VASCULAR SMOOTH MUSCLE CELLS IN CULTURES ON COLLAGEN I DEGRADED BY MATRIX METALLO-PROTEINASE-13

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Vascular diseases, such as hypertension and atherosclerosis, are accompanied by degradation of extracellular matrix by enzymes produced in vascular cells, as well as in leukocytes, macrophages or mastocytes infiltrating the damaged vessel wall (1). We studied the effects of degradation of collagen I by matrix metalloproteinase-13 (MMP-13) on adhesion, growth and viability of vascular smooth muscle cells (VSMC) *in vitro*. Collagen I was digested with MMP-13 (Calbiochem; 5 min, 37° C, pH 7.5), adsorbed on polystyrene culture dishes, and seeded with rat aortic VSMC (2547 cells/cm^2, passage 8) derived from the rat aorta by explantation method (2). Cells were incubated in Dulbecco-Modified Eagle Minimum Essential Medium with 10% of foetal calf serum for 1 to 7 days. We found that VSMC on MMP-13-treated collagen adhered at about 1.5 times lower initial number than those on unmodified collagen ($p<0.01$). The concentration of β_1-integrins (i.e., receptors for collagen) and β-actin in these cells was by 35% lower ($p<0.05$). The concentration of vinculin, talin and α-actin was unchanged. However, the clustering of vinculin and talin into focal adhesion plaques, as well as the assembly of α- and β-actin into microfilaments, were lower. VSMC on the modified collagen showed a slightly shorter cell population doubling time (by 7 ± 3 %, $p<0.01$), longer exponential phase of growth (at least by 2 days), a higher concentration of heat-shock protein 60 (by 20 ± 7 %, $p<0.05$), and were more prone to cell death (more than 3 times higher number of trypan-blue stained cells, $p<0.01$). These results suggest that cells on MMP-13-degraded collagen could escape more easily the extracellular matrix-mediated growth control and could increase their turnover.

(1) Novotná J., Herget J.: Life Sci. 62:1-12, 1998.
(2) Bačáková L. et al.: Virchow's Archiv 440:50-62, 2002.

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GENDER DIFFERENCE IN PULMONARY VASCULAR HANDLING OF INTRACELLULAR CALCIUM IS ABOLISHED BY ORCHIECTOMY

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Recently, we found gender differences in pulmonary vasoconstrictive responses mediated by calcium release from endoplasmic reticulum. The present study was therefore designed to look for the source of this variability. Adult male and female Wistar rats were either gonadectomized in deep ether anesthesia or left intact. Four weeks later, ventilated lungs were isolated under thiopental anesthesia and perfused ex vivo with Krebs-albumin solution. To minimize possible confounding influence of endothelial factors, the perfusate contained cyclooxygenase and nitric oxide synthase blockers. Thapsigargin or its solvent (DMSO) were added to the perfusate. Thapsigargin is a highly selective inhibitor of endoplasmic reticulum calcium ATP-ase, and is known to irreversibly deplete the reticulum of calcium. After measuring the vasoconstrictor responses to angiotensin II (A-II, 0.2μg bolus) and hypoxia (0% O_2), the concentration of thapsigargin in the perfusate was increased from 10^{-9}M to 10^{-8}M and the measurements were repeated. While 10^{-9}M thapsigargin had little effect on pulmonary vasoconstrion in all groups, 10^{-8}M significantly inhibited the responses to A-II and hypoxia in females but not in males. Ovariectomy did not alter this finding. However, unlike in intact males, in lungs of orchiectomized males thapsigargin inhibited the responses to both A-II and hypoxia. Thus, the effect of thapsigargin in lungs of castrated males was similar to that in all females. We conclude that calcium from the endoplasmic reticulum participates in the pulmonary vasoconstriction much less in males than in females and that the testes are responsible for this gender difference.

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FREQUENCY DEPENDENCE OF I_{TO} AND I_{NA} BLOCK IN VENTRICULAR CARDIOMYOCYTES: COMPARISON OF THE EFFECT OF AJMALINE AND PROPAFENONE

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The side effect of ajmaline (an antiarrhythmic drug of class Ia according to Vaughan-Williams classification) on the transient outward current I_{to} was studied and compared with the principal effect on the fast sodium current I_{Na} in experiments on rat ventricular myocytes (1). The block of these currents was found to be frequency independent in the range of 0.33 - 3.3 Hz. In contrast, propafenone (classified as class Ic) showed significant increase of I_{Na}-block with increasing frequency in the same range (2). The present study was aimed to explain the observed differences between the frequency dependence of block induced by both antiarrhythmics. In experiments on isolated rat ventricular myocytes, I_{Na} and I_{to} were recorded in response to imposed standard depolarizing pulses preceded by variable preconditioning using the whole-cell patch-clamp technique. Similar differences between the frequency-dependence and frequency-independence of, respectively, propafenone- and ajmaline-induced I_{Na}-block were surprisingly observed also in the case of I_{to}. To explain these differences, the variations of the degree of block evoked by a depolarizing impulse were reconstructed. The responses of the propafenone-induced block to a depolarizing and a repolarizing voltage step could be approximated by two exponentials corresponding to fast (of the order of 1 - 10 ms) and slow (100 ms - 1s) processes. The slow processes that are likely to account for the observed frequency-dependence of a steady state block were not significant in the case of ajmaline-induced block. Consequently, the repeated depolarizations did not induce cumulative block within the explored frequency range. The present results are consistent with the hypothesis that the apparent affinity of the drug to its receptor is high in the open state and low in the resting state. The differences in the values of rate constants related to the block of inactivated channels may account for the observed differences in frequency dependence of I_{Na}- and I_{to}-block induced by ajmaline and propafenone.

(1) Bahníková M. et al.: Physiol. Res. 51(4):1P, 2002.
(2) Šimurdová M. et al.: Scripta medica 70(6):263-275, 1997.

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EEG REGISTRATION IN CEREBELLAR NEURO-DEGENERATION MODEL DURING ITS EXPOSITION TO THE HIGH-FREQUENCY ELECTROMAGNETIC FIELD

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An exposure to high-frequency electromagnetic field (HF EMF) corresponding to mobile phone signals influences brain function but neither the biological nor the clinical significance of the findings is clear at present (1). This work is focussed on the technical aspects of direct registration of brain electrical activity during the influence by the HF EMF. All experimental procedures were done in Lurcher mutant mice, healthy animals were used as controls. This mutation represents a natural model of genetically determined olivocerebellar degeneration (2). Experimental animals were exposed to the HF EMF with frequency of 870 MHz. Output power of the generator was 10 W. Mouse was placed into a plastic box just before the orifice of the waveguide. Serious problem in the use of classical EEG technology is the presence of conductive (contact) electrodes in the brain tissue resulting in the discontinuity of HF EMF and possible electrolytical processes caused by the nonhomogeneity of the boundary line of metall-tissue. Our original method is based on the use of gel electrodes (silicon tubes filled by the agar). The corresponding conductivity is achieved by the supplementation of saline and final biophysical quality is adequate to the brain tissue (3). The connection with platine electrodes is performed out of HF EMF space. Brain electrical activity was registered as a spontaneous EEG with evaluation of frequency spectra (by the Fourier analysis). All measurements were performed simultaneously with HF EMF exposition. Based on our previous results (some neurons of Lurchers are more sensitive to neurotoxic substances; higher degree of excitability of the CNS in Lc/+ when compared with +/+ using a metod of audiogennic epilepsy; higher degree of brain cortical activity after previous electrical and drug stimulation; change of hippocampal activity - LTP), we expect that some brain structures in Lurchers should be more sensitive to HF EMF.

(1) IEGMP: UK Ministry of Public Health, 2000
(2) Vožeh F. et al.: J. Physiol. 511, 69P, 1998
(3) Žalud V. et al.: 14th Cong. of Pathol. and Clin. Physiol., Abstract Book,79P, 2002

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ADRENERGIC REGULATION OF LIPOLYSIS IN PATIENTS WITH ANOREXIA NERVOSA DURING EXERCISE

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Introduction: Anorexia nervosa (AN) is a severe disease characterized by hard malnutrition and fat stores loss, especialy. Sympathetic nervous system (SNS) is the main regulator of metabolism of adipose tissue. The aim of the present study was to examine whether there is any change in adrenergic regulation of lipolysis and in the rate of lipolysis in patients with AN.
Methods: *In vivo* microdialysis (CMA, Sweden) of the subcutaneous adipose tissue was used for the assessment of norepinephrine (NE), dihydroxyphenylalanine (DOPA) and glycerol concentrations in microdialysate samples of interstitial fluid, obtained from 10 patients with AN and 10 controls. HPLC was used for measurement of NE and DOPA, colorimetric method for measurement of glycerol. Aerobic exercise at 1,5 $W.kg^{-1}$ lean body mass was used for stimulation of SNS.
Results: The extracellular NE and glycerol concentrations in patients with AN were higher in course of experiment ($P < 0,01$, ANOVA) and reaction on exercise was markedly different in compare to healthy volunteers. Extracellular DOPA was significantly higher by about ten times during basal, exercise and post-exercise conditions in patients with AN but in plasma levels weren´t found differences. Plasma NE, DOPA and glycerol didn´t differ between patients with AN and controls too.
Conclusion: The results suggest that patients with AN have higher sympathetic tone, higher NE outflow and subsequently higher rate of lipolysis in adipose tissue together with higher reaction on exercise.

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

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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 7000m, for 8 hours a day. At the age of 18 days, animals were given a single i.p. injection of KA (2,5 mg/kg). Aged 22 or 90 days, 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.
Results from young animals (22-day-old) show that chronic hypoxia and KA given to the normoxic animals increases the density of the NADPH-d positive neurons in the hilus, CA1, CA3 areas and in the auditory cortex, compared to the control group. In 90-day-old ones, such increase is not significant. In contrast, KA given to the hypoxic animals lowers the density of these neurons in the hilus and in the dentate gyrus of both age groups.
This study suggests that hypoxia stimulates the nitric oxide (NO) production because of its influence on nitric oxide synthase (NOS) gene expression. KA then possibly causes NO synthesis enhancement due to its binding on KA channels (subpopulation of non-NMDA receptors). Our results show that hypoxia also lowers the density of KA receptors that may indirectly contribute to the low NO production followed by KA application.

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TIME PROFILE OF BREATH NO RELEASE DURING THREE WEEK HYPOXIA

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Nitric oxide concentration may serve as a marker of NO production in lungs and airways. Expression of NO synthase (NOS) in airways and lung vessels increases in chronic hypoxia. Exposure to chronic hypoxia increases NO synthesis in lung vessels. As the effects of NO depend on its concentration, the current study was designed to determine a time profile of NO release during a 3 week exposure of rats to hypoxia.
Adult male Wistar rats were exposed to hypoxia (10%O2) for up to 19 days. NO concentration in exhaled breath was measured on days 1, 4, 11, 19 of the exposure. For each measurement, the rat was removed from the hypoxic chamber, placed in a body pletysmograph for 20 min to measure NO exhalation and then returned to the hypoxic chamber. Exhaled NO was measured by sampling the air from the pletysmograph into a CLD 77 AM chemiluminescence analyzer (EcoPhysis, Duernten, Switerland) at the end of the 20-min sojourn of the rat in the pletysmograph.
Exhaled NO rose dramatically during the first 4 days of the hypoxic exposure (from 0.183 ± 0.011 to $3,6 \pm 0.925$ ppb/min). With continuing hypoxia, the exhaled NO production fell to a level still higher than that found in normoxia (0.906 ± 0.225 ppb/min on day 7). Thereafter, the NO production did not change any more (0.769 ± 0.182 ppb/min on day 19).
We conclude that chronic hypoxia elevates NO production into the exhaled breath. This rise in breath NO levels are most prominent during the first week of hypoxia.

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EXPRESSION OF NHERF ISOFORMS IN RAT COLONOCYTES DURING SECONDARY HYPERALDOSTERONISM AND METABOLIC ACIDOSIS

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Electroneutral sodium transport across the intestinal and renal brush border membrane represents sodium-hydrogen exchange via NHE3 and NHE2 isoforms of the Na^+/H^+ exchanger. The regulation of Na^+/H^+ exchangers involves multiple processes including response to intracellular pH, corticosteroids, cell volumes etc. The regulatory mechanisms operate at the level of transcription, internalization of the protein in the membrane and activation or inactivation of the transporter. It has been demonstrated recently that PDZ domain-containing proteins called Na^+/H^+ exchanger regulatory factors (NHERF 1 and NHERF 2) play an essential role in signal transduction and function as scaffold proteins clustering NHE3/NHE2 and cytoskeletal ezrin. The present study was undertaken to determine whether the physiological stimuli such as hyperaldosteronism or metabolic acidosis are associated with changes of NHERF1/NHERF2 expression. These stimuli were studied because hyperaldosteronism is known to inhibit sodium transport via NHE3 in distal colon whereas it stimulates this transport pathway in the proximal colon and metabolic acidosis increases colonic acid-base transport via NHE2 and NHE3.
The transport activities of NHE isoforms were detected in isolated rat crypt colonocytes as Na-dependent, amiloride-sensitive pH_i recovery using ratiofluorimetric, pH-sensitive dye BCECF and NHERF1/NHERF2 mRNA levels were evaluated by real-time quantitative PCR using a LightCycler rapid thermal cycler system, respectively.
Hyperaldosteronism activated NHE3/NHE2 transport activity in the proximal colon but decreased the activity in the distal colon. In contrast, the metabolic acidosis increased NHE3/NHE2 transport activity in the proximal colon. NHERF1 mRNA levels were not affected by either secondary hyperaldosteronism or metabolic acidosis but NHERF2 mRNA level was increased in the proximal colon of acidotic rats and decreased in the distal colon of the animals with secondary hyperaldosteronism.
This study demonstrates that NHERF2 is involved in the adaptive changes of colonic sodium and acid-base transport.

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MORPHOLOGICAL CHANGES AFTER NEONATAL ADMINISTRATION OF N-ACETYL-L-ASPARTYL-L-GLUTAMATE

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N-Acetyl-L-aspartyl-L-glutamate (NAAG) is an agonist at Group II metabotropic glutamate receptors (mGluR II) and also activates the *N*-methyl-D-aspartate (NMDA) type of ionotropic glutamate receptors, particularly at high μM concentrations. Acting through these receptors, NAAG can display both neurotoxic and neuroprotective effects. We have demonstrated NAAG-induced neurotoxicity in the adult rat hippocampus that appeared to be mediated principally throught the NMDA receptors (1). In the present study, NAAG (250 nmol/ventricle) or saline (as a control) were applied intracerebroventricularly (icv) to rat pups at postnatal day 12. The subsequent appearance of pycnotic neuronal nuclei in the hippocampus was quantitatively evaluated 24 or 96 hours after the injections. The damage to neurons was also assessed by Fluoro-JADE-B/DAPI staining, observed by confocal or deconvolution fluorescent microscopy. In addition, the TUNEL staining was used to investigate possible presence of apoptotic death. The findings have indicated that the administration of NAAG to early postnatal rats results in extensive death of neurons especially in the dentate gyrus of the dorsal hippocampus.

(1) Pliss et al., NeuroReport 11 3651-3654 (2000).

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QUANTAL LATENCIES AND PROTEIN KINASES AT FROG MUSCLE ENDPLATE

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We have demonstrated that long-latency uniquantal endplate currents (EPCs) are removed when intracellular cAMP is increased by β1 activation by noradrenaline (NA)(1), by the db-cAMP(2), forskoline or by inhibition of cAMP hydrolysis(3). This makes the evoked release of quanta more synchronous and, as the model shows, the amplitude of reconstructed multiquantal currents increases. These effects depend on the degree of asynchrony along the nerve terminal. In the proximal part, many EPCs are released with delays greater than 2 ms and they are eliminated to a large extent during the periods of increased cAMP levels. In distal parts, EPCs are more synchronous *per se* and upon cAMP application, minimal latencies and the dispersion level were not changed. Protein kinase A (PKA) is the target of this regulation as a specific inhibitor, Rp-cAMP prevents the synchronizing action of NA and cAMP in proximal parts and makes the latency dispersion greater in distal parts of the axon. Inhibition of PKA leads to the appearance of the longest latencies which can be encountered at the frog muscle endplate. In distal parts, EPCs have shorter minimal latencies and a more compact release in distal parts, where saturating concentrations of cAMP probably exist due to smaller axon volumes.

(1) Bukcharaeva E. et al.: J. Physiol. (Lond.) 517:879-888, 1999.
(2) Bukcharaeva E. et al.: Physiol. Res. 49:475-479. 2000.
(3) Bukharaeva E. A. et al.: J. Physiol. (Lond.) 538:837-848, 2002.

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THE EFFECT OF LONG-TERM HIGH-FREQUENCY ELECTROMAGNETIC FIELD EXPOSITION ON NEURAL FUNCTIONS IN NORMAL AND NEURODEFECTIVE MICE

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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. Our previous experiments showed only insignificant changes of spatial learning in mice influenced by HF EMF (2). Mice were exposed to HF EMF (880 MHz) or control conditions for 3 hours a day during early postnatal development (from 1 to 30 days) that involves the rapid brain growth spurt. After the exposition learning and motor ability as well as CNS excitability were examined. Learning ability was tested using the Morris water maze (3) and step down (passive avoidance) method. CNS excitability was tested by the method of audiogenic epilepsy. In wild type mice we found deterioration of spatial learning after HF EMF exposure in comparison with controls. In Lurcher mutant mice there were no differences between irradiated animals and controls. In the step down procedure exposed mice, both Lurcher mutant and wild type, had slightly worse results than controls. In motor function and CNS excitability there were no significant differences between irradiated and control animals. The results showed negative effect of long-term HF EMF influence on learning ability especially in wild type mice. In Lurchers, characterized by the common worse results, marked changes of examined neural functions were not observed.

(1) Zuo J. et al.: Nature 388: 769-773, 1997.
(2) Cendelín J. et al.: Physiol. Res. 51 (4): 6P, 23, 2002.
(3) Morris R.G.M.: J. Neurosci. Meth. 11: 47-64, 1984.

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LOW STATIC MAGNETIC FIELDS APPLIED AT EARLY AND LATE POSTNATAL PERIODES *IN VIVO* MEDIATE CHANGES IN HIPPOCAMPAL CHOLINERGIC SYSTEM OF YOUNG ADULT RATS

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Biological effects of static magnetic fields (SMF) are intensively investigated during the past two decades as a result of the growing number of their applications in research, industry and medicine. However, biological effects especially of weak SMF and detailed interaction mechanisms are not elucidated yet. The aims of the study are to evaluate possible alterations in hippocampal tissue of young adult male Wistar rats exposed to ± (0.14 - 0.40) T for 0.5 – 2 hours during their postnatal development (postnatal days 7 and 14).
Results indicate SMF-mediated alterations in the activity of presynaptic cholinergic nerve terminals of young adult animals and marked differences either between rats exposed at postnatal days 7 and 14 or between effects of positive (oriented paralelly with the vertical component of the static geomagnetic field of the Earth) and negative fields (opposite orientation). Using both acute expositions *in vivo* and experiments *in vitro*, the possible interaction mechanisms between SMF and polypeptide of hemicholinium-3 sensitive choline carriers are discussed in accordance with data in literature (1-3) (e.g., electrodynamic and magnetomechanical effects, electric currents induction).

(1) V. Kose and J. Sievert: Non-Linear Electromagnetic Systems, IOS Press, Amsterdam, 1998.
(2) S. Turner: Measurement and Alignment of Accelerator and Detector Magnets, CERN, Geneva, 1998.
(3) W. R. Hendee: Biomedical Uses of Radiation. Wiley-VCH, Weinheim, 1999.

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DEVELOPMENT AND OPTIMIZATION OF A REAL-TIME PCR METHOD FOR MONITORING OF RHO GDI EXPRESSION IN CELLS OF HUMAN ORIGIN

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Small Rho G-proteins have been implicated in rearrangements of cytoskeleton. We focused on expression of Rho GDP dissociation inhibitor (Rho GDI) and attempted to elaborate a method for its monitoring on the mRNA level using CEM T-lymphoblastic leukemia human cell line.
Cell lysates were prepared and purification of mRNA was done by magnetic separation method. Four primers for Rho GDI were designed. One-step RT-PCR was compared with a two-step procedure where the 1st strand cDNA was synthesized at first. Amplification reactions were effected in SYBR Green as well as hybridization probe detection format. Purity and length of PCR products as well as those of the source mRNA were checked electrophoretically.
As the result, the best combination of two of the primers giving a product of adequate degree of purity and expected chain length has been selected.
The method will be utilized for monitoring of Rho GDI mRNA levels in cytoskeleton rearrangement studies that are currently in run in our laboratories.

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HYPEROXIA PREVENTS CARRAGEENAN-INDUCED ENLARGEMENT OF FUNCTIONAL RESIDUAL CAPACITY IN RATS

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Experimental pneumonia induced by intratracheal application of carrageenan or paraquat increases the lung functional residual capacity (FRC) in rats. The mechanism of this increase is not clear, but a decrease in PO_2 may be involved. To test this possibility, we attempted to eliminate the PO_2 decrease in carrageenan-treated rats by exposing them to hyperoxia. 29 rats were randomly assigned to one of 4 groups. Animals of the first group were after intratracheal application of carageenan (0.5ml of 0.7% in saline) exposed to 7 days of hyperoxia (F_IO_2 0.78-0.84, *group Car+O_2*), animals of the second group were given the same dose of carrageenan but breathed air (*group Car+A*), the third group of rats was kept for seven days in hyperoxia (*group O_2*), and the fourth group were controls (*C*). Animals were then anesthetized and intubated and their ventilatory parameters and FRC were measured during air breathing.
Carrageenan application induced an FRC increase (*Car+A* 2.0±0.2ml, *C* 1.6±0.1ml, mean±SEM), which was not seen in carrageenan-treated rats exposed to hyperoxia (*Car+O_2* 1.6±0.1 ml). Hyperoxia alone did not affect the value of FRC (*O_2* 1.5±0.1ml). These results support the hypothesis that a decrease in PO_2 plays an important role in carageenan-induced increase of FRC in rats.

