible for the low performance of Rhod-5N.

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THE RELATIONSHIP BETWEEN VARIABILITY IN BLOOD PRESSURE AND PULSE INTERVALS IN HYPERTENSIVE ADOLESCENTS

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The aim of the present study was to determine the interrelationship among the baroreflex sensitivity (BRS), the variability in systolic blood pressure (SBP) and pulse intervals (PI) at the frequency of 0.1 Hz in hypertensive adolescents. Blood pressure was recorded in 86 healthy controls and 16 hypertensive adolescents (16-19 years of age) for 5 min (Finapres, metronome controlled breathing at a frequency of 0.33 Hz). BRS was determined by a spectral method. The power spectra of SBP (in $mmHg^2/Hz$) and PI (in ms^2/Hz) were calculated. Adolescents were divided into groups according to the spectral power at the frequency of 0.1 Hz. The following limits of power were used: high – hSBP≥100, hPI≥12500; middle – 100≥mSBP≥55, 12500≥mPI≥5400; low – lSBP<55, lPI<5400. We analyzed the relationships between the groups A(hSBP, hPI), B(mSBP, hPI), C(lSBP, hPI), D(hSBP, mPI), E (mSBP, mPI), F(lSBP, mPI), G(hSBP, lPI), H(mSBP, lPI), I(lSBP, lPI). The highest baroreflex sensitivity is in the group C (h PI and l SBP), the lowest value of BRS is in the group G (l PI and h SBP), both groups do not overlap. Mean values of BRS are in groups A (h PI and h SBP), E (m PI and m SBP), I (l PI and l SBP). The highest number of controls is in groups A, E, I (together 40 %), and the lowest number is in groups C (8 %) and G (6 %). The hypertensives are concentrated into the triplet subgroups with hSBP (88 %); the maximum hypertensives were in subgroup A; and hypertensive adolescent was in groups C, F, H, I (0 %). Subjects in the group C might have expressive dominancy of parasympathetic reflex activity, and those in the group G of sympathetic reflex activity. The majority of children in the groups A, E and I have well-balanced relationship between reflex activity in sympathetic and parasympathetic nervous system. Even thought absolute values SBP and PI in groups A, E and I were very similar, their variability differed. It means that children in group A had very high reflex parasympathetic and sympathetic activity, but their BRS, SBP and PI is the same as in group I with very low reflex parasympathetic and sympathetic activity. The high systolic blood pressure variability as a sign of an increased sympathetic vasomotor activity could be a risk factor for hypertension.

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POSTNATAL FUNCTIONAL MATURATION OF BLOOD PHAGOCYTES IN PIG

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The aim of this work was to establish the ability of phagocytes to produce the reactive oxygen species (ROS) during the postnatal development. Moreover the expression of surface CD18 and CD45 molecules as well as the velocity parameters of blood polymorphonuclear leucocytes was determined. The production of ROS was established by the luminometric technique using luminol. The whole peripheral blood from piglets aged 1, 7, 18, 31, 66 and 100 days after birth was used. The ROS production was enhanced by opsonized zymosane or phorbol-12-myristate-13-acetate. The expression of CD18 and CD45 was determined on the peripheral blood polymorphonuclear leucocytes using monoclonal angtibodies against porcine CD18 (APC conjugated) and CD45 (FITC conjugated). The piglets were aged 1, 14, 32, 60 and 100 days after birth. It was found that phagocytosis develops during the postnatal life. The production of ROS per one milliliter of blood showed neither a decreasing nor an increasing trend. Unlike the values recounted per 10^6 of phagocytes had a decreasing trend but it was not significantly different. The concentration of white blood cells (WBC) as well as the velocity of granulocytes increased during the postnatal development. The differential count of WBC was changing. The count of staffs as well as segments decreased from 1st to 17th day while the count of lymphocytes increased. The production of ROS per 0,5 μl of blood showed neither a decreasing nor an increasing trend. On the contrary, the values recounted per 500 granulocytes had a decreasing trend. The expression of CD18 and CD45 intensively increased from 1st to 14th day with a gradual decrease to 100th day.

