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DOES MAMMALIAN GLUCAGON ELICIT INSECT ANTIOXIDANT MECHANISMS?

G. Alquicer, D. Kodrík, N. Krishnan[1]
Inst. of Entomology, Biology Centre, Acad. Sci. and Faculty of Science, South Bohemian Univ., Branišovská 31, České Budějovice, 370 05-CZ, Czech Republic, [1]Dept. of Zoology, Oregon State Univ., 3029 Cordley Hall, Corvallis, OR, USA

Glucagon is well-known as a vertebrate hyperglycaemic hormone but an additional role of this peptide has been suggested in the activation of antioxidant mechanisms to protect the organism from oxidative stress (1). The involvement of metabolic hormones in response to stress situations has also been studied in insects: it was recently found in several insect models that the adipokinetic hormones (AKH) bear an antioxidant capacity in response to oxidative stress elicited by a diquaternary derivative of 4, 4´ bipyridyl - paraquat (PQ) (2, 3). Resembling the main function of glucagon, AKH mobilizes energy reserves; both peptides are synthesized as pre-prohormones and subsequently processed and stored in dense core vesicles. Vertebrate glucagon activity is directed mainly to the liver, whereas AKH function is targeted mainly in the fat body, an insect analogue of the liver tissue. Considering the similarities, this study was aimed to a) assess the effect of glucagon on mobilization of energy reserves and b) on elicitation of an antioxidant response to oxidative stress in the fire bug *Pyrrhocoris apterus*. Using the mouse anti-glucagon antibody, the presence of immunoreactive material was demonstrated for the first time in the firebug CNS and gut by ELISA. Mammalian (porcine) glucagon injected into the adult bugs showed no effect on haemolymph lipid level or on the level of AKH in CNS and haemolymph, however, it activated an antioxidant response when oxidative stress was elicited by the PQ. Glucagon elicited the antioxidant response by increasing glutathione and decreasing protein carbonyl levels in haemolymph, and decreasing both protein carbonyl and protein nitro-tyrosine levels in CNS (4). This indicates that glucagon might induce an antioxidant defence in insects. In addition, the failure of glucagon to alter AKH level in the bug's body indicates employment of an independent pathway without involving the native AKH.

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PROLIFERATION AND APOPTOSIS IN SPECIFIC, TISSUE BOUND CELL POPULATIONS EXAMINED BY FLOW CYTOMETRY OF LASER CATAPULTED SAMPLES

T. Baňoch[1,2], K. Greplová[3], L. Dubská[3], I. Míšek[1,2], D. Valík[3], K. Klepárník[4], E. Matalová[1,2]
[1]Institute of Animal Physiology and Genetics AS CR, v.v.i., Brno, [2]Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, [3]Masaryk Memorial Cancer Institute, Brno, [4]Institute of Analytical Chemistry AS CR, v.v.i., Brno, Czech Republic

The success of precise molecular analyses relies on the preparation of homogenous cell populations. Flow cytometry is widely used in cellular and molecular examinations at a single cell level. However, the ability to purify cells from solid tissues has so far lagged. Laser capture microdissection (LCM) provides a powerful tool to obtain specific cell populations as selected from tissue samples *in situ*. The technique has opened new strategies to yield a complete genome or expression profile from exactly defined cells within tissues. The major disadvantage of LCM appears in protein analysis due to minute amounts of material (just for mass spectroscopy) and limits in amplification based techniques. To overcome the protein amount limitations and combine advantages of available techniques, we have designed a novel procedure for protein analysis of specific, tissue bound cell population. The strategy is based on laser captured microdissection of cryopreserved tissue sections into the test tube followed by release of the cells by trypsinization, fluorescent labelling of molecules of interest by immunoreaction and is finalized by flow cytometry measurement. To validate the novel design we selected highly proliferative areas of mouse embryo and compared those with apoptotic populations. Proliferation was detected by PCNA (proliferating cell nuclear antigen antibody), the results were confirmed by DNA content evaluation and also by *in situ* labelling. Apoptosis examination was based on caspase-3 detection, compared by TUNEL test and labelling *in situ*. This novel approach shows promising results in developmental biology and will be further applied in disease research (e. g. tumorigenesis). Moreover, the quantum dots (CdTe semiconductor nanocrystals) as a fluorescent labelling for antibodies has been under study to increase sensitivity of molecular evaluations at single cell level.

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PHARMACOKINETIC OF THE FLAVONOID POMIFERIN AFTER ORAL ADMINISTRATION

L. Bartošíková[1], J. Nečas[1], T. Bartošík[2], P. Fráňa[3], M. Pavlík[2]
[1]Department of Physiology, Faculty of Medicine and Dentistry, Palacký University Olomouc, [2]Department of Anaesthesiology and Intensive Care, [3]2nd Internal Clinic, St. Anne´s University Hospital Brno, Czech Republic

Flavonoid pomiferin was isolated from the infructences of *Maclura pomifera*, Moraceae. The aim of the study was to determine some pharmacokinetic parameters after single oral administration of pomiferin. After sample preparation the HPLC system consisted of gradient HPLC pump Knauer 64 equipped with a LCD 2084 UV detector was used. The wavelength was set on 273 nm for pomiferin determination. The mobile phase consisted of water:acetonitrile, 15:85 (v.v). Isocratic flow was maintained at 1.3 ml/min. Data from analysis were collected and analyzed with the CSW software. The quantification of the flavonoid was achieved from areas of its peaks by comparison with calibration curves obtained using standard solution of individual flavonoids in blank serum. For determination of pharmacokinetic parameters program Kinetica 4.0 was used. The maximum plasma pomiferin concentration was 90.5±1.4 μg/l; time to maximum plasma concentration was 40 min; $AUC_{0\rightarrow last}$ was 11565 μg/min/l; elimination half-life was 101±9 min; constant of elimination was 0.006904 min^{-1}; MRT was 174±10 min.