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EFFECT OF AGMATINE ON CARDIOVASCULAR SYSTEM AND ITS LINKING TO NITRIC OXIDE SYNTHASE

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Agmatine, a biogenic amine, being an intermediate of one of the metabolic pathways of arginine, has been considered as potential factor in cardiovascular control since 1995 (1). Strong relations between individual metabolic pathways of arginine were revealed (2). Following issues were addressed: (i) the characteristic of cardiovascular response to agmatine in anesthetized rats, (ii) the outline of relation to the NO synthase activity, governing the other metabolic pathway of arginine, (iii) the response of isolated thoracic aorta to agmatine. Agmatine (30μM and 60μM i.v.) induced dose dependent long-lasting hypotension (42.6±4.6 mmHg and 70.9±6.5 mmHg, resp.). The time parameters of hypotension were as follows: peak hypotension: 33.6±4.0 s and 33.0±4.8 s; half time of return was 171.6±12.4 s and 229.2±20.4 s; time of complete return was 563.4±55.8 s and 675.7±66.0 s, always dose dependently. Inhibition of NO synthase lasting 1-3 hours increased the hypotension: 59.0±7.6 mmHg ($P<0.05$) and 95.8±8.8 mmHg ($P<0.05$); inhibition NO synthase lasting 4 weeks likely increased hypotension (82.3±12.7 mmHg, $P<0.01$, and 87.3±3.1 mmHg, $P<0.01$). However, half time of hypotension return, and time of full return were shortened significantly. Agmatine itself induced concentration-dependent relaxation of precontracted thoracic aorta. Pretreatment of aorta with agmatine (1mM) did not affect endothelium-dependent relaxation. Conclusion: Agmatine induced a long-lasting dose dependent hypotension, in anaesthetized rats and also relaxation in isolated thoracic aorta. A compromised NO production enhanced agmatine-induced hypotension which might indicate a negative feed-back relation to NO synthase activity, at least in mechanisms controlling the tone of resistant arteries.

(1) Boucher et al. Cell. Mol. Life Sci. 55:1015-1028, 1999
(2) Raasch et al. Ann. N.Y. Acad. Sci. 763:330-334, 1995

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TRANSPLANTATION OF BONE MARROW STROMAL CELLS INTO AN INJURED CNS

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Bone marrow stromal cells (MSC) are pluripotent progenitor cells that have the capability to migrate towards lesions and induce or facilitate site-dependent differentiation in response to environmental signals (1). Using *in vivo* MR imaging, we studied MSC transplantated into adult rats with a cortical photochemical lesion (2). MSC were isolated from rat bone marrow by adherence to plastic. After *in vitro* expansion, the cells were co-labeled with superparamagnetic iron-oxide nanoparticles (Endorem, Guerbert Laboratories, France) and BrdU (5 □M) 48 hours prior to transplantation and administered either intracerebrally into the contralateral hemisphere (0.3 million cells in 3□l PBS; n=12) or i.v. into the femoral vein (2 million cells in 0.5 ml PBS; n=8). A photochemical lesion was induced by rose bengal/light beam interaction 24 hours prior to transplantation. MR images were taken weekly using a 4.7 T Bruker spectrometer. Rats were sacrificed 4 weeks following transplantation, and the fate of transplanted cells in the CNS was analyzed immunohistochemically. The cells preferentially migrated into the lesion, and subsequently some of the cells that entered the lesion expressed the neuronal marker NeuN. In animals without a lesion, the majority of intracerebrally injected cells remained in the close vicinity of the needle track. Starting 7 days after transplantation and persisting for 4 weeks, MR images showed a hypointense signal in the lesion, indicating the migration of cells to the lesion. Hypointensity was also found in the lesion after i.v. injection of labeled cells. Prussian blue staining confirmed the presence of iron-oxide-labeled cells in the lesion site. The study demonstrates that iron-oxide nanoparticles can be used to track implanted stem cells in the CNS.

(1) Prockop D.J.: Science 276:71-74, 1997.
(2) Watson B.D. et al.: Ann. Neurol. 17:497-504, 1985.
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CHARACTERIZATION OF BINDING SITES OF FLUOROPHORES ON CREATINE KINASE MOLECULE

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Myofibril-bound creatine kinase EC 2.7.3.2 (CK), a key enzyme of muscle energy metabolism, has been selected for studies of conformational changes that underlie the cellular control of enzyme activity.
For fluorescence spectroscopy measurements, the CK molecule was doubly labelled with FITC (fluorescein 5'-isothiocyanate) and ErITC (erythrosin 5'-isothiocyanate). Preliminary results of fluorescence analysis were satisfactory (1), so we moved forward to identification of binding sites of fluorescent labels. The latest results were obtained with FITC modified CK. Protein bands with fluorescence under UV light were cut from the gel and trypsin digestion was performed. Tryptic mixture was subjected to mass spectrometric analysis on matrix-assisted laser desorption/ionisation reflectron time-of-flight mass spectrometer.
MALD ionization partially disrupted the bond between lysine and FITC. Therefore peaks corresponding to unmodified peptides as well as to modified are present in full MALDI spectra. From PSD experiments we were able to assign partial amino acid sequence and we also deduced amino acid composition from the presence of diagnostic immonium ions in the low mass region. Two possibly modified peptides were selected directly in this experiment.
To confirm this hypothesis we performed collision induced dissociation experiments on □LC-mass spectrometer and on nanoESI-qIT mass spectrometer from selected HPLC fractions. Full tryptic mixture was separated on reverse phase HPLC column and three fractions with fluorescence were collected. The CID experiments at various normalized collision energies confirm the presence of FITC in modified peptides and MS^3 experiments provided us with nearly complete sequence information. In these experiments the modification of two peptides was confirmed and the third modified peptide was found.
Mass spectroscopy experiments provided us 74% sequence coverage and we identified 3 FITC binding sites which does not interfere with enzymatic activity of creatine kinase molecule.

(1) Gregor M. et al.: Physiol. Res., 52 (5), 2003 (in press)

EFFECT OF LASER LIGHT AND/OR MEROCYANINE 540 ON WHOLE BLOOD PLATELETS

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Merocyanine 540 (MC540) is a lipophylic dye with a high affinity to neoplastic and virus infected cells, which may be successfully used in photodynamic treatment of tumor cells and photosterilization of blood. With respect to clinical applications of MC 540, its possible side effects on adjacent healthy blood components have to be understood.
In our study we used flow cytometry and labeling with the monoclonal antibody PAC-1 to investigate the effect of MC 540 and/or green Nd-YAG laser (532 nm, 30 mW) irradiation, on expression of GPIIb-IIIa glycoprotein complex (fibrinogen receptor) on the platelet surface. Expression of such complex corresponds to the state of platelet activation. We observed significant effect ($p<0.01$) of laser light on reactivity of the whole blood platelets. Laser light exposure times of 10 and 30 minutes caused platelet activation, while exposure time of 60 minutes resulted in attenuated platelet response to activators. The effect of MC 540 (5 μmol/l) on reactivity of the whole blood platelets was insignificant. The presence of MC 540 in the sample did not modulate the effect of laser light irradiation on the state of platelet activation.
We also monitored the effect of MC 540 and/or laser light irradiation (Nd-YAG, 532 nm, 30 mW) on the formation of the fractions of platelet microparticles and aggregates in whole blood platelets population. Neither MC 540 nor laser light irradiation had significant effect on these parameters, thus pointing that neither of these two factors affects platelet rupture/consumption and platelet clumping.
We conclude that whole blood platelets might be undesirably affected by laser light irradiation during blood photosterilisation. To confirm the insignificant effect of MC 540 alone on reactivity of whole blood platelets, more sophisticated experiments need to be done.

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THE EFFECT OF LASER LIGHT AND MEROCYANINE 540 ON HUMAN RED BLOOD CELLS

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The disease transmission is responsible for many of the complications encountered in blood transfusion therapy. Photodynamic sterilization seems to be an auspicious procedure to reduce the risk of infection. This procedure involves the light absorption by a photosensitizer selectively taken up by pathological cells and the subsequent damage of these cells. Merocyanine 540 (MC540) is a promising photosensitizer with high affinity to neoplastic, virus and malaria infected cells. In blood, the uptake of a photosensitezer is not fully specific for infected cells. Therefore, the normal cells may suffer from photodynamic action. In our work, the effect of laser light and MC540 on the red blood cells (RBCs) hemolysis was investigated.
Whole blood anticoagulated with sodium citrate was irradiated by NdYAG green laser light (532 nm, 30 mW) and incubated with MC540 at final concentrations of 1.4-10 μmol/l. The rupture of RBCs was assessed in terms of absorbance at 414 nm, which corresponds to the amount of hemoglobin released in blood plasma from RBCs after hemolysis. Laser light alone (30 J/cm^2) did not have any influence on the absorbance of hemoglobin. The presence of MC540 in blood in the dark did not cause significant changes in the 414 nm absorbance values. However, with increasing MC540 concentration (> 3 μmol/l) in blood samples, a 565 nm absorption maximum arose, which corresponds to MC540 probably associated with plasma proteins. We observed a significant ($p < 0.005$ in all cases) increase of the absorbance at 414 nm in the blood in presence of both MC540 (1.4-10 μmol/l) and laser light (30 J/cm2).
From present results it can be concluded that neither the applied light nor the MC540 photosensitizer alone did not have destructive effect on normal RBCs, but the combined effect of both caused their hemolysis. Possible side effects on the other components of blood should be under consideration.

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HYPOXIA AND REOXYGENATION INCREASE H_2O_2 PRODUCTION IN RATS

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To test changes in production of reactive oxygen species (ROS) in the lungs during transition from sustained hypoxia to normoxia, we measured hydrogen peroxide output in the expired air of rats breathing hypoxic gas mixture or air or 100% oxygen at the end of exposure to 72 hours of hypoxia. 21 male Wistar rats (200 - 280 g) were randomly assigned to one of three groups. The two experimental groups were kept for 3 days in normobaric hypoxic chamber (F_IO_2 0,1), the control group was kept in air. At the end of the exposure, rats were anesthetized, intubated, placed in the body plethysmograph and their ventilation and H_2O_2 production in expired air measured. H_2O_2 production was measured as amount of H_2O_2 in condensate collected from the expired air during 1 hour by originally designed system: in control group twice during air breathing, in first experimental group in hypoxia (F_IO_2 0,1) and then during air breathing (*SH*-H-A group), in second experimental group firstly during air breathing and then in hyperoxia (F_IO_2 1,0, *SH*-A-O_2 group),. Concentration of H_2O_2 in the condensate was ascertained by chemiluminiscence.
The H_2O_2 production of the control group was low and similar in both consecutive one hour lasting measurements (20±10 and 13±5 pmol/h, mean±SEM). Exposure to sustained hypoxia increased the H_2O_2 production to 105±18 pmol/h when measured in hypoxia. Further increase was seen after transition to normoxia (366±19 pmol/h during air breathing in the *SH*-H-A group and 421±24 pmol/h during air breathing in the *SH*-A-O_2 group). Transition from air breathing to hyperoxia did not affect the H_2O_2 production (373±25 pmol/h, *SH*-A-O_2 group).

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RISK STRATIFICATION BY FUZZY AND WEIGHTED METHODS

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Patients (pts) surviving myocardial infarction (MI) are at risk of cardiac death. The indices as ventricular ectopies per hour (VPCs) > 10; ejection fraction (EF) < 40 %; positive late potentials (LP); baroreflex sensitivity (BRS) < 3 ms/mmHg, SDNNindex < 30 ms and SDANN < 50 ms are used for prediction of a risk (1), but their predictive value is insufficient. A new method of the stratification of pts at risk using fuzzy logic and weighted approach with the structure of classifier based on neural networks was developed. Fuzzy approach takes into account that the borderline between a risky and non-risky value of a risk factor is not sharp. Sharp limits of critical values (c.v.) of factors were replaced by linear transitions between 0 and 1. Weighted approach based on Bubble sort method was used to rate a predicting quality of each risk factor. A sum of fuzzified values of risk factors was introduced as a new risk factor. We examined EF by echocardiogram (Acuson), VPCs, SDNNindex, SDANN from 24-hour ECG, BRS by spectral analysis (2) of blood pressure recording (Finapres), and LP (HIPEC) in 290 pts 7-21 days after MI; 18 pts died within 2 years. Sensitivity (Se), specificity (Sp) and positive predictive value (PPV) of all factors were determined for standard c.v. and for fuzzy c.v. C.v. of summarized risk factors (r.f.) - summa r.f. or summa fuzzy r.f. - were determined: 1) for optimal achievable Se and Sp; 2) for PPV 50%. Also predictive value of new summarized factors was evaluated. Optimal widths of fuzzy sets were 5% for EF, 3.5 ms/mmHg for BRS, 21 ms for SDANN, 7 ms for SDNNindex and 2.5 VPCs/h. The following predictive values were found: 1) for standard/optimal c.v.: summa r.f. – (c.v.; Se%; Sp%; PPV% - 1; 83.3; 75.7; 18.5), summa fuzzy r.f. (1.2; 83.3; 78.6; 20.5). 2) for PPV 50%: summa r.f. - (c.v.; Se%; Sp% - 3; 38.9; 97.4), summa fuzzy r.f. - (2.15; 44.4; 97.1). It is concluded that fuzzy method improved predictive accuracy of standard risk factors.

(1) Honzíková N., Semrád B., Fišer B., Lábrová R.: Physiol. Res. 49: 643-650, 2000.
(2) Honzikova N., Fiser B., Semrad B.: PACE 23 [Pt. II]:1965-1967, 2000.
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PREGNENOLONE SULFATE HAS A SUBUNIT-SPECIFIC EFFECT AT NMDA RECEPTORS

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Neurosteroids, endogenously occurring compounds, have been shown to exert direct modulatory effects on several types of neurotransmitter receptors. Our previous experiments indicated that 3β-hydroxy-5-pregnen-20-on sulfate (PS) havs a subunit-specific effect at NMDA receptors (1). The aim of the present study was to characterize the effects of PS on heterodimeric recombinant NMDA receptors containing combination of NR1/NR2A-D subunits. The patch clamp technique was used to record responses induced by fast application of glutamate on transfected HEK-293 cells.

Pre-application of PS (300 μM) for 39 sec. resulted in a 4 to 5-time potentiation of responses to subsequent application of the saturating concentration of glutamate (1 mM) in NR1/NR2A and NR1/NR2B receptors, however, only a small potentiation (1.2-times) of the responses mediated by NR1/NR2C and NR1/NR2D receptors was observed. This indicates that PS has a subunit specific effect at NMDA receptors. Intracellular application of PS (300 μM; 4 min.) had no effect on the degree of PS induced potentiation of NR1/NR2B mediated responses. This suggests that the binding site of PS at the NMDA receptors is located extracellularly. The degree of PS induced potentiation of responses mediated by NR1/NR2 chimeras (combination of NR2A and NR2C subunits) was dependent on the extracellular segment between the 3. and 4. transmembrane domains of the NR2 subunit. The results of our experiments indicate that PS has a subunit-specific effect at NMDA receptors with the binding sites located extracellularly. This implies that neurosteroids may affect anatomically and developmentally distinct neurons expressing only specific type of NMDA receptors.

(1) Abdrachmanova G, Chodounska H, Vyklický L Jr.: Eur. J. Neurosci. 14(3):495-502, 2001.

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NUTRITIONAL ENERGETIC INTAKE IN COMPARISON WITH BODY MASS INDEX AND WEIGHT GAIN OF PREGNANT WOMEN

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Maternal obesity carries significant risks for the mother and fetus (1). Childbirth body weight is dependent on maternal prepregnancy weight (2). The aim of the study was to describe nutritional energetic intake of pregnant women in comparison with increase of body weight to ending of 8th month of pregnancy and childbirth body weight.

The study population included 226 pregnant women at the age of 24,6 ±3,2 y. Women were divided into four groups in accordance to their pregravid body mass index. These groups were compared so that the influence of pregravid body mass index and nutritional energetic intake on gestational body mass index gain and childbirth body weight. General evaluation demonstrates the increasing of body weight about 13.32 kg with 84,9% (9406,3 kJ) of recommended daily caloric intake (11 000 kJ) with 3,52 kg of childbirth body weight (3.71 kg for boys and 3,32 kg for girls). With increasing of pregravid body mass index lower nutritional energetic intake have been demonstrated. The highest value of energetic intake in pregnancy was observed in the first group of underweight women (9763 kJ) and also weight gain was high – 13.04 kg. On the other hand weight gain in obese women was 10.77 kg with energetic intake 8990 kJ.

Results suggest that nutritional energetic intake of pregnant women is lower that recommended value. Sufficient nutritional energetic intake of pregnant women is 10 000 kJ.

1. Sebire NJ et al.: Int J Obes Relat Metab Disord 25:1175-1178, 2001.
2. Swensen AR et al.: J Am Diet Assoc 101:903-905, 2001.

ACTIVATION OF THE AUDITORY CORTEX ANALYSED BY INDEPENDENT COMPONENT ANALYSIS

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Activation of the cerebral cortex evokes a transient and local changes in charge distribution as a response to applied stimulus. Such changes can be recorded by an electrode placed near the activation sites as field potential (FP). Each FP reflects mainly superposition of the synaptic sites activated in the nearby cortex. Independent component analysis (ICA) is a statistical method for extraction of individual signals from their linear mixture without any knowledge about the properties of particular signals. Applying ICA to a set of FPs recorded from different sites in the cortex it is possible to acquire a set of single FPs (independent components) each with its specific profile in the cortex.

Charged-balanced biphasic pulses (200 μs/phase, 2 Hz repetition rate) were used for electrical stimulation of the auditory nerve in cats. ICA was applied to a set of FPs recorded from 78 sites in the auditory cortex (6 cortex positions, 13 recording depth). To acquire information on a single transmembrane currents flowing in the cortex we have computed current sourse density (CSD) for each single FP (independent component) depth *profile*. ICA of FPs results in a 2 basic patterns of independent components. The first pattern is characterized by clearly distinguishable mainly monophasic wave confined in time (duration 2-6 ms). Components belonging to this pattern were considered to reflect synaptic activation. The other pattern was characterized by low amplitude unstructured activity and was considered as noisy pattern.

ICA allows us to track the subsequent relevant components comparing their time sequence and analysing relations of their distributions. Furthermore, ICA can reveal components which are hidden in the former signal giving us an additional information about cortex activation.

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EFFECTS OF TWO TYPES OF RESTRAINT STRESS ON BEHAVIOR OF RATS IN THE Y-MAZE AND OPEN FIELD

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Various stressors have been shown to exert modulatory effects on animal cognition. The exposure of rats to inescapable stress has been demonstrated to disrupt cognitive processes in several models of dissociative or spatial learning tasks. The aim of this study was to examine the differences in response to two types of restraint stress in two behavioral tests. Rats were exposed for 1 hour to two types of restraint stressors (1), namely restraint stress alone (MO) or restraint combined with cold-water stress (IMO+C). In the first series of experiments, learned discriminatory avoidance response in Y-maze was used as a model of memory; in this device we tested the differences between Wistar and Lewis rats, the latter having decreased activity of HPA axis. After exposure of rats to stressors the latency to enter the safe arm was recorded in 10 daily trials. In the second series of experiments we used "open field" test as basic information on behavior of Wistar rats. Rats were gently places in the right rear corner of the box and allowed to explore the arena for 15 min. The following behavioral parameters were recorded: a) crossing, b) immobility, and c) rearing. All data were analyzed by ANOVA and post-tests.
In the Y-maze both stressors prolonged the avoidance latencies for 2 or 3 days in Wistar and Lewis rats, respectively. In Lewis but not in Wistar rats the latency increased more after IMO+C compared to IMO stress. In contrast to minor differences between the effects of IMO and IMO+C on the performance of rats in the Y-maze, the open field experiments showed strong suppression of the exploratory behavior after IMO+C and the weaker effect of IMO itself. The exposure to IMO+C, terminating 240 or 60 min before the open field testing, practically abolished all motor activities. In the second open field test, performed without application of stress 7 days later, rats displayed a comparable exploratory activity. In summary, our data indicate substantial differences in response of rats to two types of restraint stressors in two behavioral tests. These findings can be utilized in other behavioral tests following interactions of stress with effects of drugs on cognitive functions.

(1) Klenerová V., Šída P.: Physiol. Res. 43: 289-292, 1994.
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COMPARISON OF BIOTRANSFORMATION OF ROSCOVITINE AND OLOMOUCINE IN MICE *IN VIVO*

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The purpose of the present study was to investigate the biotransformation and elimination of two selective cyclin-dependent kinase inhibitors, roscovitine and olomoucine, in mice *in vivo*.
^{3}H-labeled parent compounds were administered intravenously (1 μmol/25 g animal body weight). Animals were anesthetized by intraperitoneal pentobarbital (0.05–0.08 mg/g body weight). ^{3}H-radioactivity in blood, liver, kidney, urine and gut was monitored by liquid scintillation. Levels of the parent compounds and those of their metabolites were assessed by thin-layer chromatography and autoradiography. The chemical structure of the main metabolites was elucidated by mass spectrometry. The level of roscovitine in blood decreased from 5.9 % of the administered dose (2 min after the administration) to 0.7 % (30 min after the administration). The level of olomoucine declined more rapidly (from 4.3 % in 2 min to 0.1 % in 30 min). The biotransformation of both compounds occurred predominantly in liver and kidney. A carboxylic acid was produced by oxidation of the aliphatic hydroxyl group of roscovitine and shown to be the main metabolite of the parent compound. Olomoucine carboxylic acid was also identified but this was not the main product of olomoucine biotransformation. Based on the comparison of the present data with those published previously it can be concluded that i) olomoucine is cleared from blood in mice *in vivo* with the same velocity as another cyclin-dependent kinase inhibitor bohemine (1) and that ii) the clearance of roscovitine is about twice as lower then that of olomoucine and bohemine. The results can facilitate designing compounds of potential practical use.