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DAMPED AMPLITUDE OF PLASMA MELATONIN RHYTHM IN HYPERTENSIVE PATIENTS WITH NON-DIPPING PROFILE IN BLOOD PRESSURE

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Several parameters of the cardiovascular system exhibit distinct circadian rhythms. The blood pressure and heart rate represent most pronounced rhythmicity. In diurnal animals, including man, higher values are observed during the day e.g. during the active phase of the day. Attenuation of amplitude in blood pressure, or higher values during the night than during the day (non-dipping profile) in hypertension patients are connected with a higher risk of cardiovascular complications. Plasma melatonin concentrations express circadian rhythms and higher concentrations are observed during the dark phase of the light-dark cycle. Melatonin receptors were found in many peripheral organs including the cardiovascular system and melatonin can act as an internal Zeitgeber that integrates the time organization of different function systems. Disturbances of rhythmic production of this hormone may result in disturbances of circadian rhythmicity of different functions. The aim of our study was to found if there are disturbances in the 24 h rhythm of melatonin in hypertension patients with a reversed blood pressure pattern. Totally 190 patients were investigated by ambulatory continues blood pressure monitory with ABPM 04. Measurements were performed in 15 and 30 min. intervals during the day and night, respectively. Systolic, diastolic and mean arterial blood pressure was evaluated automatically during the daytime, night time and all 24-hour cycle. Patients without the night decline in BP during the night were classified as non-dippers. In the subgroup of 65 patients plasma melatonin concentrations were measured in the middle of the dark and light phase, e.g. at 14.00 and 02.00 hour. Melatonin was measured by direct radioimmunoassay. The circadian rhythm of plasma melatonin concentration was preserved on both dippers and non-dippers. The ration of nighttime to daytime values was significantly lower in non-dippers than in dupers. The ratio was higher for diastolic than for systolic blood pressure. Our results suggest some disturbances in circadian melatonin production in hypertensive patients exhibiting circadian irregularities in circadian rhythms of blood pressure. The lability of the circadian organization in connection with hypertension may represent a risk factor for cardiovascular diseases.

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ESTIMATION OF THE EMBRYONIC KIDNEY FUNCTION

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Parameters characterizing development of function of the embryonic kidney during its morphogenesis were estimated in the mesonephros of chick embryos between days 5 and 10. Glomerular filtration (GF) was estimated using the passage time of the 2 % lissamine green through the individual nephrons after the intracardial injection of the matter to young embryos (1). But GF could also be detected in parallel with examination of tubular absorption of the 1.5 % trypan blue by proximal tubular epithelium after either intraamniotic, suprachorioallantoic or intravenous administration of the dye. Time and space pattern of distribution of the colored nephrons and the color density in tubules was characteristic for different developmental stages of mesonephros. The trypan blue storage was shown to depend 1. on the capability of the tubular epithelia of the dye absorption, 2. on the blood supply to individual glomeruli and 3. on the speed of transfer of the trypan blue to the blood stream from the place of its administration. The method was elaborated to change it to a semiquantitative tool capable to reveal the functional state of proximal tubules with respect to the age of the embryo and to experimental conditions evoking tubular disorders. A special attention was paid to the functional characteristics of abnormally distended tubules, so called cystic dilations of tubules (CDT), which appeared in consequence of the toxic effects and were already studied histochemically (2). Electrogenic transport in the proximal and distal tubules was assessed using a method of measurement of the transepithelial potential difference across their wall (3) on days 6 and 7. The quantities were used for the evaluation of the kidney function during development and in experimentally treated embryos - either by the nephrotoxic whole calf histone, or with the surgically induced unilateral renal agenesis (URA). The functional analysis revealed a slight delay in a functional maturation of nephrons in histone-treated embryos, more serious delay in development of the embryos with URA and a functional defect in the dilated nephrons, CDT.