NEUROVASCULAR COUPLING IN EPILEPTIC RATS

C. Brožíčková, T. Jason, P. Mrázová, G. Tsenov, H. Kubová, J. Otáhal
Institute of Physiology of the Academy of Sciences of the Czech Republic v.v.i., Prague, Czech Republic.

The function of the nervous tissue is strictly dependent on aerobic metabolism. Thus during functional activation the regional cerebral blood flow (rCBF) is increased to balance oxygen demands. Clinical data suggest that regulation of the rCBF in epilepsy might be altered. Thus we performed in vivo measurement of blood flow in rats which underwent status epilepticus at P12 and in which developed spontaneous epileptic seizures. To assess alteration of regulation of the rCBF and after status epilepticus LiCl-pilocarpine model was used. Briefly, males (n=13) of wistar rats were intaperitonealy injected with pilocarpine (40 mg/kg) at P12. Peripheral side effect was minimized with scopolamine (1 mg/kg, 1hr before). Day before were animals injected with LiCl (127 mg/kg, i.p.) to decrease the dose of pilocarpine. SE was stopped 1 hour after its development by application of the paraldehyde (0,3 ml/kg, i.p.). Controls (n=12) obtained all treatment except pilocarpine. Part of the animals from both groups was EEG monitored to confirm presence of spontaneous seizures. To assess rCBF rats were 3-6 months after initial SE anesthetized with urethane (1.2 g/kg, i.p.). Two stimulation silver electrodes were implanted epiduraly over the right senzorimotor cortex. Four recording electrodes were implanted over the both hemispheres to monitor spontaneous and evoked cortical EEG. Part of the animals were monitored with 16channel electrode stick (Michigan probe) to obtain laminar profile of the cortical field potentials. Laser Doppler and oxygen probes were placed contralateraly to stimulating electrodes. Changes of the rCBF

transients were monitored during transcalosal stimulation (20 Hz,10s) with increasing currents (1-8 mA). The rCBF transients were monophasic with latency of maxima 9,8±1,2 s in controls and 9,8±0,8 s (P=0,72) experimental group. Slopes of the I/O curve reveals significant increase in pilocarpine group (P<0.05).

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ACTIVITY OF HYPOTHALAMIC TYROSINE HYDROXYLASE SYNTHESIZING NEURONS AFTER ALPHA2-ADRENOCEPTORS STIMULATION OR INHIBITION

J. Bundzikova, Z. Pirnik, J.D. Mikkelsen[1], D. Zelena[2], A. Kiss
Laboratory of Functional Neuromorphology, Institute of Experimental Endocrinology SAS, Bratislava, Slovak Republic, [1]Department of Translational Neurobiology, NeuroSearch A/S, Ballerup, Denmark, [2]Institute of Experimental Medicine, HAS, Budapest, Hungary

Tyrosine hydroxylase (TH) is the key enzyme for catecholamine synthesis. It is co-expressed with vasopressin (AVP) in the neurons of the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei. Untreated Sprague Dawley rats exhibited only a single TH-immunopositive neurons in the SON, while AVP deficient Brattleboro rats (di/di) rats showed a marked increase in the number of SON TH-immunoreactive cells. The aim of the present study was to reveal the involvement of alpha2-adrenoceptors in the regulation of TH neurons activity in the SON of Long Evans (+/+) and di/di rats. Animals were i.p. injected with saline (SAL, 0.1 ml/100 g), xylazine (XYL, α2-agonist, 10 mg/kg), idazoxan (IDX, α2- antagonist, 10 mg/kg), atipamezole (ATIP, α2- antagonist, 1 mg/kg), and IDX or ATIP followed by XYL treatment. Ninety min later animals were sacrificed by transcardial perfusion. Activity of TH neurons was assessed by the presence of Fos protein immunoreactivity and Fos/TH co-labelings were analyzed on 30 µm thick sections using computerized light microscope. In all treated +/+ rats the SON exhibited only a few TH immunopositive neurons. In SAL treated +/+ rats, no Fos labeled TH neurons were found and XYL did not turn over this state. IDX activated 23 % of TH cells in +/+ animals and its effect was completely suppressed by XYL. ATIP had no effect at all. In the SON of all treated di/di rats more immunoreactive TH neurons were generally found in comparison with +/+ groups. As much as 90 % of TH neurons revealed Fos immunoreactivity in the SON of SAL treated di/di rats and neither of the subsequent treatments further significantly influenced their amount. These results indicate that in the SON of +/+ rats alpha2-adrenoceptors are involved in the regulation of TH synthesizing neurons whereas their stimulation inhibits while their inhibition activates the TH neurons. However, this phenomenon is difficult to aplly to di/di rats since extremly high TH neuronal activation even under basal conditions.