(1) Chmela Z. et al.: Drug Metab. Dispos. *29*, 326-334, 2001.

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QUANTIFICATION OF CHANGES IN ASTROCYTE MORPHOLOGY AFTER CORTICAL STAB WOUND USING SINGLE-CELL CONFOCAL 3D MORPHOMETRY

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A cortical stab wound is a well-characterized model of brain injury and astrogliosis. Our previous studies revealed the presence of two populations of astrocytes around a cortical stab wound. Cells in the first population, identified as reactive astrocytes, are characterized by a long-term up-regulation of delayed outwardly rectifying K^+ currents, an increase in GFAP expression and the hypertrophy of glial processes (H1). The second population is characterized by a short-term up-regulation of inwardly rectifying K^+ currents only (H2). In the present study we quantified changes in the cell morphology of both astrocyte populations by image analysis of Lucifer yellow-filled astrocytes obtained by confocal microscopy in rat brain slices 7 days after a cortical stab wound. The image of a cell filled during electrophysiological measurements with the fluorescent dye Lucifer yellow was sectioned into a uniformly spaced (0.12 □m) set of 2-dimensional parallel images. The cell surface was found in each image using an edge-detecting algorithm, and the area of the image surrounded by the edge was calculated for each image. Values of cell surface and volume for individual cells were obtained by integrating the values of the edge length and area from all images in a set. The morphometric data were normalized for normal, H1 and H2 astrocytes and expressed as two parameters: surface to volume ratio (S/V) and the percentage of the cell soma volume from the total volume of the cell (V_s/V_{tot}). Our analysis revealed that S/V of astrocytes in normal brain was 5,37±0,62 (n=5); however, in the vicinity of the stab wound it was significantly lower, 2.54±0.19 (n=11) in H1 cells and 3.54±0.42 (n=6) in H2 cells. V_s/V_{tot} did not significantly differ between normal, H1 and H2 astrocytes and was 28.74±3.10, 23.95±5.24 and 29.02±9.30, respectively.
Our morphometric quantification clearly demonstrates that astrogliosis is accompanied not only by changes in membrane currents and GFAP expression, but also by the shortening and hypertrophy of glial processes, which may contribute to increased diffusion barriers in wounded nervous tissue and may affect the process of regeneration.

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HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS - A SUITABLE MODEL FOR STUDYING MODE OF ACTION OF CATECHOLAMINES DURING NONSHIVERING THERMOGENESIS ?

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Recent experiments performed in our laboratory (1,2) demonstrated existence of nonshivering thermogenesis (NST) in humans. The clasical method for estimating quantity of NST is based on thermogenic effect of catecholamines in a whole organism. This method, when applied to man, represents a health risk and, therefore, indirect methods should be find to study the amount of NST and the detailed mode of catecholamine thermogenic action in humans.
Human peripheral blood mononuclear cells (PBMC) isolated by gradient centrifugation were used for experiments. It was found that the amount of nonspecific β-adrenergic receptors corresponded to 14,11+2,27 fmol.mg protein. Since data from other laboratories (3,4) indicated existence of the uncoupling protein 2 in PBMC, the effect of catecholamines on metabolic rate was studied in our experiments. Oxygen consumption of PBMC cultivated in RPMI-1640 medium was measured by the Clark oxygen electrode. Resting metabolic rate of PBMC was found to be 0,75 +0,39 nmol 0_2 /min.10^6 cells. Adrenaline, as well as noradrenaline, in concentrations ranging from 5.10^{-5} - 10^{-4} M, increased oxygen consumption of PBMC by more than 500 %.
Results indicate that the PBMC, when stimulated by catecholamines, can produce a considerable amount of heat and can be used, therefore, as a model system for studying the detailed mode of catecholamine thermogenic action in humans. Further studies in this direction could contribute to clarifying mechanisms preventing obesity in humans.

(1) Lesná I. et al.: J. Therm. Biol. 24: 63-69, 1999
(2) Šimečková M. et al.: J. Therm. Biol. 25: 437-42, 2000
(3) Fleury et al.: Nat. Genet. 15: 269-272, 1997
(4) Gimeno et al.: Diabetes 46: 900-906,1997

CHANGES OF BRAIN NEURONAL ACTIVITY AFTER ACUTE EXPOSURE TO HIGH FREQUENCY ELECTROMAGNETIC FIELD IN HEALTHY AND NEURODEFECTIVE MICE

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Our previous results have shown that high frequency electromagnetic field (HF EMF) affected spatial learning ability in mice that also exhibited histochemical changes in the brain tissue (1).
The aim of the present study was to ascertain the effect of HF EMF acute exposure on expression of c-Fos and NADPH diaphorase activity in the brain of neurodefective mice (Lurcher mutant Lc/+) and healthy mice (wild type +/+). Experimental animals were subjected to acute exposition of HF EMF (3 hours, 880 MHz). After the exposition the mice were anesthetized (Thiopental 2,5mg/kg) and intracardially perfused by 4% paraformaldehyde. The brains were sliced using cryostat and 40μm thick sections were histochemically and immunohistochemically processed to detect NADPH-diaphorase (2) and c-Fos (3) positive neurons.
Our results showed that HF EMF increased c-Fos–like immunoreactivity in irradiated animals when compared with control mice. Labeled cells were located mainly in hippocampus, paraventricular hypothalamic nucleus and ventrolateral thalamic area. Lower density of NADPH diaphorase positive cells in mice after HF EMF exposure was found in analogous areas of the brain in comparison with control animals.

(1) Cendelín J. et al.: Physiol. Res. 51 (4): 6P, 23, 2002.
(2) Wehby R.G., Frank M.E.: J Histochem Cytochem. 47(2):197-207, 1999.
(3) Sumová A. et al.: Brain Res. 801(1-2):254-8, 1998.

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SYNTHESIS AND DEGRADATION OF HEART COLLAGEN IN PATIENTS AFTER ACUTE CARDIAC INFARCTION

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Ischemia of heart has remodelated structural, biochemical and functional properties of extracellular matrix compartment of cardiac muscle. Whereas many results have been published from experimental studies, much less in known about human myocardium after acute cardiac infarction. Aim of present study, therefore, was to determine metabolism of heart collagen in patients with acute cardiac infarction (ACI) and then to correlate biochemical values with functional parameters.
Three groups of patients were in this study: A) patients after acute cardiac infarction treated by PCI, n= 30, B) patients after ACI without PCI, n=5 (either due to either hospitalization later then 12 h from ischemia or due to failure of PCI), C) patients only for coronarography without ACI, n= 10. Metabolism of cardiac collagen I and III was determined by Orion kits ([1]) in blood samples taken 1st, 2nd, 4th, 7th, and 30th day from acute ischemia. Functional parameters has been calculated from echocardiographic measurements; necrotic damage of myocardium was estimated by CKMB.
It has been shown (for A group) that degradation of cardiac collagen, in first days after ACI, is, later, accompanied by massive synthesis of this protein; collagen III proceeded synthesis of collagen I. Similar character of biochemical changes was in group B, however, synthesis of collagens was much more pronounced (period between 7th and 30th day from ischemia). On the other hand, metabolism of collagen was not significantly affected in patient group C. It could be, therefore, concluded that formation of scars is very dynamic procedure. Recently, complex correlations of biochemical data and functional parameters (e.g. improvement of ejection fraction) have calculated. It could aimed in new prognostic markers for cardiologists.

(1) Pelouch et al. Physiol. Res. 51:33 P, 2002

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QT DISPERSION IN PSYCHIATRIC PATIENTS TREATED WITH DOSULEPIN IN CORRELATION WITH DOSULEPIN PLASMA LEVEL

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The aim of the study was to detect changes of the QT dispersion (QTd) due to cardiotoxicity of tricyclic antidepressant dosulepin. Electrocardiographic and vectorcardiographic recordings were obtained using Cardiag 128.1 diagnostic system as a part of Body Surface Potential Mapping from 28 outpatients of the Department of Psychiatry treated with dosulepin and compared to those obtained from 37 healthy volunteers. From these recordings following parameters were evaluated: the QTd, a dispersion of heart rate-corrected QT interval QTc and space QRS-STT angle (1). Acquired data were statistically correlated by Spearman rank order correlation coefficient with dosulepine plasma levels. The average QTd (±SD) in the dosulepin group was significantly higher [70 (±21) ms] than in the normal subjects [34 (±12) ms] ($P< 0.001$). Moreover the correlation between QTd and the dosulepin plasma level was statistically significant as well ($P< 0.001$) with the value of correlation coefficient 0,7871. Similar results were obtained when QTc dispersion was used. On the contrary the QRS-STT space angle was not correlated to the dosulepin level in spite of fact that the QRS-STT angle calculated just in transversal and left sagittal plane is in significant correlation with the dosulepin level, $p < 0,05$ (2). This fact could be explained by the angle deviation in the frontal plane. According to above mentioned results we can conclude that the QTd could be used as a simple measure of the dosulepin effect on the myocardium.

(1) Lechmanová M., Kittnar O., et al.: Physiol. Res. 51: 121-129,2002
(2) Kitzlerová E., Slavíček J., et al.: Physiol. Res.: in press

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INTERACTION OF RESTRAINT STRESS AND AMPHETAMINE ON RAT BEHAVIOR IN THE PASSIVE AVOIDANCE TEST

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Stress is understood as environmental pressure on a living organism that disrupts homeostasis and evokes a cascade of adaptive responses. Many studies demonstrate some similarities in stress responses and actions of drugs of abuse. The aim of this study was to investigate the interaction between stress stimuli and the action of amphetamine (AMPH) on behavior of rats in the passive avoidance task. Restraint stress combined with water immersion (DVIO+C) of male Wistar rats was used as a strong stressor. AMPH was given i.p. as a single dose of 1 mg at various intervals in relation to exposure to the stressor and aversive stimulus (AS). Passive avoidance learning and retention (1) was studied in the apparatus from Coulbourn Instruments (PA, USA), and AS was a foot-shock (0.3 mA, 3s). Retention of memory was tested 24 hours after AS, as latencies of rats to enter the preferred dark (shock) compartment. Our protocol used a one trial avoidance task followed by a post-training amnestic treatment by AMPH at various intervals before or after the training trial. All data were analyzed by ANOVA and post-tests.
IMO+C[-2/-l] (data in brackets indicate the start and end of stressor application in relation to time of application of AS) induced very strong amnestic response that was not influenced by the application of AMPH[-1] (given immediately after termination of stressor). A relatively weaker stress, IMO+C[-3/-2] or IMO+C[-4/-3], produced less pronounced amnestic response that was removed by the application of AMPH immediately after AS (AMPH[0]). The non-significant amnestic reaction produced by IMO+C[-5/-4] was increased by AMPH[0]. Finally, very strong amnestic response due to IMO+C[-2/-l] was significantly attenuated when AMPH was added 1 hour before retention testing AMPH[+23]. Our studies indicate that there exists a significant interaction between actions of IMO+C and AMPH, which depends strongly on intensity of the stressor and time of application of AMPH, namely on its time relationship to the application of the aversive stimulus.

(1) Klenerová V., Kaminský O., Šída P., Krejčí L, HliňákZ., Hynie S.: Behavioural Brain Research 136: 21-29, 2002.

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LIPOPROTEIN (A) - AN IMPORTANT BIOMARKER OF ACUTE AND CHRONIC CORONARY HEART DISEASE

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Lipoprotein (a) - Lp(a) is a variant of apolipoprotein B - containing lipoproteins, rich in cholesterol, with pre-beta motility on agarose gel electrophoresis and chemically similar to LDL. Its level in serum is genetically determined.
Lp(a) is an independent risk factor of atherosclerotic vascular disease. Exist the strong association between raised Lp(a) levels with the presence or severity of coronary heart disease.
In recent time was published that individuals with high plasma Lp(a) levels (more than 25 mg/dl) suffer from premature atherosclerosis and myocardial infarction (1).
To the study were admited 45 patients of medium age with coronary heart disease - CHD (53±11 years), 41 patients with acute myocardial infarction - AMI (58±9 years) and 23 healthy probands - C (43±10 years). Besides Lp(a) the serum levels of apo A-I, apo A-II and apo B were estimated. The Lp(a), apo A-I and apo A-II levels were assessed by the method of radialimmunodiffusion and apo B levels were detected by the method of electroimmunoassay.
The mean value of Lp(a) ($p<0,001$) and apo B ($p<0,001$) in patients with CHD and AMI ($p<0,001$, $p<0,01$) was significantly arising. Our patients proved to have significantly lowered levels of apo A-I ($p<0,001$) and apo A-II ($p<0,001$). Elevated values of the atherogenic index apo B/apo A-I also were found.
In summary, the serum level of Lp(a) is very stabile and don´t decreased after acute heart incident. Lp(a) is involved in an atherosclerotic process and its elevatrd serum level is the most significant indicator for the development and progression of atherosclerosis (2).

(1) Fujino,A., Watanabe,T. et al.: Jpn. Circ. J., 64 ,1:51-56, 2000.
(2) Chang,S.M., Ting,A.C. et al.: Eur.J.Vasc.Endovasc.Surg., 14, 1: 17-23, 1997.

GEOMETRY AND STRUCTURE OF CONDUIT ARTERIES IN OFFSPRING OF HYPERTENSIVE NO DEFECTIVE RATS

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The aim of the study was to characterize structure of conduit arteries of offsprings of parents hypertensive due to NO synthase inhibition. Parents consisted of 5 females and 5 males at the age of ten weeks. The animals were administered NO synthase inhibitor N^G-nitro-L-arginine methyl ester (40 mg/kg/day) in drinking water, for a period of 5 weeks. Fertilisation ocurred in the course of the fifth week of L-NAME administration. In females L-NAME administration continued during the whole pregnancy and breast feeding, up to the fourth week of offspring age. Thereafter six experimental and nine control age matched offsprings were taken for the study. Blood pressure (BP) was measured by tail plethysmographic method. After sacrifying, the cardiovascular system was perfused with a glutaraldehyde fixative under the pressure 120 mm Hg. The thoracic aorta (TA) and carotid artery (CA) were excised and processed according to standard electron microscopic procedure. Geometry of the arteries was determined on semithin sections using light microscopy. Volume densities of smooth muscle cells (SMC) and extracellular matrix in arterial wall (tunica intima+tunica media) of CA were examinated in electron microscopy. BP of experimental group was higher than of control group (150.0±2.3 vs. 104.6±2.1 mm Hg, $p<0.01$). Inspite of this, heart/body weight ratio indicated hypotrophy of the heart 3.9±0.1 vs. 4.4±0.2 in controls, $p<0.05$). The inner diameter (ID) of the TA in experimental offsprings increased ($p<0.01$), not in CA. Contrary to the ID, the values of wall thickness (WT), cross section area (tunica intima and media), and WT/ID ratio of the TA and CA were in experimental offsprings lower then in controls ($p<0.01$). The volume densities of SMC in tunica media of control carotid arterial wall was 44.76±1.06 per cent (it represented 19991±1024 μm^2 of the CSA), in experimental artery their volume density was only 37.62±0.98 per cent ($p<0.01$) (it represented 14678 ± 609 μm^2 of the CSA). Conclusion: In spite of the remarkable high blood pressure the hypotrophy of both heart and arterial wall in experimental offsprings was found. The later is due to remarkably hypotrophy of SMC, not however by a decreased extracellular matrix production.

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INFLUENCE OF SHORT BURSTS OF STIMULATION PULSES ON POSTICTAL INHIBITION.

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Postictal inhibition (PI) is a decrease in excitability that follows an epileptic seizure and decreases probability of new seizure occurrence. PI may involve both increased inhibition and persisting elevated excitation. Our experiments tested whether shorter trains of weak stimuli are able to unmask this residual increase of excitability during the PI.
Under pentobarbital anesthesia (70 mg/kg i.p.), bipolar stimulation electrode and registration electrodes were placed on the cortex and fixed in place with acrylic. Indifferent electrode was located on the nasal bone. Seven days recovery period followed the operation. Four epileptic afterdischarges (AD) were evoked by intense electrical stimulation (20 s, 8 Hz, current intensity at 5x of threshold) of the neocortex in two groups (A, B) of Wistar rats. Before the first AD and during the 10 min interictal period, 8 Hz trains of four weak pulses (half of the intensity used for the AD triggering; 4P) were applied every 20 s in group B and a single pulse with similar parameters in group A.
Interictal epileptiform events evoked by 4P in the group B were significantly more numerous than in the group A (evoked by single pulses) except after the 2nd AD. Epileptic events were triggered by 4P also immediately after the AD termination.
Apparently, weak stimulation can trigger epileptic phenomena during PI. Our results indicate that it is no longer possible to perceive PI only as persisting extreme and active inhibition. An appropriate stimulation can reveal more subtle (but important) excitatory events contributing to the functional status during the postictal period.

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PEPTIDERGIC INNERVATION OF THE DIABETIC RAT HEART

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Vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY) and calcitonin gene-related peptide (CGRP) serve as neurotransmitters and/or neuromodulators of the parasympathetic, sympathetic and sensory innervation of the mammalian heart, respectively. However, VIP and NPY were identified also in the nonadrenergic noncholinergic local circuit intrinsic neurons of the cardiac nerve plexus. This study was aimed to determine putative effects of streptozotocin (STZ) - induced type I diabetes on VIP, NPY and CGRP levels in the heart compartments in the course of the disease. Diabetes was induced in 2-month-old female rats (65 mg/kg STZ, i.v.) and heart compartments were dissected and extracted for subsequent radioimmunoassay at 1, 2, 4, 6 and 10 months after the onset of the disease (STZ1, SZ2, STZ4, STZ6 and STZ10, respectively).
VIP and NPY concentrations showed similar patterns of changes in all heart compartments in the course of the disease. They did not differ from the age-matched control values until the 6th month of diabetes when they significantly declined. In contrast, changes in CGRP levels differed in the atria and ventricles. Compared to the respective controls, CGRP concentrations significantly increased in STZ1 ventricles, remained high in STZ2 and STZ4 samples and then slightly declined. CGRP tissue levels did not differ from control values in STZ1, STZ2 and STZ6 atria and they were slightly increased in the STZ4 tissue extracts.
In conclusion, neuropeptide concentrations in the rat heart compartments undergo differential changes in the course of STZ-induced diabetes. The functional relevance of these changes will be further investigated.

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THE EFFECT OF MEMBRANE NOISE ON THE TRANSMISSION OF INFORMATION ALONG ARBORISED MYELINATED AXON

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Differential conduction of action potentials (AP) at branching points of arborised axons could be responsible for spatiotemporal filtering of spike pattern propagating through dendritic tree. The inhomogeneity in membrane properties of axonal branches, caused by the accumulation of potassium ion in periaxonal space, by the varying branching geometry and by the membrane noise (mainly the channel noise), affects the AP conduction and decrease the amount of information originally contained in neuronal spike train at the beginning of axon.
We applied methods of computer modeling and built the multicompartmental model of an arborised myelinated axon with K ion concentration in the periaxonal space dynamically linked to the activity of axonal fast K channels in the paranodal region. The effect of membrane noise on the amount of information transmitted along arborised axon was investigated, assuming transmission of information by the temporal coding of various spike timing precisions. The mutual information between spike train at the beginning of axon and spike trains at the axonal terminals was estimated by the information theory. To quantify the filtering of spike patents by axonal tree, we estimated the mutual information between spike trains at different axonal terminals and related it with previous information measurements, providing representational specificities of various terminals attributable to abilities of axonal branches to relay selectively various parts of information contained at the beginning of axon.
Our simulations have shown spike-pattern dependent selectivity of axonal tree, permitting or avoiding individual APs to propagate thorough individual branches. This selectivity was weakened by membrane noise, especially at terminal branches of small diameter. Despite the effect of membrane noise on the AP propagation at individual branches, the information contained in spike pattern at the beginning of the axon, did not dissipate during propagation significantly, but was rather distributed into individual axonal terminals, suggesting that branch points of myelinated axons may play important roles in signal integration in an axonal tree.

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DIFFERENCES IN CIRCULATORY PARAMETERS BETWEEN HEALTHY AND HYPERTENSIVE ADOLESCENTS, AND ADOLESCENTS WITH WHITE COAT EFFECT

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The hypertension, which is a common cardiovascular disease in adults, could originate in childhood. The aim of the study was to show differences in circulatory parameters between healthy and hypertensive adolescents and adolescents with white coat effect. We examined 56 subjects (11-21 years) who had repeatedly high causal blood pressure. Basing on 24-hour blood pressure monitoring, the subjects were divided into groups: 24 subjects with hypertension (Hy) and 32 subjects with white coat effect (WhC). Hy and WhC subjects were compared with age-matched healthy controls for both groups: 48 controls for hypertensive subjects (CoHy) and 64 for subjects with white coat effect (CoWhC). Systolic blood pressure (SBP) and pulse intervals (PI) were recorded in all subjects for 5 min (Finapres, metronome controlled breathing at a frequency of 0.33 Hz). The power spectra of SBP and PI were calculated. Indices of baroreflex sensitivity (BRS [ms/mmHg] and BRSf [Hz/mmHg]) were determined by cross-spectral method. The SBP variability was determined as SBP spectral power in the range of 10-second rhythm ($SBP_{0.1Hz}$). Results see in table.

	SBP [mmHg]	PI [ms]	$SBP_{0.1Hz}$ [$mmHg^2$]	BRS [ms/ mmHg]	BRSf [Hz/ mmHg]	BMI [kg/m^2]
CoWhC	115±14.6	753±117	107±96	10.7±6.0	0.018± 0.008	20.1± 2.5
WhC	112±14.9	737±105	123±128	7.2±3.2 **	0.013± 0.005 **	22.9± 5.8 *
CoHy	114±16.2	772±131	94±82	9.5±3.8	0.016± 0.006	20.4± 2.8
Hy	128±15.4 ***	744±116	142±95 **	6.0±2.6 ***	0.011± 0.005 ***	24.6± 5.9 ***

Significance: *p<0.05; **p<0.01; ***p<0.001

Overweight in adolescents was associated with an increase of SBP. BRS and BRSf decreased in both, WhC and Hy adolescents. In Hy, BRS and BRSf were very low, dampening effect of baroreflex on SBP variability was insufficient and SBP variability was significantly higher comparing to controls.