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CHARACTERIZATION OF GLYCOPROTEIN CD36 FROM HEART MUSCLE AND THROMBOCYTES OF SHR RAT BY IMMUNOCHEMICAL TECHNIQUES

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The integral membrane protein CD36 is a hydrophobic protein on mammary epitelial cells, platelets, capilary endothelial cells and other cell types. Protein CD36- Glycoprotein IV of blood platelet, is recently best known also as a receptor for thrombospondin-1 and collagen and, as such, may have an important role in platelet activation and thrombus formation. CD36 also functions as a signal transduction molecule. A spontaneous deletion in the gene for Cd36 that encoding a fatty acid transport is located directly at the peak of QTL linkages on chromosome 4.A spontaneous deletion of this gene has been directly linked to the transmission of insuline resistance, defective fatty acid metabolism, and increased blood pressure. Spontaneously Hypertensive Rat (SHR) displays many of the features of human metabolic disease syndroms, thus SHR can be used as a model of mutation in CD36 and study of its CD36 protein (1). CD36 has been isolated from both SHR and the WKY as a control by six step isolation with subsequent ion exchange chromatography on HPLC from heart muscle. CD36 has been also isolated from purified platelet membranes of SHR, WKY as a control and also transgenic rat platelets. Monoclonal anti-CD36-thrombospondin receptor by Biodesign Int. US has been used for monitoring of rat CD36. Rabbit polyclonal antibodies raised against purified rat muscle CD36 gave a strong band on immunoblot and on immunoprecipitation with solubilized human and rat platelet membranes. Immunoblotting also identified CD36 on whole platelets and on the surface of U937. Preparation of row CD36 from rat platelets was subsequently purified on Protein G – agarose using specific interaction with monoclonal antibodies anti human CD36. Analysis of purified platelet CD36 by two-dimensional electrophoresis has revealed formation of multimeric CD36. There have been differences between preparations from SHR and control WKY platelet CD36. Posttranslation modifications phosphorylation and mainly glycosylation of CD36 from rat platelet has been studied. There has been substantial higher number of CD36 positive platelets in control strain of rats. Platelest of different strain also differ in number of receptors per cell. Results with transgenic line of rat SHR 10 and SHR 19 were presented.

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CALCIUM INFLUX IN REGULATION OF BLOOD PRESSURE IN RATS WITH DIFFERENT FORMS OF EXPERIMENTAL HYPERTENSION

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Elevated cytosolic free calcium plays a key role in vascular smooth muscle contraction. Its increase can be achieved by different mechanisms, but enhanced Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCC) was suggested to be involved in the pathogenesis of various forms of hypertension. We have therefore determined the contribution of VDCC to the maintenance of blood pressure (BP) in particular forms of experimental hypertension, namely in spontaneously hypertensive rats (SHR), in salt hypertensive Dahl rats and in rats with NO-deficient hypertension induced by chronic L-NAME treatment. The acute administration of nifedipine (0.5 mg/kg iv) in conscious chronically cannulated rats was used to estimate VDCC-dependent BP reduction. Nifedipine reduced BP by 58±4 mm Hg in SHR, 67±8 mm Hg in salt hypertensive Dahl rats and by 61±7 mm Hg in L-NAME hypertensive rats (vs. 30±2, 30±3 and 18±3 mm Hg in the respective controls). Compared to normotensive controls nifedipine-resistant BP remained to be elevated in SHR only. Our results indicate that BP reduction caused by acute VDCC blockade was proportional to basal BP values (SHR: r=0.84, n=33, Dahl rats: r=0.95, n=36; L-NAME rats: r=0.91, n=15, $p<0.001$ both). On the contrary, the residual (nifedipine-insensitive) blood pressure was almost independent of initial BP levels. Nifedipine-sensitive BP component seems to be responsible for the major part of BP elevation seen in salt and/or NO-deficient forms of experimental hypertension, but this was not true in spontaneous form of genetic hypertension. Our results fully confirm the importance of enhanced Ca^{2+} influx through voltage-dependent Ca^{2+} *channels in the pathogenesis of high blood pressure. Our further effort will be focused to the relationship between nifedipine-sensitive and agonist-dependent BP components.*

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