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IDENTIFICATION OF nNOS IMMUNOREACTIVITY IN PHRENIC NUCLEUS OF DOG, RABBIT AND RAT

Ľ. Capková, A. Dávidová, A. Schreiberová, N. Lukáčová
Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic

The aim of this study was to find out, whether the bulbospinal pathway connecting neurons of respiratory centres in medulla oblongata with the premotor neurons in the phrenic nucleus located in lower cervical segments is realized through NO/cGMP signalization. Spinal cord hemisection was performed at C2-C3 level in the dog, rabbit and rat**.** Experimental animals survived for seven days (rabbits and rats) and dogs survived for nine days. The expression of neuronal nitric oxide synthase (nNOS) was determined by immunocytochemical method. nNOS was quantitative measured by densitometry. The detail characteristic of changes was performed in upper and lower portions of the respiratory pathway. In all above mentioned experimental animals the distribution of nNOS was found in neuropil of phrenic nucleus. Motoneurons of the phrenic nucleus located in the ventral gray matter in the extent of C3-C5 segment were nNOS immunonegative. Spinal cord hemisection performed at C2-C3 segment level followed from seven to nine days of survival greatly decreased the density of punctate nNOS immunoreactivity in the neuropil of the phrenic nucleus ipsilaterally to the site of hemisection. A large number of double-labeled neurons was found in both respiratory groups at the contralateral side, and in contrast to this, a considerably reduced number of such neurons was detected in the ipsilateral dorsal and rostral ventral respiratory groups of the medulla. These results provide evidence that the premotor bulbospinal respiratory pathway connecting the bulbar respiratory centers with the motor neurons of the phrenic nucleus is nNOS-IR, and that the crossed premotor bulbospinal respiratory pathway contains high number of nNOS immunopositive fibers terminating in the phrenic nucleus. It is expected that the respiratory pathway is provided by NO-cGMP signalization.

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TWO MODELS FOR STUDY OF NEURONAL L-TYPE CALCIUM CHANNELS: PC12 CELL LINE AND CULTURED HIPPOCAMPAL NEURONS

A. Caro, M. Zana, B. Sedlakova, O. Krizanova, L. Lacinova
Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia

We have compared PC12 cells and cultured rat hippocampal neurons as models for studying contribution of L-type calcium channels to neuronal excitability. PC12 cell line was purchased from DSMZ. Treatment of the cells with neuronal growth factor forced them to differentiate into neuronal-like cells (1). Cell morphology changed, but calcium current density did not increase significantly. On Day 4 current density was -7.2±1.5 pA/pF, on Day 6: -8.2±1.1 pA/pF, on Day 13: -15.7±3.7 pA/pF and on Day 15: -12.9±6.1 pA/pF. Cells also express two types of potassium channels (A-type potassium current and delayed rectifier potassium current), but, in contrast to some other reports (2), we did not observe sodium current except for 2 cells out of 103. In conclusion, PC12 cells can be used for study of L-type calcium channels but not for study of neuronal action potentials. Primary culture of hippocampal neurons was established from hippocampi of newborn rats at a postnatal day 1 or 2. Expression of L-type calcium channels increased with time. Calcium current was measurable starting with Day 4 of cell culture. On Day 4 the current density was -8.6 pA/pF, on Day 5: -7.2 pA/pF, on Day 6: -11±2.1 pA/pF, on Day 7: -23.4±2.2 pA/pF, on Day 13: -42.8±15.4 pA/pF. This increase was significant starting with Day 7. Sodium and potassium channels were expressed as well. Cells were capable of generating single action potentials as well as series of action potentials. L-type calcium channel agonist BayK 8644 and L-type calcium channel antagonist nimodipine did not affect significantly analyzed action potential parameters, i.e. input resistance, thresholds for firing single and multiple action potentials, peaks of the action potentials, duration of the action potentials. In conclusion, hippocampal neurons are a suitable model to study neuronal L-type calcium channels as well as neuronal excitability manifested by action potential firing.

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SPATIAL ORIENTATION IN LURCHER MUTANT AND WILD TYPE MICE OF THE STRAINS B6CBA AND C3H

J. Cendelín, I. Korelusová, F. Vožeh
Department of Pathophysiology, Faculty of Medicine in Pilsen, Charles University, Czech Republic

Lurcher mutant mice represent a model of olivocerebellar degeneration. They suffer from cerebellar ataxia and deterioration of cognitive functions. The aim of this work was to compare spatial orientation ability in Lurcher mutant and wild type mice of the strains B6CBA and C3H with respect to retinal degeneration affecting some of the C3H individuals. Spatial orientation was tested in the Morris water maze with a hidden platform in stable position for 10 days (D1-10). For next two

days (D11-12) the platform position was changed. After 9 days pause, navigation to a visible platform in stable position was examined for 5 days (D22-26). Lurchers, both B6CBA and C3H without retinal degeneration, revealed significantly worse results than wild type control mice during both tests with hidden and visible platform. Prolongation of latencies and trajectories was observed in both types and strains of mice after the change of the platform position on D11. Wild type animals improved their results on D12 to almost the same values as before the change, while Lurchers did not. Both wild type and Lurcher mutant mice of the B6CBA strain achieved better results compared to C3H mice of the same type without the retinal degeneration in both tests with hidden and visible platform. Wild type mice of the C3H strain suffering with the retinal degeneration reached significantly worse results in the maze during all phases of the experiment than unaffected C3H wild type mice. In Lurchers, significant differences between animals with and without the retinal degeneration were found only when the platform was visible. C3H Lurchers affected with the retinal degeneration showed longer latencies and trajectories than affected wild type mice, when the platform was hidden. The translocation of the platform did not lead to marked change of the latencies and trajectory lengths neither in Lurcher nor in wild type mice affected with the retinal degeneration. In the last part of the experiment there were no significant differences between the mice of these two groups. The experiment confirmed the deficit of spatial orientation in Lurcher mice and the differences between the C3H and B6CBA strains, which were marked even in the navigation to the visible goal. In C3H mice with severe sight affection we found also differences between wild type and Lurcher mutant mice, which are probably due to different strategy of maze exploration after learning the existence but not the position of the escape platform.