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NEUROPROTECTIVE EFFECT OF (R,S)-4-PHOSPHONOPHENYL-GLYCINE AGAINST NEURONAL DAMAGE ASSOCIATED WITH HOMOCYSTEIC ACID-INDUCED SEIZURES IN IMMATURE RATS

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To evaluate anticonvulsant activity of the group III metabotropic glutamate receptor agonist, (R,S)-4-phosphonophenylglycine ((R,S)-PPG) was administered prior to the induction of DL-homocysteic acid (HCA) seizures. DL-homocysteic acid (HCA) was infused to 12-day-old male Wistar albino rats by means of bilateral intracerebroventricular (i.c.v.) infusions. Glutamate receptor agonist was given in the same way 15-20 min before the DL-HCA infusion. Control pups received corresponding volumes of normal saline.
Brains were fixed by intracardial perfusion one day later. Cryostat sections were processed for Fluoro-Jade (FJ) staining to visualise the dying cells. Material was evaluated and photographed under OLYMPUS Provis fluorescence microscope.
Our earlier experiments have shown in similarly treated rats significant changes in the hippocampal structure, namely the reduction of numbers of cells in the dentate gyrus and fragmentation of the neuronal nuclei.
In the present study of the HCA administration effects, many FJ positive cells were identified in almost whole hippocampus (CA1, CA3) and in the dentate gyrus. Preventive administration of (R,S)-PPG brought about significant decrease of the FJ positive cells in the hippocampal region.
Our finding indicate that the death of the majority of pyramidal and granular cells in the hippocampal region can be attributed namely to the HCA induced seizure activity and only partly due to its direct neurotoxic effect.

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INFLUENCE OF HYPOXIA ON FLUROTHYL SEIZURES

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Studies of the threshold for epileptic seizures were mostly performed on the model of electrical kindling. Intensity and duration of chemically elicited seizures is usually related to the plasma concentration of the substance and the duration of its metabolisation. Changes of threshold are related to the repetition of seizures and partly to their intensity. In the flurothyl model the intensity of seizures is related to the flurothyl concentration in the inspired air and seizures ceased immediately after cessation of its inhalation. Our present experiments are focused on the threshold changes in animals after short period of hypobaric hypoxia (30 min) and repetition of the seizures. Intervals between seizures were 30 min or 3 days. The same intervals were used between hypoxia and seizure.For seizure testing, the rats (adult, male Wistar) were challenged with flurothyl (30 µl/min constant flow rate) in an airtight chamber (14 l) until clonic seizures occurred. We determined the latency to the onset of seizures. Because the flurothyl was administered in the chamber at constant rate the latency to onset of seizures is related to the threshold amount of gas. Immediately after onset of the seizures were the gases in the chamber replaced by the fresh air. In the group of animals with 30 min between the two seizures we did nod observe significant changes in the threshold. In the group with 3 days interval the threshold decreased significantly. Previous hypoxia did not influence the thresholds for flurothyl seizures. Requirement of the long interval between seizures to decrease the threshold could be explained by invovlment of time consuming metabolic processes and/or substantial morphologic changes. Our results could be also explained by postictal inhibition period. We excluded the possibility of involvement of the recurrent seizures after the first convulsion by the experiment performed on the group with implanted electrodes. The EEG in these animals was without any signs of epileptic activity during 7 day after flurothyl seizure.

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GROWTH INHIBITION- AND ENHACED DIFFERENTIATION-ASSOCIATED ACTIVATION OF Γ-GLUTAMYLTRANSFERASE (GGT) IN G6 GLIOMA CELLS IN CULTURE

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γ–glutamyltransferase (GGT) hydrolyses γ–glutamyl peptides, including glutathione, and transports amino acids into the cells. In the brain, GGT is a specific component of the hematoencephalic barrier. Its functional significance and regulation in the parenchymal cells of the brain, including normal and tumour transformed astrocytes, are still not understood enough. In this study we examined activity of GGT in the transformed astrocyte-like C6 glial cells in cultures modulated in growth by different concentrations of serum (10 % and 0.1%) and Db-cAMP supplement (1.66 x 10^{-3}M). Compared to cultures with 10% serum, the total activity of GGT in cells grown with 0.1% serum complement increased 2 and 4 times, when expressed per μg DNA and mg protein, respectively. The increase was mainly (90%) due to up-regulation of the membrane bound fraction of GGT. In cultures with 0.1 % serum+Db-cAMP, activity of GGT further increased. The changes were accompanied by a stepwise inhibition of proliferation, progressive changes in morphology of cells, increased expression of GFAP and heat shock proteins 60 and 70 in the major part of the population. In cultures with reduced serum+Db-cAMP supplement, some cells were loosing attachment to the growth support and undergoing apoptotic-like cell death. The GGT up-regulation associating growth inhibition and enhanced differentiation induced by Db-cAMP-mediated phosphorylation and growth factors deprivation is supposed to reflect alterations in redox potential, and/or, oxidative stress of cells compensated by GGT-GSH co-operation.

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REPEATED ADMINISTRATION OF ZINC CHANGES EXCITABILITY OF CORTICAL NEURONES IN YOUNG RATS EXPOSED TO HYPOXIA

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Zinc is the second most abundant trace element in the body. The primary function of zinc in nonneuronal cells is to stabilize the content of secretory granules. The nervous system contains high concentration of zinc. It represents an important component of many proteins and serves as a signaling messenger that is released from nerve terminals during activity at many excitatory synapses. Cytotoxic amounts of zinc may induce selective neuronal death as a consequence of the ischemia or seizures.
In rats exposed to stress (intermittent hypobaric hypoxia at a simulated altitude of 7 000 m since the day of birth till the 11th or 17th day for 8 hours a day, with their mother) significant prolongation ($p<0.01$) of evoked cortical afterdischarges (Ads) (elicited by repeated electrical stimulation of sensorimotor cortex – frequency 8 Hz, duration of stimulation 15 s, intensity 3 – 5 mA, repetition of stimulation 5 times, always 1 min. after the end of previous epileptic seizure) 1 day or 8 days after the end of hypoxia were registered. 18 days after the end of hypoxia, prolongation or shortening of epileptic seizures were observed. In the present study, we tried to reveal changes in the excitability of brain in rats exposed to hypoxia with zinc pre-treatment (zinc chloride, 5 mg/kg s.c., administered just before each exposition to hypoxia). Rats exposed to hypoxia treated with physiological saline instead of zinc were used as controls. Experiments were performed on rats 12, 25 and 35-day-old - one, eight and eighteen days after the end of hypobaric hypoxia. In 12-day-old animals, the pre-treatment with saline but not with zinc shortened the duration of epileptic seizures elicited by the repetitive stimulation of the sensorimotor cortex. In 25-day-old rats, the pre-treatment with saline did not change the duration of epileptic seizures. The pre-treatment with zinc blocked elicitation of seizures after the 2nd and 3rd stimulation ($p<0.01$). In 35-day-old animals, 18 days after the end of hypoxia, the pre-treatment with zinc blocked elicitation of epileptic seizures in the same way as in 25-day-old rats. Saline application shortened (after the first stimulation) or prolonged (after five stimulations) cortical afterdischarges.
Activation of glutamate receptors or induction of selective necrosis of neurons may explain different reaction to the stimulation of sensorimotor cortex in rats.

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FAS MEDIATED APOPTOTIC CELL DEATH IN TOOTH EMBRYONIC DEVELOPMENT

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The development of different types of teeth and their correct positions in the jaws is genetically controlled and specified early in embryogenesis. Apoptosis represents one of the morphogenetic mechanisms involved in tooth formation.
Apoptotic cells appearing in embryonic development of dental primordia were detected and localized by TUNEL test - terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (Roche Diagnostics) using POD/DAB (peroxidase/diaminobenzidine) modification for light microscopy detection. Apoptotic morphological changes were evaluated after haematoxylin&eosin staining and used as a further criterion of apoptotic death at the level of individual cells. Fas receptor molecules were detected using mouse antibody (BD Pharmingen), Fas ligands were labeled by rabbit polyclonal antibody (Santa Cruz). Field vole (*Microtus agrestis*) embryos (formol fixation) from an embryological collection were employed.
Serial tissue sections were photodocumented after immuno-histochemical staining and Fas receptor and Fas ligand molecules were localized. The temporo-spatial correlation with TUNEL positive cells in epithelium and mesechyme was evaluated. Other steps in the Fas mediated apoptotic cascade and their roles in tooth development are a challenge for our future work.

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11β-HYDROXYSTEROID DEHYDROGENASE (11HSD) IN THE HEART OF NORMOTENSIVE AND HYPERTENSIVE RATS

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Recent studies have shown that not only increased blood pressure but also various humoral factors including corticosteroids play a role in the cardiac fibrosis and hypertrophy secondary to hypertension. The effects of glucocorticoids and mineralocorticoids on the target cells depend on coexpression of glucocorticoid (GR) and mineralocorticoid receptors (MR) and 11HSD which catalyzes interconversion of glucocorticoids to 11-oxo metabolites that have different affinities for GR and MR. The type 2 isoform (11HSD2) converts glucocorticoids to their inactive 11-oxo metabolites and thus protects MR from binding of glucocorticoids. In contrast, 11HSD type 1 (11HSD1) operates in vivo in an opposite direction and increases local concentration of glucocorticoids. To ascertain whether cardiac hypertrophy during hypertension is associated with changes of 11HSD we performed real-time RT-PCR analysis and enzyme activity measurement of 11HSD1 and 11HSD2 in two genetic models of hypertension: spontaneously hypertensive (SHR) and Dahl salt-sensitive (DS) rats and their normotensive counterparts Wistar-Kyoto (WKY) and Dahl salt-resistant (DR) rats.
The relative heart weights of Dahl rats kept on high-salt diet (8 % NaCl) was higher in DS than in DR rats. Similarly, heart weight was higher in SHR than in WKY rats. 11HSD2 mRNA abundance was lower in SHR than WKY rats but not between DS and DR rats. In contrast, 11HSD1 mRNA expression was similar in SHR and WKY rats but was lower in DS than in DR rats. 11HSD1 activity studies correlated with 11HSD1 mRNA expression.
In conclusion, the cardiac metabolism of glucocorticoids varies between normotensive and hypertensive rats. These findings open the possibility for changes in autocrine and paracrine actions of corticosteroids during cardiac remodelling.

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INCUBATION WITH GLUCOSE INITIATES EXTENSIVE OXIDATIVE DAMAGE IN CULTURED PIG EYE LENS

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The reducing sugars such as glucose interact with free amino-groups of proteins in a complex multistep process called non-enzymatic protein glycation. Reactive oxygen species participate in these reactions as intermediates. In patients with poorly controlled diabetes mellitus these reactions may eventually lead to increased oxidative damage to eye lens and formation of cataract.
We studied the effects of glucose concentration (5mM, 10mM, and 50mM) on pig lens in culture in the course of 5 days. We measured production of H_2O_2, leak of LDH, accumulation of lipophilic fluorescent products (LFP) of lipid peroxidation, and the levels of α-tocopherol. Maximum of H_2O_2 production was observed after 48 hours and at the end of incubation it returned to the initial level. The production in the presence of 50mM glucose was approximately double than in the presence of 5mM (control) glucose. The production of H_2O_2 did not correlate with the leak of LDH, that did not depend on the glucose concentration, increased steadily during the time of incubation and was maximum at the end of incubation. LFP were analyzed in the fraction of soluble proteins, in the chloroform-methanol extract, and in the non-soluble protein fraction after digestion with a proteinase. Production of LFP was related to the decomposition of α-tocopherol, the principal lipophilic antioxidant. At 50 mM glucose concentration the destruction of of α-tocopherol was apparent already after 6 h, however, the oxidative damage measured on the basis of LFP concentration was different in individual compartments.
This study documents the complex nature of free radical damage to lens in the presence of increased glucose concentration. It appears that H_2O_2 production is not directly related to the oxidative damage.

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ONTOGENY PATTERN OF NUCLEOTIDE DRIVEN SECRETION IN RAT DISTAL COLON

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Extracellular nucleotides act through purinergic receptors to evoke a wide variety of biological responses in many tissues including intestinal epithelium. P2Y subtypes of these receptors are present also on enterocytes and mobilize Ca^{2+} from intracellular stores. The increase of $[Ca^{2+}]_i$ is followed by activation of K^+ channels and leads subsequently to Cl^- secretion. Autocrine/paracrine effect of nucleotides might contribute to severity of diarrhoea observed especially during early ontogenetic periods. *Aims*: The aim of the present study was to evaluate the developmental changes of secretory response to purinergic and pyrimidinergic nucleotides. *Methods*: Distal colons were isolated from suckling, weaning, and adult rats. Part of the colon was stripped, mounted in Ussing chambers and bathed in Krebs & Ringer solution. Short-circuit current was measured in the absence or presence of various concentrations of ATP, UTP and UDP. All experiments were performed in the presence of amiloride (10^{-5} M) and tetrodotoxin (10^{-6} M) in order to inhibit electrogenic sodium absorption and neuronal regulatory pathways. Microfluorimetry was used to measure the intracellular Ca^{2+} concentration. *Results*: The developmental pattern of secretory response to UDP and UTP was similar, but the later compound had somewhat higher effect. Response to both nucleotides increased from suckling to weanling period and dropped in adult rats under the values of suckling pups. The sensitivity to UDP/UTP was unchanged during the ontogeny. Increase of secretory response induced by ATP reached lower values comparing to UDP/UTP and did not differ markedly during ontogeny. All nucleotides evoked the increase of $[Ca^{2+}]_i$ but ATP was much more effective than UDP/UTP. The ATP-dependent increase of $[Ca^{2+}]_i$ was more profound in the base crypt cells than in the middle or surface cells. Although the effect of ATP in the middle part of crypt was lower in colon of weanling than adult rats, the $[Ca^{2+}]_i$ increase induced by ATP of weanling rats was nevertheless more prolonged. *Conclusions*: Our results suggest that maturation of the rat distal colon secretory response to purinergic and pyrimidinergic nucleotides differs. Major development undergoes secretory response to UDP/UTP while the development is less obvious in the case of ATP. Further exploration is necessary to relate the developmental changes of extracellular nucleotide response to their signal transduction pathways on one hand and the co-operation with other secretagogues on the other hand.

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THE EFFECTS OF DEXAMETHASONE ON HEART MUSCARINIC RECEPTORS AND □ADRENOCEPTORS DIFFER FROM THAT OF HYDROCORTISONE

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Steroid hormones are known to change the expression of huge amount of proteins including receptors. We have reported previously that hydrocortisone brings about increases in the densities of muscarinic receptors, □$_2$- and □$_1$- adrenoceptors without alteration in subsequent steps of signal transduction in the heart (1). Here we present new data on the effects of other steroid, dexamethasone.
Male Wistar rats (aged from 46 to 55 days) were infused by dexamethasone (30, 100 and 300 □g/kg/24 hours) for 1, 3 and 7 days using Alzet osmotic minipumps implanted subcutaneously. At the end of the infusion the animals were killed, hearts were removed, divided into atria, left and right ventricles, homogenised and the receptors were determined using saturation binding experiments with ^{3}H-QNB, ^{3}H-CGP 12177 and competition experiments with ^{3}H-CGP 12177 and CGP 20712A or ICI 118.551.
Dexamethasone caused increase in muscarinic receptors in atria and left ventricles, followed by decrease on day 3 in atria only. After 7 days there were the dose-dependent increases in the atria and left ventricles but the dose-dependent decrease in the right ventricles. Treatment also decreased □$_1$-adrenoceptors number in the atria after 3 days and in the left ventricles after 1 and 3 days. After 7 days there was the dose-dependent increase in □$_1$-adrenoceptor number in atria and left ventricles and decrease in their number in the right ventricles. □$_2$-adrenoceptor number decreased after 1 and 3 days of dexamethasone treatment in left ventricles and in the atria. Dose-dependent changes have been revealed for □$_2$- adrenoceptors in atria and left ventricles (increase) and in the right ventricles (decrease) after 7 days of treatment.
We can therefore conclude that the effects of dexamethasone are more complex than that of hydrocortisone (increase in receptor number only). Our observations are consistent with the hypothesis (2) that certain steroid ligands can act as antagonists in some cells and agonists in others.

1) Mysliveček J., Říčný J., Tuček S.: Physiol. Res. 51,2002
2) Kumar R., Thompson E.B.: Steroids 64: 310-319,1999

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EFFECT OF STRONTIUM ON CA - DEPENDENT CHLORIDE CHANNEL

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By electrophysiological experiments strontium is used for substitution of calcium ions. Whereas some functions of calcium can be supplied by strontium, for example contraction, other functions are unaffected in presence of strontium (for example calcium induced inactivation of calcium channel). The aim of our study was to analyze influence of strontium on Ca - dependent chloride channel.
We used papillary muscles from the right chamber of adult rabbit myocardium. Electrical phenomenons were measured by means of glass microelectrodes. In control solution calcium concentration was 2 mmol/l. We used these modified solutions: a) calcium was substituted by strontium, concentration of strontium was 1,6 mmol/l and concentration of calcium was 0,4 mmol/l, b) sodium chloride was equimolary substituted by iodium chloride (ICl_{Ca} is known to be more permeable for iodide ions than for chloride ions), c) combination of a) and b). The steady state stimulation frequency was 1Hz. In experiments we used stimulation frequencies 3, 2, 1, 0.5, 0.3, 0.2, 0.1 Hz.
In steady state stimulation regime application of strontium leaded to distinct prolongation of action potential (AP) inversely proportional to stimulation frequency. In control conditions action potential became shorter with decreasing stimulation frequency. After application of strontium AP was longer in low stimulation frequencies. Strontium is not able to inactivate ICaL channel, whereby the phase plateau of action potential is prolonged. After substitution of chloride ions by iodide ions action potential was shortened.
Shortening of AP in presence of iodide ions is evidence, that ICl_{Ca} is fully functional when calcium is substituted by strontium. Our results suggested that strontium is able to substitute calcium like opener of ICl_{Ca}.

THE USE OF THE MODIFIED ELISA METHOD FOR DETERMINATION OF ANTIBODIES AGAINST CA MAMMAE AND LDH VIRUS

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The communication is linked up with the previous work in which we were engaged in the utilization 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). The procedure is suitable for early diagnosing and monitoring antibodies in a malignant breast tumor simultaneously with senological examinations which include clinical examinations and mammography (3). We determined a titer of total antibodies in blood of 153 women patients with a various degree of a non-malignant disease of the breast and in 227 samples of blood in women patients with a malignant breast tumor. The examination was extended by us onto determination of a titer of IgG and IgM class antibodies in all samples tested. In both groups of patients, a titer of antibodies was determined against the antigen prepared from the malignant breast tumor and against the 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. In a control group (n=50), various behavior of immunoglobulins was ascertained in relation to antigens used, dilutions used and a potential hidden infectious viral disease of blood donors. Results obtained until now have indicated differences in an immune response of B lymphocytes to a used antigen prepared from human malignant breast tumor and to that obtained from blood of C3H/H2K strain inbred mice infected with the mice LDV. In our further work, we will focus on a quality of the control group of blood donors.

(1) Nedbalová M. et al.: Physiol. Res. 50:P20, 2001.
(2) Jandová A. et al.: Praktická gynekologie 1: 9-12, 2000.
(3) Riley V. et al.: Science 200:124-126, 1978.

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PROXIMAL TO DISTAL GRADIENT OF TEMPERATURE SENSITIVITY OF QUANTAL ACETYLCHOLINE RELEASE AT FROG MUSCLE ENDPLATE

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The conduction velocity of the nerve terminal, mean quantal content and release latencies of uni-quantal endplate currents (EPCs) were recorded in proximal, central and distal parts of the terminal by extracellular pipettes located 5, 50 and 100 □m from the end of myelinated nerve trunk at frog cutaneus pectoris muscle. The spike conduction velocity, minimal latency, modal value of the latency histograms and time interval during which 90% of EPCs are released (P_{90}) at distal, central and proximal part of the frog nerve terminal have different temperature dependency between 10 and 28 ºC. As shown by the size and time course of reconstructed multi-quantal EPCs (1), the better synchronization of quanta release (which is greatest in distal parts), compensates at least partly for the progressive slowing down of spike conduction velocity in the proximo-distal direction, in particular at lower temperatures.

(1) Samigullin D. et al.: J. Neurochem. 2003. (in press).

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EFFECT OF ACUTE ISCHEMIA ON PKC ISOFORMS EXPRESSION IN RAT MYOCARDIUM ADAPTED TO CHRONIC HYPOXIA

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Prolonged exposure to intermittent high altitude (IHA) hypoxia increases cardiac tolerance to an acute ischemic injury (1). We examined whether chronic hypoxia affects the expression and distribution of protein kinase C (PKC) isoforms α, δ, ε and ζ in ischemic and non-ischemic rat ventricular myocardium. Adult male Wistar rats were exposed to IHA hypoxia of 7000 m in a barochamber for 8 h/day, 5 days/week; total number of exposures was 24. Control (normoxic) animals were kept at the altitude of 200 m. The day after the last exposure, the regional ischemia was induced in anesthetized open-chest animals by occlusion of the LAD coronary artery for 9 min. The immunoanalysis of PKC isoforms was performed in particular fractions enriched by nuclear, mitochondrial and microsomal membranes and in the cytosol obtained from the ischemic area of the left ventricle and from the septum (non-ischemic tissue). Adaptation to IHA hypoxia increased the relative content of PKC α, δ, and ζ in all particulate fractions while PKC ε was increased only in the microsomal fraction as compared with corresponding normoxic control values. Acute ischemia decreased content of PKC δ in both normoxic and hypoxic groups. Nevertheless, the values of isoform δ still remained significantly higher in the hypoxic than in the normoxic groups. Contents of PKC α, ε and ζ were decreased in particulate fractions of hypoxic group only. Our results suggest that PKC isoforms α, δ, ζ and ε are upregulated in various cellular compartments of the chronically hypoxic rat myocardium. Their potential role in increased ischemic tolerance of these hearts remains to be determined.

(1) Neckář J. et al.: J. Basic Res. Cardiol. 97:161-167, 2002

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DESENSITISATION OF SIGMA RECEPTORS IN GUINEA PIG ATRIA

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Previous studies have shown a modulatory effect of sigma receptor activation on cardiac contractility and its desensitisation. These experiments were performed in isolated rat ventricular myocytes. Since the rat myocardium is well known for a number of quite specific properties, this study was aimed at verifying the effects of sigma ligands in a more typical model, i.e. guinea pig atria.
Nineteen adult male guinea pigs (295±28 g) were sacrificed under ether anaesthesia. The hearts were rapidly removed, left atrium was cut and placed in thermostatically controlled horizontal perfusion bath (30°C) containing 95% O_2, 5% CO_2 aerated Krebs-Henseleit solution (1.25 mM Ca^{2+}). The preparation was attached to a transducer and stimulated at the rate of 1Hz (1ms pulses of twice the current threshold). Contractions were recorded isometrically. After 30-45 minutes of stabilisation, the typical sigma ligand haloperidol in 10nM concentration was administered to the bath for 30 minutes. Then, a period of 15 min washout and another 30 min period of haloperidol perfusion followed.
The changes of amplitude of contraction and of mechanical restitution were investigated. In all cases, marked positive inotropic effect was observed (increase in the amplitude ranging from 36 to 123%, with maximal effect in the 5th minute of perfusion). The mechanical restitution pattern under the effect of sigma receptor ligand was not altered. This positive inotropic effect was attenuated after the second haloperidol administration (an increase in the amplitude ranging from 9 to 93%, with the maximum effect in the 3rd minute).
In conclusion: the positive inotropic effect of haloperidol, previously described in rat myocardium, was confirmed also in guinea pig atrial preparations. Its attenuation after the repeated exposure indicates that sigma receptors in guinea pig heart undergo a desensitisation process previously reported in rat myocardium. According to the effect of haloperidol on the mechanical restitution it is possible to speculate that it is mediated via increased availability of cytoplasmatic calcium.

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DETERMINATION OF BAROREFLEX SENSITIVITY IN YOUNG SWIMMERS AFTER ONE YEAR OF PHYSICAL TRAINING

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Increased heart rate and decreased baroreflex sensitivity are correlated with higher cardiovascular mortality and morbidity. Heart rate can be decreased by physical training, evaluation of the changes of baroreflex was the aim of the present study.

Baroreflex heart rate sensitivity (ms/mmHg:BRS, Hz/mmHg:BRSf) was determined in a group of sportsmen (swimmers: 9 girls and 10 boys, age 13-15 years) before and after 1 year of training (10 MET; 1 MET is defined as the energy expenditure for sitting quietly, which for the average adult is approximately 3.5 ml of oxygen . kg body weight $^{-1}$. min $^{-1}$) by spectral analysis of spontaneous fluctuations of cardiac intervals and blood pressure (Finapres Ohmeda, 5 min recording, metronome controlled breathing 0.33 Hz). The study was approved by the local ethics committee. The mean values (±s.d.) of BRS (as cardiac interval dependent parameter) and BRSf (as cardiac independent) for the whole group were 9.57±3.12 ms/mmHg and 0.0153±0.005 Hz/mmHg before, and 10.13±4.76 ms/mmHg and 0.0142±0.006 Hz/mmHg after 1 year. Significant differences between boys and girls as well as between the values before and after were not observed. Significant increase of cardiac intervals (before:788±88ms, after:889±105ms) was observed in boys only. The correlations between the first and second values were observed in cardiac intervals (boys: $r = 0.798$, $p<0.01$, girls: $r = 0.842$, $p<0.01$) and in systolic blood pressure (boys: $r = 0.639$, $p<0.05$) but not in BRS (boys: $r = 0.357$, girls: $r = 0.252$) and in BRSf (boys: $r = 0.109$, girls: $r = 0.057$). It is concluded that a 1-year physical training decreased heart rate in boys and did not change BRS and BRSf in adolescents of either sex. Cardiac interval and systolic blood pressure, but not BRS and BRSf, remained relatively constant after 1 year in individual subjects.

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TUMOUR NECROSIS FACTOR ALPHA (TNF-α) AND LEPTIN IN SUBCUTANEOUS AND INTRA-ABDOMINAL ADIPOSE TISSUE.

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INTRODUCTION: Abundantly expressed TNF-α and leptin in various departments of adipose tissue were repeatedly referred to play a considerable role in developing insulin resistance-related metabolic syndrome, an essential pathophysiologic component of obesity (1, 2). METHODS: Two kinds of fat tissue, subcutaneous and intra-abdominal, were obtained during intra-abdominal operations of 13 patients of Surgery Clinic. The protein concentration of homogenates of these tissues was measured by Bradford-technique (3). The concentration of TNF-α and leptin was determined using standard kits. RESULTS: The concentration of leptin in subcutaneous adipose tissue in both gender was significantly higher than in intra-abdominal adipose tissue ($p=0,002$), while the concentration of TNF-α in these departments did not differ significantly. In subcutaneous adipose tissue of women the concentrations of both leptin and TNF-α were randomly higher than in men; both factors correlated with BMI ($R^2=0,71$ and 0,78, respectively), and leptin highly correlated with TNF-α ($R^2=0,932$, $p<0,01$). In men only leptin ($R^2=0,67$) – but not TNF-α ($R^2=0,000$) – correlated with BMI, and the concentration of leptin behaved abstractedly from TNF-α ($R^2=0,0005$, $p<0,6$). In intra-abdominal adipose tissue of women the concentrations of leptin were randomly higher and of TNF-α lower than in men; in both genders only leptin correlated with BMI ($R^2=0,72$ and 0,79, respectively) and correlation between leptin and TNF-α was absent ($R^2=0,01$ and 0,04, respectively). CONCLUSION: The significant gender-related differences in production of leptin and TNF-α in subcutaneous and intra-abdominal adipose tissue were described that could contribute to understanding of obesity-related insulin resistance.

(1) DPS-study group: Horm. Metab. Res. 31: 616-619, 1999.
(2) Kern P.A. et al.: J. Clin. Invest. 95: 2111-2119, 1995.
(3) Bradford M.: Anal. Biochem. 72: 248-254, 1976.

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THE EFFECT OF SPECIFIC LIGANDS OF 5-HT / DA RECEPTORS ON LOCOMOTION IN MK-801 TREATED ANIMALS

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In experimental setting various animal models of the psychosis have been assessed. The blockade of NMDA glutamatergic receptors by MK-801 is thought to be the most appropriate pharmacological animal model of schizophrenia-like psychosis. MK801 alone is known to produce marked hyperlocomotion when administered to rats in doses lower than those producing anesthesia. The involvement of D_2 receptor blockade in counteracting this stimulatory effect is well known. The role of brain serotonergic system on MK-801 induced hyperlocomotion is less clear.In our study we examined the role of combined blockade of $5\text{-}HT_{2a}$ receptor (ritanserin) with blockade of D_2 receptor (haloperidol) and combined activation of $5\text{-}HT_{1a}$ receptor (8-OH-DPAT) with blockade of D_2 receptor (haloperidol) in animal model of psychosis (using NMDA antagonist MK-801). The total locomotion and exploratory behaviour of rats in the open field test (Ethovision, Noldus) was examined. The combination of $5\text{-}HT_{2a}$ and D_2 blockade was more efficient than D_2 blockade alone in adjusting the total locomotor activity to controls in the open field test as well as in normalizing exploratory behaviour. Combination of $5\text{-}HT_{1a}$ activation and D_2 blockade led to an increase of total locomotor activity in the open field test, depending on the dose of 8-OH-DPAT used, and also changed the exploratory behaviour compared to separated D_2 blockade. The exploratory behaviour was different compared to controls as well as to animals with haloperidol-induced D_2 blockade alone.According to our results, the mechanisms within 5-HT receptors may play an important role in modifying the hyperlocomotion induced by MK-801. As MK-801 induces psychosis-like behaviour, our results can serve a new point of view in understanding the role of 5-HT system regarding schizophrenia and its treatment.

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BIOCHEMICAL REMODELATION OF CARDIAC MUSCULATURE IN CHILDREN WITH CONGENITAL HEART DISEASE

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Congenital heart disease (CHD) is cause by developmental abnormalities in the first six to eight weeks of fetal life. Two groups were described: normoxemic and hypoxemic CHD; both ones resulted from aberrant organogenesis due to dysfunction of genes. Aim of this study, therefore, was to analyze protein (P) and phospholipids (PL) profiles of human myocardium (tissue samples - atrial and ventricular musculature obtained during surgery of children with different CHD). We have shown that P profiles of atrial and ventricular parts of myocardium in children with CHD were different; higher concentration of myofibrillar proteins and lower one of extracellular matrix proteins were in ventricles. Furthermore, profiles of both light (L) and heavy (H) chains of myosin were different as well; beta H chain is typical for ventricular and alpha H for atrial part of musculature. Moreover, atrial L were contaminated by ventricular L and vice versa. Furthermore, activities of ventricular glycolytic and mitochondrial enzymes correlates with higher amount of both cardiolipin (mitochondrial PL) in ventricular musculature. Concentration of major PL was lower in atria; however, in both cardiac parts PL-choline and ethanolamine occupied about 70% of total PL. Hypoxemia did not significantly affected both P and PL profiles and compartmentation of troponinT between myofibrillar and cytosolic pools; however, significantly lower myosin ATPase activity myosin was in hypoxemic ventricles only. Electrophoretic pattern of H and L myosin was not affected by hypoxiemia, however, second weak band appeared in both H myosin (alpha and beta H chain in both hypoxemic ventricles and atria, resp.).Moreover, hypoxemia induced in atria both higher synthesis of pepsin-soluble collagen (predominantly collagen III) and activation of metalloproteinase's; total collagen was unchanged. On the other hand, proportion of PL in both atrial and ventricular musculatures were not affected by lower blood oxygen saturation.

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DOWN-REGULATION OF DELAYED OUTWARDLY RECTIFYING AND A-TYPE K^+ CURRENTS IN ASTROCYTES AFTER EXPOSURE TO ELEVATED K^+ CONCENTRATION

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Astrocyte swelling induced by elevated extracellular K^+ is accompanied by morphological changes in astrocytes and increased GFAP staining, ultimately leading to astrogliosis (1). Using the patch-clamp technique in the whole-cell configuration, we studied changes in astrocytic membrane currents in spinal cord slices from 10-day-old rats after incubation of a lumbar spinal cord segment for 3 hours in artificial cerebrospinal fluid containing 50 mM K^+. Cells were identified as astrocytes after electrophysiolgical measurements by positive immunostaining for GFAP or S100β.

Astrocytes characterized by symmetrical, passive K^+ currents were divided into 2 groups according to the expression of additional voltage-activated currents: astrocytes (A1) with inward rectifier K^+ currents (K_{IR}), and astrocytes (A2) with A-type K^+ currents (K_A) and delayed outward rectifying K^+ currents (K_{DR}). Incubation of spinal cords in 50 mM K^+ did not affect the membrane properties of astrocyte precursors or A1 astrocytes. In A2 astrocytes, however, it caused a shift in membrane potential from -88.4 ± 1.62 to -77.6 ± 2.7 mV and a shift in reversal potential after depolarization from –72.2 ± 2.2 mV to –58.5 ± 2.4 mV. Furthermore, inward conductance (G_{in}) decreased from 15.89 ±1.98 pS to 7.14 ± 1.22 pS, outward conductance (G_{out}) decreased from 20.10 ± 1.92 pS to 10.71 ± 1.93 pS and G_{out}/G_{in} increased from 1.63 ±0.15 to 2.82 ± 0.56. A2 astrocytes also showed a significant decrease in the amplitude of both K_A and K_{DR} currents. The amplitude of K_A currents decreased from 1535 ± 208 pA to 879 ± 171 pA and K_{DR} currents from 469 ±71 pA to 240 ± 47 pA.

We conclude that incubation of the spinal cord in 50 mM K^+ selectively affects only one population of astrocytes characterized by K_A and K_{DR} currents, while the electrophysiological properties of astrocytes with K_{IR} currents and astrocyte precursors remain unchanged.

(1) Syková E., Vargová L., Prokopová Š., Šimonová Z.: Glia 25: 56-70, 1999.

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DOPAMINERGIC NEUROTRANSMISSION AND LEVELS OF CAMP IN BLOOD PLASMA OF YOUNG ADULT RATS

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Cyclic adenosine 3´, 5´-monophosphate (cAMP) should no longer be regarded only as an intracellular second messenger but also as a first messenger responsible for coordination of cerebral and hepatorenal functions (1). In mammals, cAMP is probably bound to not yet identified G-protein-coupled receptor(s) unrelated to brain binding of [^{3}H]cAMP to protein kinase A in psychotics (2). Perinatal ischemia, infections or pathological degeneration in the brain may lead to a perturbation of glutamate catabolism in nerve cells with a relation to later dysfunction of glutamatergic neurotransmission observed in schizophrenics. Resulting hypofunction of this system alters functional activities of dopaminergic (and serotonergic) neurons, which are linked with cAMP and IP_3 signaling systems. Because blood platelets (neuroectodermal derivatives) exhibit some similarities to serotonin synapses (3), we collected blood samples into S-Monovette (with K^+-EDTA) from control and experimentally manipulated rat males on postnatal day 50. Isolated platelets were resuspended, counted and bound IP_3 was determined using D-myo-inositol 1,4,5-trisphosphate [^{3}H]assay system (Amersham, UK), whereas plasma cAMP was assayed by RIA cAMP kits (Immunotech, France).

Plasma levels of cAMP in adult rats, being a 10-fold higher than those in humans, were not changed by an hour cerebral ischemia in 12-day-old rat pups. Ischemia did not change the platelet levels of IP_3 as well. However, degeneration induced by a bilateral intracerebroventricular (i.c.v.) infusion of quinolinic acid (250 nmol QUIN/lateral ventricle), an immune-like metabolite of tryptophan with an NMDA receptor affinity, caused a moderate increase of the plasma cAMP (but not platelet IP_3). This increase was statistically significant after i.p. administration of 10 mg GBR12909 (an inhibitor of dopamine reuptake). Haloperidol (0.1 mg/kg, s.c.), partly selective D_2 receptor antagonist, inhibited DA-induced hyperlocomotion, but it did not suppress the increased levels of plasma cAMP. We shall discuss functional importance of the findings.

1) Bankir L. et al., Am. J. Physiol. 282:F376-392, 2002.
2) Dwivedi Y. et al., Am. J. Psychiatry 159:159-166, 2002.

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PROGRESSION OF KAINIC ACID NEUROTOXIC EFFECTS IN THE RAT HIPPOCAMPUS

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Extent and intensity and probably also the time schedule of neurotoxic effects of the kainic acid depend on the distribution of specific types of receptors and on the wiring diagram of the neurons within the affected neuronal circuits. While distribution of receptors is a condition of direct effects, the organization of interconnection may be responsible for the progression of the cell extinction. The detailed description of the progression of nerve cell extinction after the kainic acid administration may thus help to understand the mutual relation of individual components of neuronal circuits. Kainic acid was administered intraperitoneally to adult Wistar male rats in the dose of 10mg per kg, diluted in PS. Brains were fixed by intracardial perfusion 2, 4, or 6 days later. Cryostat slices were processed for Fluoro-Jade staining to visualise the dying cells. Material was evaluated and photographed under OLYMPUS Provis fluorescence microscope. Two days after the kainic acid administration large numbers of neurons in CA1 and CA2-3 were stained. Few scattered neurons were stained in the hilus (interneurons of the hilar regions) Very few neurons were stained in the adjacent part of CA3. Neurons were well preserved and some of dendritic shafts were visible. Four days after the injection of kainic acid the most of the labelled cells were found in the hilus and in the adjacent part of CA3. CA1 region was affected in its distal part only, the region CA2 had no dying neurons. In the six days interval, only few stained cells were present in CA1, CA3 and in the hilus. Most of the cells manifested signs of a longer disintegration process. The dynamics of the development of kainic acid effects indicate, that the mechanism of cell death is related not only to the direct effect of this excitatory molecule, but it may result also from the complexity and specific sensitivity of the neuronal circuits involved.

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CYCLIC FOUR-STATE MODEL DOES NOT EXPLAIN THE CONJUGATED SLOWING OF THE ON/OFF RATES OF ORTHOSTERIC LIGANDS BY MUSCARINIC ALLOSTERIC MODULATORS

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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 (1,2). Most of them decrease both rate of association and rate of dissociation. There are seeming paradoxes: an increasing concentration of the prototypical positive modulator ALCURONIUM slows gradually more even the association rate for the competitive antagonist N-methylscopolamine at M2 subtype of muscarinic receptor. Similarly, negative modulator GALLAMINE slows the dissociation rate in a concentration-dependent fashion. To overcome the problem we hypothesized that a compulsory order of ligand binding proceeded. It means, when allosteric modulator is bound to the receptor, association/dissociation of the orthosteric ligand onto/from the receptor is prevented (3).

In general, the ternary complex allosteric model is described by a cyclic kinetic scheme. The system of differential equations cannot be, in general, solved explicitly. Its correct numerical solution is difficult, with respect to a stiffness of the equations. Presumably, this is the reason, why most of authors still speculate upon a cyclic four-state kinetic scheme. Up to now, there is lack of studies which would analyse the suggested model with respect to the qualitative behaviour. Using Laplace transformations on the system of differential equations we found out simple solutions for the convenient initial conditions. We can visualise the behaviour of the cyclic and opened four-state system (for a ternary complex). Using proof by contradiction, we demonstrate a physical inconsistency of the model with cyclic reaction scheme.

1) Christopoulos A., Lanfazame A., Mitchelson F.: Clin. Exp. Pharmacol. Physiol. **25**:184-194, 1998.
2) Christopoulos A., Kenakin T.: Pharmacol. Rev. **54**:323-374, 2002
3) Proška J., Tuček S.: Mol. Pharmacol. **45**:709-717, 1994.

PHARMACOLOGICAL REMOVAL OF SARCOPLASMIC RETICULUM (SR) IN ADULT RABBIT INDUCES NEONATAL-LIKE MECHANICAL RESPONSES OF VENTRICULAR MYOCARDIUM

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The characteristic property of adult mammalian myocardium is a relation of contraction force to the rhythm and stimulation rate. This frequency dependence is missing in the underdeveloped myocardium. The most pronounced phenomenon of the frequency sensitivity to the premature excitation is a postextrasystolic potentiation (PEP). We assume that frequency and rhythm sensitive contractions depend on the normal (mature) function of SR. To confirm this hypothesis, we have to functionally eliminated adult SR and compare the mechanical behavior with the health (pharmacologically untreated) adult myocardium.
The experiments were performed on the right ventricle papillary muscles from newborn and adult rabbits. By means of two-chamber stimulation and mechano-electrical transducer the pseudoisometric contractions were measured. The block of SR-calcium release channels (by means of tetracaine or by means of supra-micromolar concentrations of ryanodine) caused at the beginning depression and than the disappearance of PEP. The permanent depletion of calcium ions from SR by caffeine (from 2 to 10 mmol.l^{-1}) inhibited PEP. The block of sarcoplasmic Ca-ATPase by cyclopiazonic acid caused the negative inotropic response and avoided PEP.
Our results confirm the assumption that the crucial cause of frequency sensitivity of contractions is normal function of SR. The normal mature SR serves for the optimal intracellular calcium handling in adult heart tissue and it is responsible for reticular type of ECC. The functional block of SR (pharmacological elimination of SR) in adult papillary muscles causes the functional conversion of ECC from the reticular to the sarcolemmal type. Neonatal myocardium is unable to optimize the contraction regime and exhibits only small perceptible relationships between rate and rhythm of activity and contraction force.

INFLUENCE OF THE BODY POSITION ON THE MAXIMAL SPACIAL T-VECTOR IN BOYS AND YOUNG MEN WITH INCREASED DIASTOLIC BLOOD PRESSURE

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The purpose of this study was to analyze variations in responses of repolarization parameters to head up tilt, with respect to initial and reactive blood pressure (BP) values. Changes. The study population consisted of 30 young men and boys 11-36 years old, mean age=17,7±3,9 years, with either increased systolic blood pressure values (EBPs) N=23, or diastolic BP values (EBPd), N=17, or both N=10, (high normal or hypertension I, according the classification WHO/ISH 1999), without ECG signs of ventricular hypertrophy. There were 36 age matched normotonic subjects in the control group (CG). Standard ECG and VCG were recorded in sitting and supine position as well as during head up tilt to 60º. The following electrocardiologic characteristics were evaluated: the maximal spacial T vector (sTmax), spacial angle between integral QRS and T vectors, R-R and QT intervals. BP was measured simultaneously in each of the body position.
Resting sTmax values in seated persons were higher ($p<0.05$) in CG, but in supine position the differences were not significant. Values of the spacial angle, R-R and QT intervals as well as their reactive changes were comparable in all subgroups, regardless of body position. The decrease of the sTmax amplitude due to head up tilt was significantly greater (by 31,2%, $p<0.001$) in EBPd group than in the others (15-20%). There were thight correlations between the magnitude of sTmax and R-R, respectively QT intervals, ranging from r=0.52 to r=0.79, which were not altered either in CG or in EBPs by changing body position. The magnitudes of the orthostatic Tmax changes were proportional to those of R-R and QT interval. None of these relationships were found in EBPd.
Whereas increased systolic BP was not associated with different pattern of repolarization as compared with normotonics in none of the examined body positions, head up tilt induced in EBPd quantitatively different changes of sTmax, it altered the mutual relationship between repolarization parameters, as well between their initial and reactive values.