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FOS EXPRESSION RESPONSE TO ISCHEMIA-REPERFUSION INJURY OF LIVER IN SELECTED BRAIN AREAS

E. Cibulova, Z. Pirnik, J. Bundzikova, L. Lackovicova, B. Mravec[1], A. Kiss
Laboratory of Functional Neuromorphology, Institute of Experimental Endocrinology, Slovak Academy of Science, Brastislava, and [1]Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Liver ischemia-reperfusion injury (IRI) may influence the activity of neurons in the brain. To verify this assumption, we tested two experimental models of liver IRI: IRI1 - single ligation of the hepatic artery for 30 min and IRI2 – combined ligation of the portal triade (combined ligation of the hepatic artery, portal vein, and bile duct) for 15 min with aim to reveal changes in Fos protein expression, as a marker of neuronal activity, in selected brain areas. Fos immunoreactivity was analysed on 30 μm sections in the subfornical organ (SFO), suprachiasmatic (SCH), paraventricular (PVN), supraoptic (SON), arcuate (ARC), and ventromedial (VMN) hypothalamic nuclei, locus coeruleus (LC), parabrachial nucleus (PBN), nucleus of the solitary tract (NTS) and A1/C1 hindbrain catecholaminergic cell groups, 90 min, 5, and 24 hr after liver IRIs. Ninety min after surgeries, all brain areas studied exhibited stronger neuronal activity in both IRI groups of rats in comparison with sham operated controls. More distinct response in Fos expression occurred after IRI2 then IRI1 in the SON, PVN, VMN, and NTS. Five hours after surgery, Fos expression was still increased in the PBN and NTS in both IRI models. However, the extent of Fos occurrence sharply declined 24 h after surgeries in both IRI models. These data indicate that the activity of neurons after both types of IRIs is monitored by the brain. However limited amount of Fos appearance also in sham operated rats speak out for a possible involvement of some non-spocific factors associated with the process of surgery.

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INTRATHECAL DELIVERY OF BONE MARROW MESENCHYMAL STEM CELLS FOLLOWING SPINAL CORD INJURY

D. Cizkova[1], L. Slovinska[1], M. Cizek[2], M. Nagyova[1], I. Novotna[1], J. Rosocha[3], J. Radonak[4], J. Hlucilova[5], S. Juhas[5], I. Vanicky[1]
[1]Institute of Neurobiology, Slovak Academy of Sciences, Kosice, Slovakia, [2]University of Veterinary Medicine, Kosice, Slovakia, [3]Associated Tissue Culture Bank, Medical Faculty, University of PJ Safarik, Slovakia, [4]2nd Surgical Clinic, University of PJ Safarik, Slovakia, [5]Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Libechov, Czech Republic

Transplantation of bone marrow mesenchymal stem cells (MSCs) has been shown to improve functional recovery after various models of spinal cord injury (SCI) (1,2). However, definition of the optimal dose, time point and route of MSCs application is importand to achieve beneficial therapeutic outcome (3). The objective of this study was to standardize and optimize the intrathecal (IT) transplantation of MSCs after a moderate (12.5 μL) balloon-compression SCI in adult rats. In vitro labeled MSCs with green fluorescent PKH-67 cell linker kit were IT administered to rats (at lesion site) at 3 or 7 days after SCI as: (a) a single injection ($5x10^5$ MSCs/rat) or (b) three daily injections ($5x10^5$ MSCs/rat/day, total of $1.5x10^6$ MSCs/ rat. Animals were behaviorally tested for 3 weeks using the Basso, Beattie, Bresnahan (BBB) locomotor rating scale and histologically assessed for MSCs distribution. Rats treated with a single injection of MSCs at 3 or 7 days, showed only modest improvement in hindlimb function (BBB=9±2) and low penetration of grafts. Scattered PKH-67 fluorescence cells could be found adjacent to the dura or pia mater rostrally to the lesion site. In contrast, rats treated with three consequent daily injections of MSCs injected at 7 days, showed higher recovery (BBB=16±2) of hindlimb movements from 2 to 3 weeks. MSCs formed an apparent migrating PKH-67 green fluorescence cell tract continuously extending from the tip of the catheter dispersing into the lesion cavity. The majority of PKH-67 labeled cells were found within damaged white and gray matter with maximum spreading 2 mm rostrally, but not caudally to the lesion. These results suggest that IT transplantation, which imposes a minimal burden on animals, may improve behavioral function, when the optimal dose, timing and direct IT grafting of MSCs towards lesion cavity is utilized.

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NESTIN – AN IDEAL MARKER FOR MUSCLE DEVELOPMENTAL STUDIES

D. Čížková[1], J. Mokrý[1], J. Říčný[2], T. Soukup[2]
[1]Department of Histology and Embryology, Charles University in Prague, Faculty of Medicine in Hradec Kralove, and [2]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague , Czech Republic

Under physiological conditions, nestin has been detected in muscle tissues during limited developmental periods. In general, nestin can be identified in mature tissues under situations that reproduce developmental phases, e.g. physiological renewal of certain cell types, tissue regeneration and healing, revascularisation, production of undifferentiated elements in tumours etc. Here we describe the expression and distribution patterns of nestin in rat normal and regenerating skeletal muscles. Regeneration was achieved by heterochronous isotransplantation (1) of extensor digitorum longus (EDL) or soleus muscles from 15-day-old rats into EDL muscles of adult female inbred Lewis rats. Recipients were sacrificed by anaesthetic overdose and the host muscles with the graft were excised after 7-, 16-, 21- and 29-day survival, fixed in 10% formalin solution and immunohistochemically stained. In regenerating skeletal muscles maximal nestin expression was observed in newly formed myoblasts and myotubes and its expression decreased in the course of skeletal

myofibres development (2). We demonstrate a peripheral accumulation of nestin called 'lateralisation', which may represent a mechanism of nestin elimination during the development of skeletal myofibres. Special attention was given to regeneration of muscle spindles (3). Nestin expression in intact spindles in host muscles was restricted to Schwann cells of sensory and motor nerves. In transplanted muscles, however, nestin expression was also found in regenerating "spindle fibres", 7 and 16 days after grafting. From the 21st day onwards, the regenerated spindle fibres were devoid of nestin immunoreactivity. Desmin was detected in extrafusal and intrafusal spindle fibres at all stages examined both in intact and regenerating muscles. Furthermore, nestin has been detected in newly formed endothelial cells of blood vessels growing into the regenerated area and in peripheral nerves innervating the graft as well as the host muscle. In conclusion, according to our results, nestin represents an ideal marker for skeletal muscle regeneration, as its expression reflects processes of development, revascularisation and reinnervation of new myofibres (2,3).

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TEMPORAL CHARACTERISTICS OF INTRACEREBRAL P3 WAVE IN A VISUAL ODDBALL TASK

A. Damborská[1], M. Brázdil[2], I. Rektor[2], M. Kukleta[1]
[1]Department of Physiology, Faculty of Medicine, Masaryk University, Brno, [2]1st Department of Neurology, St. Anne's Faculty Hospital, Masaryk University, Brno, Czech Republic

The P3 wave as the most prominent component of the event-related potential (ERP) elicited during the visual oddball task is generally believed to reflect cognitive processes. According to several studies, however, in some cases this P3 wave appears to represent processes rather engaged in movement triggering. Here we report on evidence supporting the view of the P3 wave to be a heterogeneous phenomenon. We searched for the relationship between the stimulus-response interval and the peak amplitude latency of intracerebral P3 waves recorded on EEG in a visual oddball task. In 14 epileptic patients we examined 93 cerebral sites in which a P3 wave was detected. It was elicited in response to the target stimuli in a latency range of 250-650 ms after the stimulus onset. In each patient 29–58 target trials were distributed into 5–9 subgroups according to the stimulus-response (SR) interval. For each patient separately subgroups were sorted into short and long ones using median split of SR interval duration. The latency of the P3 wave was measured on an averaged EEG record of each subgroup. The position of P3 peak latency with respect to the SR interval was assessed. Three types of P3 waves were identified: Type A -the P3 peak latencies showed no significant correlation with SR intervals across the subgroups. This type was found in 51 cerebral sites of all 14 patients. Across all 51 sites the P3 peak latencies were longer in short subgroups (mean 90.3±15.2 % of SR; N=119) than those in long subgroups (mean 84.3±13.6 % of SR; N=111). This difference was statistically significant (Mann-Whitney *U* test, $p<0.0039$). Type B - the P3 peak latencies showed a significant correlation with the SR intervals at $p<0.01$ ($r>0.88$). This type was found in 18 cerebral sites of 9 patients. Across all 18 sites the P3 peak latencies in short subgroups (mean 87.0±10.7 % of SR; N=54) did not significantly differ from those in long subgroups (mean 88.4±8.2 % of SR; N=46). Type C – the P3 peak latency was longer than the SR interval in all subgroups. This type was found in 9 cerebral sites of 8 patients. Across all 9 sites the P3 peak latencies in short subgroups (mean 121.5±11.7 % of SR; N=21) did not significantly differ from those in long subgroups (mean 115.2±14.7 % of SR; N=18). The remaining 15 cerebral sites of 6 patients showed P3 waves whose latencies did not meet the criteria for classification into any of the three types. We suggest that visual P3 waves include different potentials which vary in their temporal characteristics with respect to the stimulus and movement onset. Thus, the P3 wave probably represents different processes, some of which are closely related to the movement.

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POSTCONDITIONING PREVENTS OF DELAYED NEURONAL DEATH IN THE HIPPOCAMPAL CA1 REGION

V. Danielisová, M. Gottlieb, M. Nemethová, P. Kravčuková, I. Domoráková[1], J. Burda
Institute of Neurobiology , Slovak Academy of Sciences, Košice, [1]Faculty of Medicine, University of P.J. Safarik, Košice, Slovakia.