COMPARATION OF IMMUNE RESPONSE OF DIFFERENT SPECIES OF RODENTS AFTER THE ANTIGENIC STIMULATION OF BBSL

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In 1999 there were for immunization purposes trapped wildly living rodents from the tribe Muridae (*Apodemus flavicollis*, *Mus musculus*) in 2 areas of the Czech Republic. The process of immunization was practised by whole-celled antigens *Borrelia afzelii* and *Borrelia garinii* in 3 phases and after each application of the antigen the blood samples were taken. The acquired sera were analysed by the Western blott technique (WB).
With the help of this method antigens were applied on the polyacrylamid gel and transferred on the nitrocellulose membrane. The acquired replica was further used for the detection of same components by a specific antibody. After the reaction with the antibody bands of bacterial proteins appeared on the membrane. Their molecular weights were determined by the programme Molecular analysts, with the help of which were also determined for individual molecular weights their optic density (OD) and percentage of their volume (%V), which are the most conclusive parameters while comparing the immune response of different species of animals. The data were successively processed by a nonparametric ttest with levels of signification □= 0,05 and □= 0,1.
If we applied antigen *B. afzelii* on the gel, immune response aren´t statistically significantly different (with □= 0,05) only in 2 cases from total 8 ones. On the other hand, if we used antigen *B. garinii* in WB, the significant difference between experimental rodents was proved only in 2 from total 8 cases on the level of significance = 0,1. The results are identical in both types of entrance data (OD, %V) and they show important distinctive differences between the immune response of *Mus* and *Apodemus* if we use the race *B. afzelii*, while using the race *B. garinii* rodents immune responses differed insignificantly.
Significant conclusions can be also confirmed by a visual judgement of the membranes, where much more intensive immune responses of *Mus* are perceptible. More over, at *Apodemus* there is a lack of bands with higher molecular weights. This can be explained by the presence of immunocomplexes, that obstruct the reaction with the bacterial antigen.

DOES CIRCULATING CORTICOSTERONE AFFECT THE EXPRESSION OF PROOPIOMELANOCORTIN, INTERLEUKIN-1BETA, AND INTERLEUKIN-6 IN ADENOPITUITARY OF NORMAL AND ARTHRITIC RATS?

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Adjuvant arthritis (AA) is a rat model of rheumatoid arthritis in humans. In the acute phase of AA the hypothalamo pituitary adrenocortical axis is activated with chronically elevated corticosterone (CORT) levels. There are feedback regulations between CORT and inflammatory cytokines, namely interleukin-1 beta (IL-1beta) and interleukin-6 (IL-6) released from the inflammatory cells. These interleukins have been produced also in the brain and adenopituiutary (AP). To find out a possible regulatory relationship between circulating CORT and adenopituitary POMC, IL-1 beta and IL-6 we analyzed the 24-hour pattern of CORT and that of POMC, IL1-beta and IL-6 mRNA in AP in normal and arthritic rats The experiment was performed in male Long Evans rats in the acute phase of AA i.e. on day 23 of the disease. Intact animals served as controls. The animals were kept under a strict 12:12 h light/dark cycle with free access to pelleted diet and water. The rats were killed by decapitation in 4-h intervals started at 14:00 and finishing at 10:00 on the following day. Trunk blood was used for CORT determination, by radioimmunoassay. Adenopituitaries (AP) were extracted for total RNA and the message of interest was quantitated by real – time PCR using specific primers and Taq-man probes. Parameters of rhythms were evaluated by cosinor analysis. In normal rats, serum CORT showed a circadian rhythm with the peak at 18:00 h. Arthritic rats had suppressed rhythmic secretion with lower afternoon and higher morning values. POMC mRNA showed inverse pattern to CORT levels in normal animals. Similar profile was found in arthritic rats with higher mesor and lower amplitude. IL-1 beta and IL-6 expressions in normal rats showed clear cut circadian rhythms which inversely correlated with CORT levels. In arthritic rats the rhythm of IL-1 beta was muted and that of IL-6 persisted with higher values of mesor. These results suggest a regulatory relationship of CORT and the expression of POMC, IL-1 beta and IL-6 in the AP in normal rats. In arthritic rats the correlation between CORT and IL-1beta is eliminated.

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MATURATION OF RAT DISTAL COLON SECRETORY RESPONSE

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The developing colon undergoes morphogenesis and cytodifferentiation that are precisely regulated and highly organised, both spatially and temporally. These events are associated with dramatic changes of epithelial transport processes including secretion. It is a common clinical observation that the incidence and severity of infectious diarrhoea decreases with age, but the mechanism of these developmental changes of intestinal secretion is unknown. *Aims:* As enteric, immune and neuronal cells produce important modulators of intestinal transport, the aim of the present study was to examine the effect of some secretagogues in immature and mature rat colon. *Methods:* Distal colons were isolated from suckling, weaning, and adult rats. Part of the colon was stripped, mounted in Ussing chambers and bathed in Krebs & Ringer solution. Short-circuit current (Isc) was measured in the absence or presence of various concentrations of 5-hydroxytryptamine (10^{-8} to 10^{-4}M), histamine (10^{-8} to 10^{-3}M) and cholinergic agonist betanechol (10^{-8} to 10^{-3}M). Theophylline (1 mM), the inhibitor of phosphodiesterase, was used to measure the basal cAMP-dependent secretory tonus. All experiments were performed in the presence of amiloride (10^{-5} M) and tetrodotoxin (10^{-6} M) in order to inhibit electrogenic sodium absorption and neuronal regulatory pathways. *Results:* 5-hydroxytryptamine produced a concentration-dependent increase in Isc; the maximum response was slightly higher in adulthood than in suckling and weaning rats but the sensitivity to the transmitter was higher in immature than in mature colon. Maximum response to bethanechol also increased from suckling to weanling period but decreased in adults. Suckling pups were more sensitive to this secretagogue than the older animals. In contrast, the response to histamine was identical in young and adult animals. Although the basal cAMP-dependent secretory tonus was smaller in sucklings and weanlings than in adult rats, the shift in sensitivity to 5-hydroxytryptamine and bethanechol does not occur on the level of cAMP and $[Ca^{2+}]_i$ respectively. *Conclusions:* The results demonstrate that colonic secretory pathways are not fully matured during suckling and weaning periods and that there are different developmental patterns for various secretagogues. Our observation that immature rat colon is more sensitive to 5-hydroxytryptamine and bethanechol can explain a part of the increased secretory response to enterotoxins in early postnatal life of human and animals.

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THE ROLE OF FREE RADICALS IN ALZHEIMER'S DISEASE: A STUDY OF THE END-PRODUCTS OF LIPID PEROXIDATION IN DOG

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The pathogenesis of Alzheimer`s disease is still unknown. In the recent time oxidative stress is being discussed as an important contributor. In the present study we investigated the role of free radicals in the canine model of Alzheimer`s disease. We analysed the end-products of lipid peroxidation, lipofuscin-like pigments, protein carbonyls, and vitamin E to obtain more complete description of the oxidative damage in brain of demented dogs. When the generation of free radicals is intensive, the toxic products of lipid peroxidation can diffuse out from the site of the primary formation and can attack erythrocytes. Therefore we also determined the level of lipid peroxidation in red blood cells.

In brain of demented animals compared to age-matched controls the level of LFP increased (to 247 %, $P<0.05$) as well as protein carbonyls (to 438%, $P<0.01$) while the vitamin E concentration was lowered (34%, $P<0.01$). The end-products of lipid peroxidation have been increased also in erythrocytes of demented dogs (235%, $P<0.05$). In this case a new, disease-specific fluorophore was observed.

The present results indicate intensive production of free radicals in brain of animals with Alzheimer type dementia which induces damage to erythrocytes. Detection of the specific products of free radical damage in erythrocytes could be used for diagnostic purposes.

DOSE-AND PLASMA LEVEL-DEPENDENT DOSULEPINE CHANGES OF HEART ELECTRIC FIELD IN DEPRESSIVE WOMEN

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The tricyclic antidepressant Dosulepine (D) is often used in depressive patients, but it has some cardiovascular side effects. In our previous works D in therapeutic doses caused tachycardia and changed some parameters in ECG depolarization and repolarization surface isointegral and isoarea maps (1,2). The aim of the present work was to confirm the direct effect of D on these parameters due to its therapeutic and plasma levels. In 18 female outpatients with recurrent depressive disorder, without cardiovascular diseases, age 46 ±12 years, treated with D daily doses 25-125 mg for 4-8 weeks, 30 parameters of heart electric field (ECG, VCG, body surface maps) were measured with Cardiag 128.1 diagnostic system. Plasma levels of D were determined by high performance liquid chromatography (HPLC). The higher heart rate (84.4±11.7/min) and shortening of R-R interval (725±102.8 ms) were observed. The Spearman correlation coefficient showed 1) QRS right axis deviation in frontal plane from +10° to +92°, 2) opening of QRS-STT planar vector angles in transversal (horizontal) from -2° to -150° and left sagittal from -20° to -100° plane, 3) the maximum (extreme) in 40th ms of QRS depolarisation isoarea map (DIAM max 40) decreased proportionally from 27.7 to 4.0 μVs to the increase of D plasma level from 10-70 ng/ml. The spatial QRS-STT angle was higher in D patients (64.3±45.4°) than in controls (44.6±20°).The results confirmed the direct dependence of the parameters on plasma level in D due to the activation of adrenergic system.

(1) Paclt I. et al.: Homeostasis 36: 223-227, 1995
(2) Slavíček J. et al.: Physiol. Res. 47: 297- 300, 1998
(3) Andrásyová D. et al.: Physiol. Res. 47: 1P, 1998

TRANSPLANTED HEART AND IT'S ELECTRICAL CHARACTERISTICS, USE OF TIME AND FREQUENCY DOMAIN ANALYSES

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The standard method for acute rejection detection is endomyocardial biopsy (EMB). It has several limitations involving the reliability in determining the degree of rejection based on morphologic characteristics of a small number of bioptic specimens acquired from a small localized area in the right ventricle, sampling error, specimens from the scars after previous biopsies. Thus intensive search is underway to find an appropriate and reproducible noninvasive methods, such as signal-averaged ECG {SA ECG}. For SA ECG recordings portable bedside measuring unit ART 1200 EPX was used. Obtained recordings were analysed in time-domain (LP) and frequency domain (FFT, Wigner's distribution) analyses. The study group consisted of 6 female and 33 male patients after HTx, age ranging from 18 to 65 years (46.92± 6.94), median 47. The follow-up duration ranged from 2 days to 18 months (9.63 ± 3.47). Our study revealed, that time-domain analysis is not an appropriate method for the detection of rejection when used alone, and can not totally replace EMB. Negative predictive value is relatively high (93.5%), but the positive predictive value is low (32%).Wigner´s distribution gives a more complex and exact information about the characteristics of cardioelectric signal as FFt, and enables more precise detection of potentials in the range of high frequencies (70-130), which seems to be more important in evaluation of the state of myocardium after transplantation. Even though the results of several investigators studying the possibilities of SA ECG in detection of cardiac allograft rejection are conflicting, there is an agreement that most interesting are the changes in high frequencies, which can be useful in long term follow up and evaluation of these patients. Further investigations are needed to obtain more detailed information. Time and frequency domain analyses could be appropriate complementary methods for long term follow up of cardiac transplant recipients in combination with other diagnostic procedures.

DIFFERENCES IN ADENYLYL CYCLASE ACTIVITY INMYOCARDIUM OF VARIOUS INBREAD STRAINS OF RATS

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In recent years, cardiovascular research concentrated on the theoretical basis of rational prevention and therapy of most serious cardiovascular problems, such as primary hypertension, ischemic heart disease, etc. In experimental cardiology there are used various rat strains as experimental models for the study of genes underlying various cardiovascular diseases. Since adenylyl cyclase is one of the principal targets for drugs affecting heart rate and intensity of myocardial contraction, we extended our previous experiments on inbred rat strains and tested adenylyl cyclase activity in several frequently used strains. We used these strains: Wistar rats (WI), Sprague-Dawley rats (S-D), Lewis rats (LE) spontaneously hypertensive rats (SHR), normotensive Brown-Norway rats (BN) and congenic strain BN-Lx.
Adenylyl cyclase (AC) activity was determined in crude tissue homogenates of left ventricles, and in some experiments also in right ventricles. AC activity was assayed by a method using ^{32}P-α-ATP; radiolabeled cAMP was purified on DOWEX X50 and isolated on aluminium oxide column chromatography (1). In all tissue samples we determined total adenylyl cyclase by forskolin activation, receptor induced activation (by isoprenaline) and activity dependent on activation of G protein by the use of guanylylimidodiphosphate (Gpp/NH/p).
The highest total adenylyl cyclase activity in left ventricles was found in S-D rats, the lowest one in LE rats and SHR. BN rats revealed higher activity than SHR rats. Similar ratio of AC activity was also observed in unstimulated samples (basal activity). In samples stimulated by isoprenaline in a wide range of concentrations we were unable to demonstrate more potent activation in SHR than in other rat strains. In summary, we have demonstrated substantial differences in adenylyl cyclase activity, however, clear relationship between adenylyl cyclase activity and possible model of cardiovascular disease were not yet determined.

(1) Hynie S., Šída P., Klenerová V., Asemu G., Ošťádal B.: Journal of Receptor and Signal Transduction Research 22, No 4: 2002.

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MORPHINE ADMINISTERED DURING PREGNANCY ALTERS TWO GENERATIONS OF RAT PROGENY

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Previous studies demonstrated that morphine administration during pregnancy impairs maternal behaviors and has long-term effects on rat progeny. It is, however, not known whether the maternal morphine administration alters also maternal behavior of adult, morphine-exposed female progeny and whether the morphine-induced effects persist into the second generation. In the present study, morphine was administered subcutaneously in the dose of 5-10 mg/kg to pregnant female rats on gestation days 11 to 18. Adult, drug-exposed female progeny were impregnated and tested for maternal behaviors. The effects of prenatal morphine exposure on nursing, maternal, non-maternal activities, and on retrieval were examined. Prenatal morphine exposure increased active nursing, while decreased passive nursing. There were no differences in maternal activities such as time spent in the nest, in contact with pups, grooming pups, and/or manipulating nest shavings. In the retrieval test, prenatally morphine-exposed mothers were quicker in retrieving pups into the nest than prenatally saline-exposed mothers. The present study demonstrates that the effects of morphine on maternal behavior is different in prenatally morphine-exposed mothers than in mothers chronically treated with morphine during pregnancy. While direct morphine treatment impairs maternal behaviors, prenatal morphine exposure opposes these effects. In addition, first and second generations of morphine-treated mothers were tested during lactation. Number of pups per litter was counted after delivery. The ano-genital distance (AGD) was measured on postnatal day (PD) 1, righting reflex was examined daily during PD 1–12, and all pups were weighed daily between PD 1–25. There were no differences in the number of pups/litter, the percentage of male and female pups/litter, or in the AGD of saline- and morphine-exposed litters. Morphine-exposed pups were slower to right in both generations. Moreover, the latency to gain upright position was even longer in the second generation compared to the first. There were no morphine-induced differences in the weight during lactation in either generation. Thus, morphine administration has long-term effects that affect even the second generation of pups, which were not exposed to the drug at all.

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NOREPINEPHRINE-STIMULATED THERMOGENESIS AND IT´S INDUCTION BY HIGH FAT FEEDING IN C57BL6/J AND A/J MICE

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Two mice strains C57BL6/J (B6) and A/J differing in obesity disposition (the first is obesity prone, the second is obesity resistant) increase the expression of uncoupling protein 1 (UCP1) in brown fat as well as in subcutaneous dorsolumbal white fat (1). To analyse the contribution of high fat (HF) feeding induced UCP1 expression on the thermoregulation of these mice strains, the B6 and A/J male mice were randomly selected at 4 weeks of age and kept at 20 °C or 30 °C. One half (control groups) was fed by a standard diet (ST; caloric intake: 25% proteins, 9% fat, 66% carbohydrates), the other (experimental groups) by HF diet (caloric intake: 13% proteins, 60% fat, 27% carbohydrates). After 2 weeks, the capacity of non-shivering thermogenesis (NST) stimulated by norepinephrine (NE) application (600 □g kg^{-1}, i.p.) was tested under total anaesthesia (thiopental in a dose 100 mg kg^{-1}, i.p.) as a difference in oxygen consumption before and after NE injection at 30 °C.
The analysis of results was performed with ANOVA or Kruskal-Wallis nonparametric test (numbers of animals in a single group 5 to 9, 8 groups, total n = 58). After application of norepinephrine, NST was significantly higher in A/J mice than B6 ($P < 0.05$) and HF diet induced significantly the NST capacity in A/J group (about 95 %, $P < 0.001$) but not in B6 group (about 21%, $P > 0.19$). The NST capacity induced by feeding was higher in mice of A/J strain kept at 20 °C compared to 30 °C ($P < 0.04$). The results show that NST induction by HF diet in A/J mice (contrary to B6) is coherent with the obesity resistance of this mice strain and with the higher content of UCP1 transcript in subcutaneous dorsolumbal white adipose tissue (1). It seems therefore that brown fat adipocytes interspersed in subcutaneous white fat and their induction by high fat diet play important role in A/J mice both in obesity resistance and their increasing capacity of non-shivering thermogenesis.

(1) Pražák T., Flachs P., Štefl B., Machová E., and Kopecký J.: Wenner-Gren Symp. Proc.: Fever, Hyperpyrexia and Thermogenesis, June 5-8, Stockholm.

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DEVELOPMENT OF CEREBELLAR PURKINJE CELLS DEGENERATION IN LURCHER MUTANT MICE

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Lurcher mutant mice are a natural model of geneticaly determined olivocerebellar degeneration (1), which is caused by δ2 glutamate receptor gene mutation (2). Heteroyzgous individuals (+/Lc) suffer from a rapid complete loss of cerebellar Purkinje cells and decrease of cerebellar granule cells and inferior olivary neurons number during postnatal development (3). We investigated two different strains of Lurcher mutants, C3H and C57B1/7.
We used 8 animals 14 days old, 4 of each strain. The mice were deeply anesthetized with Thiopental (0.17 mg/kg) and perfused with paraformaldehyde. Brain sections were processed by a fluorescent doublestaining method (4) and Nissl staining. In wild type mice only normal Purkinje cells were present. In Lurcher mutants there were also still some normal-shaped cells like in wild type mice but most of their cells showed signs of degeneration. In each individual mutant mice wide scale of stadia of cell degeneration can be found. The number of Purkinje cells was higher in wild type mice. However, cerebellar cortex of Lurcher mutant mice also contained areas with normal number of Purkinje cells.
The combination of two histological methods enables to evaluate the progression of the cerebellar degenaration in individual Lurcher mutant mouse.

(1) Phillips R.J.S.: J. Genet. 57 (1960) 35-42
(2) Zuo J., De Jager P.L., Takahasi K.J. et al.: Nature 388: 769-773, 1997
(3) Caddy K.W.T., Biscoe T.J.: Phil. Trans. Roy. Soc. Lond. B. 287: 167-201, 1979
(4) Kröger R.H.H., Wagner H.-J.: Journal of Neuroscience Methods 84: 87-92, 1998

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EXERCISE CARDIOPULMONARY PHYSIOLOGIC DATA MEASURING AND EVALUATING

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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. For 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. The curve can be filtering by least-squares data smoothing. The program KONSIL enables to find automatically some important value from the data measured. The programs and systems for automatic stress testing have been developed in cooperation with doctors for more then 18 years and are used in several function laboratories in the Czech Republic.

1. Stork, M.: The Hardware and Software for Cardio-pulmonary Exercise Testing. Symbiosis 01, VI International Conference, Szcyrk, Poland, ISBN 80-214-0893-6, September 2001, pp.149-154.
2. Stork, M.: The Laboratory and Telemetry Systems for Cardiopulmonary Exercise Testing, IF MBE Proceedings, 2nd European Medical and Biological Engineering Conference EMBEC'02, Vienna, December 2002, Part 1, pp. 518-519.
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RAISED LEVELS OF PLASMA AND CEREBROSPINAL FLUID INTERLEUKIN-1β AND γ-GLUTAMYL TRANSPEPTIDASE ACTIVITY IN ADULT RAT BRAIN

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It has been reported that in inflammatory and autoimmune diseases, peripherally released interleukin-1β (IL-1β) and other proinflamatory cytokines, have neurochemical and behavioral effects similar to those of quinolinic acid (QUIN). Whereas the intracerebral injection of IL-1β transiently increased permeability of rat blood-brain barrier (BBB) to serum protein, alteration of the BBB functioning after the systemic administration of IL-1β has not been studied. As QUIN increases permeability of the BBB to serum albumin in parallel to a decrease in brain tissue and capillary γ-glutamyl transpeptidase (GGT) activity, we decided to study changes in the enzyme activity in 50-day-old Wistar (and/or Long Evans) rats 2, 24 and 96-h after an intravenous injection of 5 μg IL-1β (recombinant rat IL-1β, Sigma) or in rats 2-h after a bilateral intracerebroventricular (i.c.v.) injection of 50 ng IL-1β/ventricle and/or in Long Evans rats with experimental rheumatoid arthritis. Brain endothelial cells are the direct target of circulating IL-1β via activation of the arachidonic acid cascade and, therefore, their membrane-bound GGT activity may reflect the BBB dysfunction. We found a significant decrease of the GGT activity in the Wistar entorhinal cortex and hippocampus, but not in the hypothalamus 2-h after the i.v. injection of IL-1β. However, 24-h after the injection, the enzyme activity increased in all studied brain regions with highly significant increase in the hypothalamus; this effect disappeared after 96-h. After the i.c.v. injection of IL-1β decreased GGT activity was observed in the entorhinal cortex and hippocampus (but not in hypothalamus) of male Wistar rats but quite opposite changes were found in Long Evans rats. Also 1-h lasting cerebral ischemia (bilateral ligation of arteria carotis comm.) in 12-day-old Wistar rats, did not change the cortical and hippocampal enzyme activity on postnatal day 50. This study demonstrates biphasic changes of GGT activity in some rat brain regions after the systemic IL-1β administration but minimal changes were observed after inflammation accompanying rheumatoid arthritis and/or cerebral ischemia.