In the present study we investigated several key components involved in apoptosis since much evidence suggests that apoptosis plays an important role in ischemic brain injury. Ischemia and reperfusion causes mitochondrial dysfunction that initiates the mitochondrial apoptosis pathway. This pathway involves the release of cytochrome c and activation of the caspase cascade. The increase of cytochrome c in the cytosol is an indicator of its release from mitochondria, activation of caspase-3 and facilitated apoptosis. Prevention of apoptosis could reduce brain damage. Delayed, protein synthesis-dependent, postconditioning is based on the fact that ischemic tolerance consists of a combination of mechanisms developing during 2 days after the initial ischemia and proteins synthesized during the first hours after the second stress. This stress, if applied before the onset of delayed neuronal death, is able to stop apoptotic process and prevent neuronal degeneration (1, 2, 3). Postconditioning as well as the initial lethal ischemia, increases the activity of SODs and catalase (4, 5). Observed increase in the activity of a mitochondrial MnSOD in the cytoplasm after lethal ischemia indicates the release of MnSOD from mitochondria during delayed neuronal death. This increase is accompanied by an increase of cytoplasmic concentration of cytochrome c. Ischemia leads to increase in activated caspase-3 level in the CA1 region 3 days after ischemia. However, application of postconditioning before the beginning of delayed neuronal death, 2 days after ischemia, significantly prevents all these changes.

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THE EFFECT OF THORACIC SPINAL CORD TRANSECTION ON THE LEVEL OF nNOS

A. Dávidová, A. Schreiberová, Ľ. Capková, N. Lukáčová
Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic

This study was designed to outline more fully the changes taking place in the spinal cord after its transection performed at the low thoracic level and survival of experimental animals for 7 days, with special emphasis on the changes in the level of neuronal nitric oxide synthase (nNOS) protein and nNOS immunoreactivity (nNOS-IR) in the rat lumbar spinal cord segments (L2-L6). Our data show that the spinal cord transection induced a change in the level of nNOS protein in segments located below the injury; in comparison with control the level of nNOS protein was strongly upregulated. Such prominent increase of nNOS protein is in agreement with the enhancement of nNOS-IR in specific spinal cord regions, such as the dorsal and ventral columns and the motoneurons. Immunohistochemical analysis discovered, that this increase may be a result of higher nNOS-IR in a large number of axons of dorsal column, located predominantly in dorsomedial margin of the dorsal horn. The densitometric profile of nNOS immunoreactivity in these axons was considerably higher in comparison with intact rats. The lower lumbar motoneurons did not express nNOS under physiological conditions. Moderatelly stained motoneurons and their processes were seen one week after thoracic spinal cord transection. Experimental studies show that transected hindlimb corticospinal tract axons sprouted into the cervical spinal cord gray matter to contact long propriospinal

neurons that bridges the lesion and extended processes to lumbar motoneurons. This created a new intraspinal circiut relaying supraspinal inuput to its original spinal targets (1). In order to investigate a pain-related behaviour, we determined the heat-induced tail-flick reaction time of experimental animals in the end of experiment. The tail-flick response was monitored in unanaesthetized animals at 15-min intervals for a total of three determinations. The animals reacted to the thermal stimulus with a typical contraction of their tails into circles. The tail-flick response was immediate in experimental animals, while control animals responded to thermal stimulus with delay. We observed a significantly higher tail-flick latency in experimental animals. The present study suggests, that thoracic spinal cord transection strongly influences nNOS-IR in neuronal circuitry that underlie the tail-flick reflex.

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MODULATION OF BEHAVIORAL CHANGES AFTER CNS INSULT IN RATS

K. Deykun, M. Pometlová, O. Raška, J. Mareš
Department of Normal, Pathological and Clinical Physiology, the Third Medical Faculty, Charles University in Prague, Czech Republic

Stimuli that cause cerebral damage can also have protective effect for the tissue further injury, when applied at close but below threshold of damage - preconditioning. One noxious stimulus can be capable to induce tolerance or cross-preconditioning for another one. It had been suggested that reactive oxygen species (ROS) may represent such stimuli. Flurothyl-induced seizure is a model of generalized epilepsy. Even though, the exact mechanism of flurothyl neurotoxicity (in the absence of morphological changes) is not well understood, we are concerned that ROS generation plays important role in this. Continuous hypoxia causes energy depletion and impairment of biological membranes. It facilitates mechanisms of cellular adaptation with eventual tolerance development. From this aspect intermittent hypoxia causes greater cerebral damage then continuous. However, there is no evidence on acute short-term intermittent hypoxia and its potential of preconditioning induction. Earlier, we have described protective effect of hypobaric hypoxia induced prior flurothyl epileptic seizures (FS). Present study was aimed to investigate whether INH would exhibit similar to hypobaric hypoxia cross-preconditioning on FS in Wistar rats. Behavioral changes were tested in (MWM). We had three experimental groups. Group 1 was exposed to INH and 3 days later to flurothyl; group 2 – to hypoxia alone and group 3 – to flurothyl alone. Results were compared with naïve control animals (MWM tested only). INH was conducted in air-tight chamber with oxygen concentrations cycling between 21 and 8 % every 30 s during one hour. FS alone, in combination with INH and in lesser extent INH alone caused increase in latency and distance moved in WMW, in comparison to control animals. However, there was also significant improvement of performance of animals preconditioned by INH in contrast to FS group. These findings confirm our hypothesis that INH has protective preconditioning effect. Despite this protection we observed worsening of learning comparing to our previous results with hypobaric hypoxia.

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INTERACTIVE PHYSIOLOGY FOR PRACTICAL TRAINING

J. Doubek, E. Matalová
Department of Physiology and Pathophysiology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1-3, 612 42 Brno, Czech Republic

Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno (FVM UVPS) has been successfully running accredited international education in English for several years. As many textbooks are generally available for theoretical studies of physiology, practical designs vary among universities. Therefore, the aim of this work was to compose methods and protocols for practical courses Physiology I, II at the FVM UVPS in an attractive interactive format. The material is available on a DVD and the content corresponds to the recent syllabus for Physiology at the FVM UVPS Brno. The topics cover blood physiology, immunology, cardiovascular and respiratory systems, neurophysiology, endocrinology, reproduction, metabolism and gastrointestinal tract. Each part starts with a comprehensive review of individual practical courses further divided into subchapters. Clicking on particular tasks opens the whole protocol structured into Introduction, Aims, Experimental design, Results, Discussion which offer also related figures, animations and video-sequences. Specific seminars and student conferences are included as independent chapters. Each part of individual chapters involves also a protocol scheme giving an opportunity for result evaluation and conclusions by students themselves. The whole material is interconnected by hypertext structure enabling knowledge integration and an easy search. The DVD can be run under any browser program, optimization was done for Internet Explorer. Web connection is not required but can be used for additional references. Animations and videos become open in extra windows after click on the hypertext reference.

The interactive DVD version was prepared in cooperation with the Institute of Animal Physiology and Genetics v.v.i., Academy of Sciences, Brno, the Department of Laboratory Medicine, Masaryk Memorial Cancer Institute, Brno and the Faculty of Science, Palacky University, Olomouc and issued in 2008: Matalová E et al.: Interactive Physiology for practical training, p. 20 + DVD, UVPS Brno. ISBN 978-80-7305-042-9.

ARE S4 SEGMENTS OF DOMAINS I, III AND IV IN CLOSE PROXIMITY OF IV S5 IN $Ca_v3.1$ CHANNEL?

M. Drigelova[1], M. Kurejova[2], N. Klugbauer[3], L. Lacinova[1]
[1]Institute of Molecular Physiology and Genetics, Slovak Academy of Science, Vlárska 5, 833 34 Bratislava, Slovakia, [2]Institute of Pharmacology, University of Heidelberg, Im Neuenheimer Feld 366, 691 20 Heidelberg, [3]Institut für Pharmakologie und Toxikologie der Technischen Universität München, Biedersteiner Str. 29, 808 02 München, Germany

Voltage-operated calcium channels consist of four homological domains, each containing six transmembrane segments S1-S6. The S4 segments, containing five to six basic amino acids, form putative voltage sensor of the channel, while S5-S6 segments form the channel pore (1). It was suggested that in Shaker potassium channels S4 segments interact with S5 segments in the same domain when the channel is in the open state and they interact with S5 segments in the neighbouring domain when the channel is in the resting state (2). In our study we tested if such interaction takes place also in the $Ca_v3.1$ channel. Uppermost arginines in the S4 segments from domains I, III and IV and the leucine located on the top of the S5 helix in the domain IV were replaced by cysteines using a PCR-based method. Resulting mutants (R180C/L1773C, R1717C/L1773C and R1379C/L1773C) were transfected into HEK 293 cells. Whole-cell patch-clamp was used for current analysis. The bath solution contained (in mM): HEPES 10, $CaCl_2$ 2, $MgCl_2$ 2, NMDG 140; pH 7.4 with HCl. The pipette solution contained: (in mM): CsCl 130, Mg-ATP 5, EGTA 10, HEPES 10; pH 7.4 with CsOH. Introduction of cysteines into IS4 and IVS5 resulted in non-functional channel. When cysteines were introduced into IVS4 + IVS5 and into IIIS4 + IVS5 channels did carry significant inward calcium current. We tested if introduced cysteines were close enough to form disulphide bonds by application of oxidizing agent DTNB and reducing agent DTT. In a channel containing cysteines in IVS4 / IVS5 segments DTT slightly inhibited the current, while DTNB fully blocked it. When cysteines were inserted into IIIS4 / IVS5 segments, DTT potentiated calcium current and DTNB inhibited the current only partly. These results suggest, that, in analogy with Shaker potassium channel (2), IVS4 and IVS5 segments may interact in the open state while IIIS4 and IVS5 segments may interact when the channel is in its closed state.

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APOPTOTIC MARKERS EXPRESSION IN DIFFERENT TYPES OF HUMAN ENDOMETRIUM

M. Dvorska[1], D. Driak[1], I. Svandova[3], K. Benkova[2], Z. Spurkova[2], B. Sehnal[1], M. Halaska[1]
[1]Department of Obsterics and Gynaecology, University Hospital Na Bulovce, First Faculty of Medicine, Charles University, Prague, [2]Department of Pathology, University Hospital Na Bulovce, Prague, [3]Department of Physiology, Faculty of Science, Charles University, Prague, Czech Republic