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THE EFFECT OF NO SYNTHASE ACTIVITY ON DEVELOPMENT OF POSTDENERVATION CHANGES IN SKELETAL MUSCLE FIBERS

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The hypothesis that changes in the NO cascade activity could play a role in the development of some postdenervation changes was tested at rat skeletal muscles. The examined postdenervation changes were: the appearance of TTX-resistant muscle action potentials, the changes of the trigger level for anodal break excitation (ABE), and the changes in AChRs distribution. M. extensor digitorum longus (EDL) and diaphragm (DF) muscles from female rats (120-150 g) were used for both, electrophysiological recordings of membrane potentials and the fluorescence detection of AChRs labeled with FITC-□-bungarotoxin.
In the first series of experiments, the control intact animals were compared with intact animals treated with i.p. injections of NOS inhibi-tors (L-NAME, ethylthiourea) for 4-7 days (daily dose 50 mg/kg and 12.5 mg/kg, respectively). In treated animals, the number of TTX resistant muscle fibers increased by 86.9% (DF) and by 80.1% (EDL) while ABE threshold potential decreased by 25.5 mV (DF) and by 23.6 mV (EDL). In the second series, the control denervated animals were compared with denervated animals treated with i.p. injections of NO donor (sodium nitroprusside) for 4-7 days (daily dose 1.5 mg/kg). The number of TTX resistant fibers decreased from 100% in controls to 20.0% (DF) and 41.4% (EDL) in animals treated with NO donor. ABE threshold potentials in treated animals were 31.9 mV (DF) and 43.6 mV (EDL) above the level observed in controls.
As observed by fluorescence, the spreading of AChRs was suppressed by the treatment of denervated animals with NO donors for 16 days. On the other hand, extrasynaptic AChRs could be detected in intact animals after 16 days of the treatment with NOS inhibitors.
The results obtained by both, electrophysiological and fluorescence methods, support the view that NO cascade activity plays an important role in the functional state of muscle end-plate.

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EFFECT OF VARIOUS INSULIN CONCENTRATIONS ON MECHANICAL RESPONSE OF MYOCARDIUM IN NORMAL AND DIABETIC ALBINO RATS

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Insulin is known to stimulate L-type of calcium channel (1) and calcium current of Na^+/Ca^{2+} exchanger (2). It is supposed that insulin can influence parameters of the cardiac contraction.
The aim of this study was to compare the effect of various insulin concentrations on normal and diabetic myocardium of albino rats. Insulin dependent diabetes was induced by streptozotocin (65 mg/kg of body weight). Experiments were performed 12 weeks after streptozotocin administration. The measurements were performed on right ventricular papillary muscles in modified Tyrode solutions and in the Tyrode solution containing insulin (8, 80 and 800 I.U./l). Two stimulation regimes were used: steady state and period of rest (steady state interrupted with pause of 10 – 300 sec). The following parameters were recorded by means of a mechanoelectrical transducer: maximum isometric tension (MG), maximum rate of isometric force increase (dF/dT_{contr}), maximum rate of pseudoisometric rate relaxation (dF/dT_{relax}), time to peak of contraction (TTP) and half time of relaxation (R/2).
Insulin weakened MG in both groups; this decrease was dose-dependent in the normal rats but almost dose-independent in the diabetic ones. The other parameters of contraction (dF/dT_{contr} and dF/dT_{relax}) were altered in the same way like MG. Time parameters (TTP and R/2) were shortened with insulin (more in higher concentration) in the control group and almost unchanged in the diabetic group. Insulin potentiated the force of the first contraction after period of rest more than in control solution in both experimental groups.
Our results suggest that insulin exerts the negative inotropic effect in the albino rat and this effect is dose-dependent in the control animals.

(1) Aulbach, F. et al: Cardiovasc. Res. 42 (1): 113 - 120, 1999.
(2) Ballard, C., Mozaffari, M., Schaffer, S.: Mol. Cel. Biochem. 135: 113 - 119, 1994.

EFFECT OF TWO ANESTHETIC AGENTS ON RAT MYOCARDIUM AT PULMONARY DISORDERS. CHRONOPHYSIOLOGICAL STUDY.

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Objectives: In *in vivo* experiments, there are many factors with the direct or additive effetcs on the electrical stability of the heart. Effect of the light and dark and effect of the used anesthetic agent can be one of them. The aim of study was to determine whether the electrical stability of the heart is influenced by the light and dark cycle or also by anesthesia in the conditions of the hypoventilation and reoxygenation. Methods: The ventricular arrhythmia threshold (VAT) and heart rate (HR) were measured in female Wistar rats (adaptation on the light regime 12 : 12 h, pentobarbital 30 mg/kg, i.p, ketamine/xylazine 100 mg/15 mg/kg, i.m., open chest experiments). The conditions of the pulmonary ventilation - normal ventilation and reoxygenation V_T = 1ml/100g, respiratory rate 40 breaths/min, hypoventilation V_T = 0.5 ml/100g, respiratory rate 20 breaths/min. In the control groups (n = 67, light control pentobarbital group; n = 50, dark control pentobarbital group; n = 90, light control ketamine/xylazine group; n = 57, dark control ketamine/xylazine group), the first value of VAT was measured after five minutes of stabilization. In the experimental groups (n = 26, light pentobarbital group; n = 16, dark pentobarbital group; n = 13, light ketamine/xylazine group; n = 18, dark ketamine/xylayine group), the animals were subjected at once to 20 minute hypoventilation followed by 20 minute reoxygenation. Results: During hypoventilation, the VAT was significantly lower vs. control group with the continual decrease to the end hypoventilation under the both anesthesias and in the both light parts of day. Reoxygenation increased the VAT vs. hypoventilation ($p < 0.001$) but only in the light part of day. In the dark part of day, the next decrease was seen. The HR was significantly lower ($p < 0.001$) in the experimental groups under ketamine/xylazine anesthesia in the both light parts of the rat regime day. Conclusions: Hypoventilation decreases the electrical stability of the heart independently on the used anesthetic agents in the both light parts of day. Reoxygenation acts proarrhythmogenic, facilitates the development of reoxygenation arrhythmias mainly in active part of day. These changes do not depend on the type of the used anesthetic agent but exclusively on the light - dark cycle. (VEGA, 1/7188/20)

TOPOLOGY OF ORGANELLES IN SLOW SKELETAL MUSCLE FIBRES OF MOUSE.

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The contractile ability of skeletal muscle cells is closely related to their highly organised structure. At the ultrastructural level, the topology of organelles is a result of continuous adaptation of the cell to the short- and long-term stimuli that shape the contractile characteristics of the muscle. The aim of this study was to compare the volume and surface densities of the cellular organelles and their spatial relations in the slow- and fast-type muscle fibres of the mouse. We have used samples of 5 soleus muscles of C57BL/6 mice, which were processed for electron microscopy, randomly sectioned, photographed and analysed using the method of vertical sections (1). In comparison to gastrocnemius muscle fibres (2), the soleus showed a higher relative volume of membranous organelles: subsarcolemmal mitochondria (SM) 3.9×, interfibrillar mitochondria (IM) 3.0×, sarcoplasmic reticulum (SR) 1.9×, and T-system 2.2×. The volume of the Z-line was also higher (2.2×) but the volume of the A-band was 4.6× less. The volume of the I-band was not different. Analysis of the mitochondrial environment revealed a 7.5x larger fraction of the SM surface near the SR, 1.8× larger near the SR cisterns, 12.3× larger near the T-system, and 2.3× larger between the mitochondria themselves. On the contrary, the surface of the SM near the myofilaments was lower, namely, 2.7×, 3.2× and 3.6× near the Z-line, I-band and A-band, respectively. Interfibrillar mitochondria of the soleus also faced significantly different environment than IM of the gastrocnemius. The relative surface area near the neighbouring mitochondria (2.1×) and near the I-band (1.9×) was lower but near the Z-line and the A-band was not changed. Substantially higher was the occupancy of the IM by the SR (3.4×) and especially by the T-system (10.4×) but not by the SR cisterns. The comparative stereologic analysis underlined morphological differences between the fast and slow skeletal muscle fibres, which have their counterpart in the contractile function.

(1) Baddeley AJ et al.: J. Microsc. 142: 259-276, 1986.
(2) Novotová M. et al.: AFM conference, Nice-Acropolis, France, Program and Abstracts P247, 2000.

Supported by VEGA 2/7191/20

QUINOLINATE LESION OF DEVELOPING HIPPOCAMPUS DISRUPTS PREPULSE INHIBITION OF ACOUSTIC STARTLE IN ADULT RATS

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Perinatal retroviral infections can disrupt hippocampal development and cause selective neuronal death by toxins, like quinolinic acid (QUIN), produced by activated macrophages/microglia. The retroviral brain activation may be involved in the pathogenesis of schizophrenia (1). We reported that increased levels of QUIN in the cerebrospinal fluid of 12-day-old rats, induced by intraventricular infusion of 250 nmol/lateral ventricle, were associated with deficits in behavioral activities in the open field testing and were correlated with a decrease in the specific [^{3}H]glutamate binding and with atrophy of the hippocampus of 50-day-old rats. Using Startle Reflex System (San Diego Instruments) the acoustic startle responses (ASR) and their prepulse inhibition (PPI) were assessed in the QUIN-treated, saline-treated (control) and intact young adult rats under the session arrangement as described by Ellenbroek (2).
First, we excluded a possibility that QUIN-induced brain lesion at or below the pons could be accompanied by a hearing deficit owing to an involvement of glutamate receptors in the primary (trisynaptic) ASR pathway (3). Then, the three groups of rats received a block of 50 trials, pseudo-randomly presented throughout the session, to measure ASR and PPI. QUIN-treated rat males had a tendency to higher basal ASR amplitude (pulse 120 dB), but their PPI was completely disrupted at the lowest prepulse intensity (73 dB) and remained depressed at prepulses of 75 and 80 dB. It suggests that PPI depends not only on the startle habituation, but also on the intensity of the prepulse.
These data, together with recently published studies (4), provide further evidence that infection/toxin-induced neuronal death and glutamate receptor loss in supra-pontine brain regions may impair sensorimotor gating in the animal neurodevelopmental model of schizophrenia.

(1) Lewis D.A., Proc. Natl. Acad. Sci. USA, 98:4293-4294, 2001.
(2) Ellenbroek B.A., Pharmacol. Biochem. and Behavior 73:177-184, 2002.
(3) Koch M., Progr. Neurobiol., 59:107-128,1999.
(4) Bubeníková V. et al., Physiol. Res., 51:5P, 2002.

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EXPERIENCES WITH FECAL ELASTASE-1 DETERMINATION IN CHILDREN WITH AND WITHOUT PANCREATIC INSUFFICIENCY

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Fecal elastase (E-1) examination represents a noninvasive, simple method assesing the exocrine pancreatic function (1).
We studied E-1 stool concentration in 78 patients with (CF) cystic fibrosis (mean age 9.8 ±6.1 years), 22 insulin dependent diabetes mellitus (IDDM) patients (12.2 ± 4.8), 16 patients with chronic pancre-atitis (10.4 ± 3.1) and 124 controls without pancreatic or intestinal disorders (7.4 ± 5.6).
Pancreatic elastase (E-1) was examined with Elastase-1 ELISA kit Bioserv Diagnostics, Rostock, Germany.

	Median content E-1 µg/g stool	Range
Controls	598 ± 227	72 - 1020
CF	64 ± 134	0 - 900
IDDM	304 ± 188	70 - 740
Chronic pancreatitis	577 ± 252	65 - 1070

In CF patients E-1 concentration positively correlated with the sweet Cl^- and to their CFTR genotype. The E-1 concentrations of CF and control group were statistically highly significant different ($p < 0,001$). Generally lower E-1 concentration was also found in IDDM (6 cases under 200 µg/g stool). The lowest E-1 concentration we found only in patients with severe exocrine pancreatic insufficiency.
The E-1 determination in stool seems to be more sensitive than other indirect test (chymotrypsin, trypsin) and pancreatic function can be asessed without interuption of an exogenous pancreatic enzyme therapy.

(1) Philips I.J., Rowe D.J.: Ann.CIin. Biochem. 36:739-742, 1999

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SIMVASTATIN IMPROVES ENDOTHELIAL FUNCTION OF AORTA IN HEREDITARY HYPERTRIGLYCERIDEMIC RATS

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Previously we have shown that hereditary hypertriglyceridemic (hHTG) rats are characterised by endothelial dysfunction which contributes to enhancement of systolic blood pressure and impairment of endothelium-dependent relaxation of conduit arteries (1). The aim of this study was to investigate the effect of long-term (4 weeks) treatment of hHTG rats with three drugs which, according to their mechanism of action, may be able to modify the endothelial function: simvastatin (an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase), spironolactone (an antagonist of aldosterone receptors) and L-arginine (a precursor of nitric oxide formation). Systolic blood pressure was measured indirectly by tail-cuff plethysmography each week. In vitro analysis of endothelium-dependent relaxation of the thoracic aorta was performed. Mean systolic blood pressure in hHTG rats before treatment was in the range of 145-149 mm Hg in all groups. At the end of 4th week blood pressure in control hHTG group was 148±2 mm Hg and in control normotensive Wistar group 117±3 mm Hg. Arginine did not significantly influence blood pressure, but after simvastatin (118±1 mmHg) and spironolactone (124±4 mmHg) blood pressure was significantly lowered ($P<0.001$). In isolated phenylephrine-precontracted aortic rings from hHTG rats endothelium-dependent relaxation was diminished as compared to control Wistar rats. Simvastatin improved acetylcholine-induced relaxation, but spironolactone and L-arginine did not significantly change endothelial function of the thoracic aorta. We conclude that long-term treatment with simvastatin has a beneficial effect in hHTG rats since it lowered elevated blood pressure and improved impaired endothelial function of the thoracic aorta.

(1) Török J. et al. Ann. N.Y.Acad. Sci. 976:469-475, 2002

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NEW PROGRESS IN QUANTITATIVE BALLISTO-CARDIOGRAPHY

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This abstract deals with the quantitative measuring of systolic force and the analysis of heart rhythm coherence through the use of quantitative ballistocardiography (Q-BCG) measurement. The use of Q-BCG makes it possible to set up the characteristics as follows: the systolic force (F) and the minute cardiac force (MF), which are related to the body mass of each examinant so as to obtain comparable values. The F is an arithmetical mean of forces that are measured from HI, IJ and JK ballistocardiographic amplitudes. This arithmetical mean represents a response of force according to heart activity and is expressed in units of force (Newton). To obtain a picture of total intensity of the heart, the MF minute cardiac force has been used equal to F multiplied by the HR heart rate, and expressed in units of force per minute (1). The target is to monitor the heart rate and carry out an analysis of heart rhythm variability. The latter contains the statistical and autocorrelation analyses, spectral analysis, assessment of the aggregated effect of the regulation of autonomous functions of vegetative homeostasis, activity of the vasomotor centre, activities of the sympathetic cardiovascular centre and the stress index. In conclusion, the exactitude and reliability of the above methods will be discussed in relation to the current monitoring of persons during their conventional working activities. This absolutely non-invasive method can be used to monitor the operators either within their examination on the ground or in flight conditions and in weightlessness. We can also mention, in addition to other applications, the continuous monitoring of staff to prevent diseases, to determine breaks during the working process, and to estimate the potential capabilities for performing duties (monitoring the mental load, fatigue, inadvertence etc.).

(1) Trefny Z., at al.: Journal of Cardiovascular Diagnosis and Procedures 14/2:109, 1997.

CELLULAR ADAPTATION OF THE SLOW SKELETAL MUSCLE OF THE MICE TO THE DOUBLE DEFICIENCY OF CREATINE KINASE

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In fast glycolytic skeletal muscle fibres with blocked gene expression of both the mitochondrial and cytosolic isoforms of creatine kinase (CK), it was shown that the lack of this key enzyme in muscle energetics triggers a tissue-specific adaptation of the function (1), which includes reorganisation of their ultrastructure (2). The aim of this study was to analyse the adaptation of the ultrastructure in slow oxidative skeletal muscle fibres (soleus) of the $CK^{-/-}$ mouse with the use of the method of vertical sections.

We have found that in comparison to control mice, the volume of the subsarcolemmal (SM, 1.6×) and interfibrillar (IM, 1.3×) mitochondria and of the lipid droplets (2.1×) was increased. In contrast, almost a 5× decrease of the sarcoplasmic reticulum (SR) volume was found. The volume of the SR cisterns and of the T-system decreased 1.6× each. Analysis of the environment of the SM and IM revealed an increase in the mitochondrial surface occupied by the A-band, 1.4× and 2.8×, respectively. On the contrary, a decrease in the SM and IM surface facing the SR (7.3× and 5.5×), the cisterns of SR (26.3× and 4.5×), and of the T-system (3.8× and 7.0×), respectively, was observed.

Similar to the fast skeletal muscle fibres, there was a significant increase in the volume of both mitochondrial populations and their relocation to the A-band in slow fibres. In contrast to the gastrocnemius, however, the volume of the SR system in soleus was greatly reduced in $CK^{-/-}$ mice with respect to the control. Interestingly, in spite of the increased mitochondrial volume, oxidation of the lipid droplets was obviously reduced. In the context of the substantial reduction in the volume of the SR system it points to a greatly reduced energy consumption in the soleus of the $CK^{-/-}$ mouse. The possible regulatory step might consist in the loss of the association of the mitochondria with the cisternal SR, that is, in a decreased exposition of the mitochondria to the regulatory calcium transients.

(1) Steeghs K. et al.: Mol. Cell. Biochem. 184:183-194, 1998.
(2) Novotová M. et al.: AFM conference, Nice-Acropolis, France, Program and Abstracts P247, 2000.

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GEOMETRY OF THE CAROTID ARTERY OF WISTAR RATS AND SHR IN THE COURSE OF THE ONTOGENIC DEVELOPMENT

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The aim of the study was to evaluate the geometry of the carotid artery (CA) of Wistar rats and spontaneously hypertensive rats (SHR) in three periods of ontogenic development. Three groups of Wistar rats of the age: 1) 3 weeks, 2) 9 weeks, and 3) 17 weeks were taken for the study and three groups of the age matched SHR. Blood pressure (BP) was measured indirectly by the tail plethysmographic method. After sacrifying the rats of all groups were perfused with a glutaraldehyde fixative under pressure 90 mm Hg (3 weeks old rats) and 120 mm Hg (9 and 17 weeks old rats). CA were excised and processed according to standard electron microscopy procedure. Geometry of the artery – wall thickness (WT), cross sectional area (CSA), inner diameter (ID) was measured on semithin sections using light microscopy. Wall thickness/inner diameter ratio (W/D) was calculated. BP of 3 weeks old Wistar rats (83±1.9 mm Hg) did not change from the age matched SHR (84±1.4 mm Hg). The difference in this respect was observed between the groups of 9 weeks (106±11 mm Hg in Wistar rats and 154±1.4 mm Hg in SHR, $p<0.01$) and 17 weeks old rats (127±1.4 mm Hg in Wistar rats and 216±5.0 mm Hg, $p<0.01$). Body weight was in all SHR groups lower than in the age matched control groups ($p<0.01$).

	3w Wistar	3w SHR	9w Wistar	9w SHR	17w Wistar	17w SHR
WT	27±0 .80	30± 0.78*	27± 1.20	34± 0.66*	25± 1.50	46± 2.02*
ID	519±9	448± 15*	743± 24	714± 21	824± 28	852± 21
CSAx10^3	47± 1.9	46± 2.3	65± 2.3	79± 1.5*	66± 3,3	130± 7.0*
WD x10^{-2}	5.3± 0.15	6.8± 0.23*	3.7± 0.30	4.8± 0.22*	3.1± 0.25	5.4± 0.25*

*P<0.01 with respect to the age matched Wistar rats.

In conclusion: BP and arterial wall mass (CSA) of CA of 3 weeks old SHR, contrary to 9 and 17 weeks old SHR, did not significantly differ from the age matched controls. We suggest close relationship between BP and arterial wall mass in CA.

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BRACHIAL PLEXUS INJURY IN YOUNG RATS

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The model of brachial plexus avulsion - the multiple dorsal rhizotomy in rats - was introduced in 1974 by Basbaum (1). Different changes following the unilateral section of 3 to 9 dorsal roots at the cervicothoracical level implicate the development of chronic pain syndrome localised in the ipsilateral limb (2). The brachial plexus is the most common site involved in upper extremity neuropathies in human neonates. Self-mutilation develops in some cases and is severe in patients with total palsy.
Unilateral extensive dorsal rhizotomy (C4 – Th1) was performed during postnatal period (up to 60 days postnatally) under pentobarbital anaesthesia (50 mg/kg; 2% solution) in male rats. The frequency of occurrence of self-mutilation behavior was studied. When rhizotomy was performed during the early postnatal period (P10 to P22 days postnatally), self-mutilation did not develop at all. There was a high mortalitiy rate in this group, especially in the youngest rats. The occurrence of self-mutilation in rats that underwent rhizotomy later than the P22 was not significantly different compared to the rhizotomy in adults. The preliminary results indicate that development of self-mutilation behavior in rats strongly depends on the period of nervous system injury and that mature nervous system is required.

(1) Basbaum A.I.: Exp. Neurol. 42: 490-501, 1974.
(2) Vaculin Š., Rokyta R.: Physiol Res 49, 2000.

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REDISTRIBUTION OF PULMONARY MAST CELLS AND THEIR MMP-13 PRODUCTION IN HYPOXIC RATS

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Chronic hypoxia results in pulmonary hypertension due to structural remodelling of peripheral lung blood vessels. We hypothesise that vascular remodelling in chronic hypoxia is initiated by increased collagen cleavage in the walls of prealveolar pulmonary arteries (1). In chronic hypoxia, the collagenolytic metalloproteinases might be released from the activated mast cells. We identified pulmonary mast cells within 50 mm^2 of lung sections collected from Wistar male rats, 10 control, 10 exposed to 4-day, and 10 exposed to 20-day normobaric hypoxia (10% O_2). Total mast cells' amount we identified using Toluidin Blue staining, mast cells expressing MMP-13 (rodent type interstitial collagenase) were detected using the monoclonal anti-MMP-13 antibody visualised by the sequence consisting of the alkaline phosphatase-labelled secondary anti-mouse IgG antibody, Fast Red and substrate, counterstained with haematoxylin. Significant increase in number of MMP-13 positive mast cells was revealed within adventitia of prealveolar arteries after 4 days of hypoxia. Number of the MMP-13 positive mast cells within adventitia of conduit arteries increased significantly in the 20-day hypoxia group. Number of total mast cells increased significantly in the 20-day hypoxia group due to both mast cells localised subpleurally and within adventitia of conduit arteries as well. Our observation supports the hypothesis that perivascular pulmonary mast cells contribute to the vascular remodelling in hypoxic pulmonary hypertension in rats by the release of interstitial collagenase.

(1) Hampl, V., Herget, J.: Role of nitric oxide in the pathogenesis of chronic pulmonary hypertension. Physiol. Rev., 80, 2000: 1337-1384.

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ROLE OF PRESSURE DIURESIS IN INTEGRATION OF BODY FLUIDS AND CARDIOVASCULAR FUNCTION. PHYSIOLOGICAL, CLINICAL, AND THERAPEUTIC CONSIDERATIONS

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Long-term mechanisms that regulate arterial pressure have been intimately implicated in the regulation of sodium balance and circulatory and extracellular fluid volume, in contrast to acute, alarm regulatory mechanisms that act primarily by influencing cardiovascular capacitance, peripheral circulatory resistance, and cardiac performance (1-4).
As long as the circulation maintains its integrity and appropriate plasma protein concentration remains in blood, the plasma volume and the interstitial fluid volume of the extracellular compartment change proportionately. A change in blood volume, as related to capacitance of the vessels, can change the degree of vascular filling that alters arterial pressure through the direct effect on venous return. Numerous mechanisms exist that can detect the degree of vascular filling. The very fundamental among them is that of pressure diuresis.
A concept of pressure diuresis states that the kidneys set the systemic arterial pressure at the level they require to maintain ion and fluid balance. Additional mechanisms can detect the degree of vascular filling and signal the kidney to modulate the rate of loss of Na^+ and water. Thus, the regulation of the total body fluid volume is indirect involving long-term regulation of systemic arterial pressure.
There is no other known basic mechanism to monitor the total body water, the extracellular fluid volume, or the blood volume besides pressure diuresis. Within the overall conceptual framework of pressure diuresis a number of physiological, clinical and therapeutic data can be discussed as illustrated by examples given in the presentation.

(1) Guyton A. C., Hall J. E.: Textbook of Medical Physiology. 10th Edition. W. B. Saunders, Philadelphia 2000.
(2) Jelínek J.: Čs. fysiol. 39, 473-486, 1990.
(3) Červenka L., Šímová M., Malý J., Heller J.: Čs. fyziol. 49, 116-133, 2000.
(4) Veselý J.: Tlaková diuréza a arteriální hypertenze. Epava, Olomouc 2002.

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NEUROBEHAVIOURAL IMPACT OF MANGANESE EXPOSURE

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The aim of this study was to monitor a) manganese penetration into several tissues of rat, including the brain, under the conditions of modelled inhalatory exposure to manganese (Mn) and b) potential neurobehavioural impact of such Mn dosage. Eventual influence of second exposure factor: sinusoid magnetic field (SMF) was observed, too. Manganese was elected because of its neurotoxic potential.
Female Wistar rats were used, average weight 200±20 g body weight, kept on standard pellets, water ad lib. Groups of rats: C - without exposure, C1 - exposure to Mn, C2 - exposure to SMF, EX - exposure to SMF + Mn. Manganese was instilled intratracheally as MnO_2 suspension in saline (control rats saline only), dosage: 0.48 mg Mn/kg body weight, 2x per week, for 12 weeks altogether, total dose of Mn at the end of exposures being 11.5 mg Mn/kg b.wt. Rats were exposed to SMF (B = 10 mT and f = 50 Hz) for one hour .
Manganese content in the brain, lung, kidney and liver was analyzed at the end of 6 weeks or 12 weeks exposures and 2 months after the end of exposures by means of atomic absorption spectrometry. Neurobehavioural impact of exposures was evaluated by means of examinations in two tiers: Functional Observational Battery (FOB) consisting from simple observations and measurements, and second tier examinations consisting from long-term locomotion activity measurements, electrocardiographic and startle reaction examinations.
The most important analytical finding is the Mn clearance from the lungs and brains of rats at the period 2 months after the end of exposures. Statistically highly significant Mn lung residual retention ($P<0.001$) as well as still significant brain Mn content ($P<0.05$) for EX and C1 groups of rats denote toxicologically endangering situation: potential redistribution of huge Mn lung depot to the brain of rats.
Analytical data for C1 and EX groups of rats correlated with neurobehavioural findings: significantly increased spontaneous motoric activity ($P<0.05$) and increased startle reaction for EX group ($P<0.05$).

(1) Vojtíšek M. et al.: 8th International Symposium: Neurobehavioural Methods and Effects in Occupational and Environmental Health, Abstracts, June 23-26, Brescia, Italy, 2002. p.151

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CHANGES IN DIFFUSION PARAMETERS IN THE BRAIN OF MICE LACKING TENASCIN-R AND HNK-1 SULFOTRANSFERASE

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We studied the influence of extracellular matrix (ECM) composition on the diffusion parameters in brain tissue. Two different methods were employed to measure diffusion changes: Magnetic resonance (MR) imaging and the real-time iontophoretic tetramethylammonium (TMA) method. The MR method determines the apparent diffusion coefficient of water (ADC_W) in the tissue; the TMA method determines the extracellular space (ECS) volume fraction (α = ECS volume/total tissue volume) and the apparent diffusion coefficient of TMA (ADC_{TMA}) in the ECS. Experiments were performed in vivo in the cortex of 8-month-old mice. Two different strains of knockout animals were used: Tenascin-restrictin-deficient mice (TN-R -/-) (1) and HNK-1 sulfotransferase-deficient mice (ST -/-) (2) along with their wild-type littermates. In TMA experiments, the animals were anesthetized with sodium pentobarbital (90 mg/kg); during MR measurements, isoflurane (1.5%) was used for inhalation anesthesia. The ECS volume fraction (α) was significantly decreased in the knockout animals: in TN-R -/- mice $\alpha = 0.118 \pm 0.009$ (n = 10) and in ST -/- animals $\alpha = 0.157 \pm 0.008$ (n = 6) as compared to the controls ($\alpha = 0.185 \pm 0.004$, n = 8 and $\alpha = 0.196 \pm 0.003$, n = 7, respectively). In TN-R -/- mice we found significantly increased ADC_{TMA} (612 ± 19 $\mu m^2 s^{-1}$, n = 10 vs. 548 ± 12 $\mu m^2 s^{-1}$, n = 8 in controls), and a tendency towards an increased ADC_{TMA} was also observed in ST -/- mice. In contrast to these elevated ADC_{TMA} values, ADC_W in TN-R -/- and ST -/- knockout animals was significantly decreased (526 ± 10 $\mu m^2 s^{-1}$, n = 10 and 564 ± 4 $\mu m^2 s^{-1}$, n = 8, respectively) as compared to the controls (576 ± 8 $\mu m^2 s^{-1}$, n = 8 and 601 ± 6 $\mu m^2 s^{-1}$, n = 4, respectively). Our results show that the extracellular matrix is important in determining the size of the extracellular space and that it forms diffusion barriers in the ECS affecting diffusion in the brain tissue. In the absence of the extracellular glycoproteins tenascin-restrictin or HNK-1 sulfotransferase, the ECS volume is greatly decreased and, in addition, there is an increase in ADC_{TMA}. The decrease in ADC_W may reflect an increased ratio between the intra- and extracellular space volumes rather than changes in ECS diffusion barriers.

(1) Weber et al.: J. Neurosci., 19 (1): 4245-4262, 1999.
(2) Senn et al.: Mol. Cell. Neurosci., 20 (4): 712-729, 2002.

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FUNCTIONAL EXPRESSIONS OF NEURODEGENERATION AND PLASTICITY IN TWO STRAINS OF LURCHER MUTANT MICE

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Mechanisms of plasticity underlie processes of learning and memory but they also play a key role in functional compensation of different neuropathological states. The activity dependent plasticity was studied in two strains of Lurcher mutant mice (Lc/+) suffered from progressive olivocerebellar degeneration. The development of motor learning was investigated using four methods (1) in the course of the first month of life, spatial learning was studied in two months old mice by means of swimming Morris water maze method (2). Healthy wild type mice (+/+) served as controls. A comparison of motor learning and topical motor skills in trained and untrained animals showed significantly worse results in Lc/+ than in +/+. However, the learning effect was still present in Lc/+ and in 30 days old animals (C3H strain) it was relatively higher when compared with +/+. In spatial learning the animals of the C57B1/7 strain proved to be a more suitable model than C3H mice. Nevertheless the learning effect was also evident in Lc/+ of both strains during the training course (6 days) though they were generally worse in comparison with +/+. The results have confirmed a new conception of the cerebellum that deals with its role in cognitive function (3). They also showed that neurodefective Lc/+ were able to learn to a certain degree, and this fact gave evidence about effective mechanisms of activity dependent plasticity and compensation despite the progessive neurodergeneration and strain differences.

(1) Vožeh F., Cendelín J., Motáňová A.: Homeostasis 39:248-250, 1999
(2) Cendelín J., Vožeh F.: Acta Physiol. Hung. 89:189, 2002-11-30
(3) Petrosini L, Leggio MG, Molinari M.: Prog Neurobiol. 56:191-210, 1998

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ANALYSIS OF THE MOLECULAR MECHANISMS OF ACTION OF PREGNENOLONE SULFATE AT NMDA RECEPTOR CHANNELS

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20-oxopregn-5-en-3beta-yl sulfate (PS; pregnenolone sulfate) is a naturally occurring neurosteroid that is produced in the central nervous system. The aim of the present study was to characterize the effect of PS on NMDA receptors.
Patch clamp technique was used to record the responses from native and recombinant NMDA receptor channels expressed in cultured hippocampal neurons and HEK-293 cells transfected with genes encoding for NR1 and NR2B subunits.
Responses mediated by native and recombinant NMDA receptors (activated by 100 μM NMDA or 1 mM glutamate) were potentiated by PS (300 μM) 3.5 and 5-times, respectively, and the deactivation of responses was slowed ~2-times. Kinetic modelling was used to analyse molecular mechanisms possibly involved in PS action. The results indicate that PS affects the probability of NMDA receptor channel opening. Experiments using 9-aminoacridine, a voltage–dependent NMDA receptor channel blocker, confirmed this hypothesis. PS increased the probability of NMDA receptor channel opening from 22% in control to ~100%. PS affinity determined from the association and dissociation rate constants ($K_{on} = 2.5\ 10^3\ s^{-1}M^{-1}$ and $K_{off} = 0.13\ s^{-1}$) of PS interaction with resting NMDA receptors was found to be $K_d = 43$ μM. The affinity of PS was reduced upon NMDA receptor channel activation. Analysis of responses mediated by recombinant receptors indicates that PS has a dual effect. At low concentrations PS potentiate the responses, while at concentrations >30 μM it has also an inhibitory effect.
The results of our experiments indicate that neurosteroids are potent modulators of NMDA receptors with a potential therapeutic use.

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PRIMARY AND SECONDARY CATARACT MODIFIES PROTEINS OF OCULAR LENS DIFFERENTLY

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Specific proteins of ocular lens – crystallins - exist in three types : α-, β- a γ-. They form more then 90% of water soluble lens proteins and their correct aggregation and arrangement is necessary for transparensy of lens. The loss of this lens transparency causes primary and secondary cataract . Degree of aggregation of crystallins was studied by SDS electrophoresis in polyacrylamide gradient (8-20%) gel. Crystallins from primary cataracts were found in more aggregated form than crystallins from health or secondary cataract. The low molecular protein (probably natural product of degradation) was observed in control healthy and primary cataract lens. In next step we studied distribution of individual crystallins. We prepared mouse monoclonal antibodies against pig crystallins which were able to detect also human ones. After identical SDS electrophoresis separated proteins were blotted on nitrocellulose membrane and individual crystallins were detected by specific antibodies. Although γ-crystallins were in healthy lenses only in monomeric form (20000Da) these crystallins from primary cataract ones were covalently attached to high molecular aggregates. On the contrary no γ-crystallins were detected in blotted proteins from secondary cataract lens. The loss of transparency in primary cataract may be therefore caused by errorous function of γ-crystallins or by different function of high molecular aggregates with attached γ-crystallins. All these results show that the mechanismus causing the loss of transparency of lens in primary and secondary cataract is caused by different reasons.

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MEMBRANE FLUIDITY – A REGULATORY VARIABLE OF CARDIAC SARCOLEMMA AND MITOCHONDRIA PROPERTIES IN DIABETIC RATS

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We have recently shown that glycoxidation present in diabetes decrease membrane lipid fluidity at both *in vitro* and *in vivo* conditions. Such a membrane remodelling is believed to be involved in the heart adaptation to the disease. To validate whether decreased membrane fluidity may be considered a necessary prerequisite for such an adaptation in rats with streptzotocin-induced diabetes, we used the membrane fluidising agent with recognised anti-glycation and antioxidant activities - resorcylidene aminoguanidine (RAG).
The fluorescence anisotropy of diphenylhexatriene, which was in a reciprocal proportion to membrane lipid fluidity, was monitored in sarcolemmal (SLM) and mitochondrial membranes (MM). For more details of the experiment, see references 1 and 2.
We confirmed the fluidising effect of RAG on heart-SLM from diabetic rats. This effect was associated with increased vulnerability of the hearts to Ca^{2+} overload (1). These results supported our hypothesis that some glycoxidation-related events might represent an important compensating factor that possibly participates in the concurring adaptation changes of the diabetic heart. Otherwise, we found the heart MM from diabetic rat significantly more fluid compared to control animals. The fluidisation might be associated with diabetes-induced adaptation in cardiac energetics (2). The adaptation includes the formation of mitochondrial contact sites which facilitate the Ca^{2+}-dependent/high-capacity energy transfer. We introduce a model describing the events that presumably led to such a change in MM fluidity.

(1) Waczulikova et al.: J.Physiol. Pharmacol. 4, 2002, in press.
(2) Ziegelhöffer A. et al.: Ann.N.Y. Acad. Sci. 967:463-468, 2002.

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CHANGES IN ERYTHROCYTE MORPHOLOGY IN CANCER PATIENTS

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The rheological properties of blood are closely related to the morphology of erythrocytes. Changes in erythrocyte morphology may occur due to certain pathological conditions such as malignant disease. In this study, erythrocyte morphology in cancer patients was studied and compared to that in healthy subjects.
The patient group consisted of 17 women (mean age ± SD, 53 ± 16 years) diagnosed with stage II or stage III ovarian cancer; the control group comprised 20 healthy women (20 ± 2 years).
Samples of venous blood, collected from each participant as part of routine hematological examination, were analyzed by scanning electron microscopy to characterize red blood cells. Two types of abnormal cell morphology were identified and classified as knizocytes (triconcave elements) and echinocytes (spherical elements with spikes). The proportion of each cell type was determined and the results were statistically evaluated by the Mann-Whitney test.
Findings of abnormal erythrocytes in venous blood (in %)

Cell	Cancer patients			Healthy controls			p <
morphology	Mean	S.D.	Range	Mean	S.D.	Range	
Knizocytes	2.45	3.72	0 -15.7	0.66	0.57	0.1-2.5	0.01
Echinocytes	1.94	1.04	0.5 -3.6	1.03	0.71	0.1 -.6	0.01

Knizocytes are not directly associated with impaired plasticity of erythrocytes, which is the quality required for passing through capillaries. However, echinocytes, due to their poor deformability, directly affect blood flow particularly in smaller capillaries and also increase blood viscosity, thus markedly changing the rheological properties of blood. This may eventually lead to microembolism in some parts of the capillary system and to ischemia of the affected tissue.
In our patients with ovarian cancer, proportions of the abnormal erythrocytes studied were significantly increased and this implies a higher risk of defects in microcirculation.

CHANGES OF THE CIRCADIAN PHASE OF TYMPANIC TEMPERATURE RHYTHM IN ANOREXIA NAD BULIMIA NERVOSA AFTER THE LIGHT TREATMENT

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Light and food are important synchronizers of biological rhythms. In eating disorders (ED) the food intake and the temperature circadian rhythms are abnormal but therapeutic effects of light treatment in bulimic patients with winter worsening of eating symptoms brought controversial results (1,2).
We tested the association between thermoregulation and abnormal pattern of food intake before and after the light treatment in a sample of 25 females patients hospitalized with DSM-IV diagnosis of eating disorders (14 bulimia nervosa and 11 anorexia nervosa) and in 6 healthy women. Light of intensity of 5000 lux was applied from 6 AM to 7 AM. Treatment was given for one week in January-February. Circadian rhythm of tympanic temperature was measured in both ears every hour before and after light therapy. Changes in appetite, mood, and sleep were monitored on visual analogue scales and in daily sleep logs. Temperature curves were analyzed with cosinor analysis; cases without significant rhythms (n=6) were excluded.
Light therapy did not influence significantly amplitude and mesor of the temperature rhythm neither in controls nor in patients, however, it reduced the interindividual variability of temperature minimum phase. After the light therapy, the circadian rhythm was normalized, phase delayed subjects were advanced and phase advanced subjects were delayed. The inflection point between advances and delays occurred near the temperature minimum. In patients, bright light exposure showed a slight antidepressant effect, without changes in their appetite and sleep. Our results support the normalizing effect of bright light in ED.

(1) Lam R.W., Goldner E.M. et al.: Am. J. Psychiatry 151:744-750, 1994.
(2) Blouin A.G. Blouin J.H. et al.: Psychiatry Res. 60:1-9, 1996.

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COMPARISON OF BAROREFLEX SENSITIVITY DETERMINED BY CROSS-SPECTRAL AND CONTINUOUS ALPHA INDEX METHODS

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Baroreflex sensitivity (BRS) determined by two methods based on spectral analysis (a continuous alpha index or a cross-spectral method) of variability in pulse intervals (PI) and systolic blood pressure (SBP) differs with respect to the coherence between variability in PI and SBP. The aim of our study was to compare BRS determined at a frequency band of 0.1 Hz by these two methods with respect to the linkage between variability in PI and SBP in time course.
Blood pressure was recorded (Finapres) in 51 subjects (21-22 years) 3 min at rest (R1), during exercise 0.5 W/kg of body weight (9 min, analyzed in two halves, periods E1 and E2), and 6 min in recovery period (R2). At R1 and R2, controlled breathing at 0.33 Hz was applied. BRS determination: 1. cross-spectral method (BRScs) - gain between spectrum of SBP variability, and cross-spectrum of PI and SBP variability in the frequency range of 0.07-0.12 Hz, at coherence >0.5, was taken as an index of BRS. The resulting BRS was given by sum of BRS of all selected components weighted by the power of these components. 2. alpha index (BRSai) - amplitude spectra of PI and SBP signals were calculated in segments (70 s duration, steps 0.5s), ratio of couples of maxima at 0.1 Hz was taken as BRS. Mean BRS was weighted by the density of presence of 0.1 Hz components in the time course of the signals. BRS decreased during exercise (Tab. 1). The values of BRSai and BRScs correlated in each period (correlation coefficients; R1:0.903, E1:0.934, E2: 0.962, R2:0.962, $p<0.001$). The reliability of BRS values was influenced by the percentage of the presence of 0.1 Hz components in the spectra of PI and SBP using the alpha index method and by coherence using the cross-spectral method. These parameters correlated (R1:0.621, E1:0.656, E2:0.726, R2: 0.568, $p<0.01$).
Table 1: BRScs and BRSai at Rest, during Exercise and a Recovery Period

	R1	E1	E2	R2
BRSai	12.5±5.3	$7.6\pm4.5^{+++}$	7.5 ± 4.3^{xxx}	11.2±4.6*
BRScs	12.5±5.8	$7.7\pm4.445^{+++}$	7.5 ± 4.2^{xxx}	11.0±4.9*

$^{+++}$ $p<0.001$, E1 vs. R1/R2; ^{xxx} $p<0.001$, E2 vs. R1/ R2; *$p<0.05$ R1 vs. R2.
BRS determined by both methods does not differ significantly

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ONTOGENETIC MATURATION OF BLOOD POLYMORPHONUCLEAR LEUKOCYTES

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Aim of this work was to establish the postnatal development of natural immunity. One of the effector mechanisms of natural immunity is phagocytosis mediated by "professional phagocytes" – neutrophils and monocytes. As a parameter of functional ability of these cells the production of superoxide anion and activity of aminopeptidase N was monitored.

It was used peripheral blood of piglets aged 11 and 17 days, two days before and two days after weaning (26 and 30 days) and further 35, 70 and 100 days in our experiment. Blood leukocytes were isolated using sedimentation in dextran. Superoxide anion was estimated by a modified technique of reduction of cytochrome c oxidized forms (absorption maximum at 550 nm) and the elimination of superoxide anion in controls was carried out with superoxidedismutase. Beside spontaneous production of superoxide anion we observed also its activation using opsonised zymosan or phorbol-12-myristate-13-acetate. Aminopeptidase activity was determined using spectrophotometry (at 405 nm) by modified method using a L-alanine-p-nitroanilide. Bestatin was used as inhibitor.

It was found out that natural immunity develops during postnatal life. The most notable increase in production of superoxide anion (with even without activation) occurs between 17th and 26th day of life. Furthermore changes in spontaneuos production of superoxide anion between 26th and 30th day indicates that it is altered by the influence of weaning. Rapid increase of production of superoxide anion after activation by PMA which occured after 70th day can bear on enhance ability of neutrophils to „kill" bacterial cells. Aminopeptidase N activity markedly decreased during the first four weeks of life.

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