Under homeostatic conditions, apoptotic cell death counteracts uncontrolled proliferation. Breakage of this highly orchestrated machinery results in pathologic conditions. We studied expression of Pro-Caspase-3, Caspase-3, PARP, Bcl-2 and Bax, e.g proteins shown to be associated with the control of apoptosis in human endometrium.
The endometrial specimens used in the study were classified as follows: normal endometrium in proliferative phase of the menstrual cycle (NE, n=8); endometrium from patients with endometrial hyperplasia (HE, n=6); atrofic endometrium (AE, n=5) and cancerous endometrium (CE, n=6). Proteins were fractionated by 15 % SDS-PAGE and their expression was measured by Western blot using antibodies against human Pro-Caspase-3, Caspase-3, PARP Bcl-2, Bax. Proteins expression was normalized to β-Actin. We detected significantly higher levels of Pro-Caspase-3 and Caspase-3 in CE and HE (1676 % and 700 % in CE resp. 375 % and 163 % in HE) compared to NE. Expression of Pro-Caspase-3 and Caspase-3 was significantly lower in HE compared to CE. Expression of both the inactive and active form of Caspase-3 was significantly lower in AE compared to NE. With respect to NE, Bcl-2 expression was significantly higher in CE and significantly lower in AE, whilst the only significant change of Bax expression was its decrease observed in AE. Decrease of Bcl-2 and Bax expression was determined in HE with respect to CE. Bcl-2/Bax ratio, the factor determining cell survival or apoptosis, was 30 % higher in CE and about 25 % lower in HE compared to NE. Interestingly, we observed slightly higher Bcl-2/Bax ratio in AE compared to NE. Difference of PARP expression was of no significance with respect to CE and HE vs. NE; in AE, its expression was significantly lower. The data demonstrate that cancer and hyperplasia should be explained by changes in both the cell proliferation and apoptosis, and correspond with greater predominance of proliferation over the apoptosis and higher number of mitoses with a risk of mutations and cancerogenesis based on hyperplasia.

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CHANGES IN Bcl2/Bax EXPRESSION IN DIFFERENT TYPES OF ENDOMETRIUM

M. Dvorska[1], D. Driak[1], I. Svandova[3], K. Benkova[2], Z. Spurkova[2], B. Sehnal[1], M. Halaska[1]
[1]Department of Obsterics and Gynaecology, University Hospital Na Bulovce, First Faculty of Medicine, Charles University, Prague, [2]Department of Pathology, University Hospital Na Bulovce, Prague, [3]Department of Physiology, Faculty of Science, Charles University, Prague, Czech Republic

Under homeostatic conditions, apoptotic cell death counteracts uncontrolled proliferation. Breakage of this highly orchestrated machinery results in pathologic conditions. We studied expression of Bcl-2 and Bax, the two members of the Bcl-2 family shown to be associated with the control of apoptosis in human endometrium. The endometrial specimens used in the study were classified as follows: normal endometrium in proliferative phase of the menstrual cycle (NE, n=8); endometrium from patients with endometrial hyperplasia (HE, n=6), atrofic endometrium (AE, n=5) and cancerous endometrium (CE, n=6). Proteins were fractionated by 15 % SDS-PAGE and their expression was measured by Western blot using antibodies against human Bcl-2, Bax and beta-Actin. We detected higher levels of Bcl-2 than Bax protein in the four groups of endometria studied. Compared to NE, Bcl-2 expression was significantly higher in CE and significantly lower in AE, whilst the only significant change of Bax expression was its increase observed in AE. Decrease of Bcl-2 and Bax expression was determined in HE with respect to CE. Bcl-2/Bax ratio, the factor determining cell survival or apoptosis, was 30% higher in CE (primarily due to the increase in Bcl-2 expression) and about 25 % lower in HE compared to NE. Interestingly, we observed slightly higher Bcl-2/Bax ratio in AE compared to NE. The data demonstrate that cancer and hyperplasia should be explained by changes in both the cell proliferation and apoptosis.

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BRAIN-REGION SPECIFIC CHANGES IN $GABA_A$ RECEPTOR LEVELS IN ACETYLCHOLINESTERASE KNOCKOUT MICE

V. Farár[1], E. Duysen[2], O. Lockridge[2], J. Brabec[3], A. Hrabovská[4] , J. Mysliveček[1]
[1]Institute of Physiology, 1st Faculty of Medicine, Charles University, Albertov 5, 128 00 Prague, Czech Republic, [2]Eppley Institute, University of Nebraska Medical Center, Omaha, NE 68198-6805, USA, [3]Institute of Anatomy, 1st Faculty of Medicine, Charles University, U Nemocnice 3, 128 00 Prague, Czech Republic, [4]Department of Cellular and Molecular Biology of Drugs, Faculty of Pharmacy, Comenius University, Kalinčiakova 8, 832 32 Bratislava, Slovakia

Although acetylcholinesterase plays a key role in the living organism and its function seems to be essential for life, genetically modified mice with missing gene for AChE are viable (1). However they have strong changes in their phenotype. One of the typical signs of their phenotype are epileptic-like seizures, that are induced by stress (2). Because impairment in GABAergic system is traditionally proposed to play essential role in production of siezures we decided to test AChE -/- mice for changes in $GABA_A$ receptors ($GABA_AR$) levels in brain. In nine specific brain sections (cerebellum, frontal, temporal, parietal and occipital cortex, striatum, hippocampus, thalamus and hypothalamus) we quantified amount of $GABA_AR$ by radioligand binding study using a specific ligand [^{3}H]SR-95531 and compared to the wildtype, which has normal AChE activity. The most of studied brain sections did not display differences in number of receptors between the two genotypes except striatum where we observed a threefold increase of the number of $GABA_AR$ in AChE -/- mice. The dissociation constant (K_D) of [^{3}H]SR-95531 was identical in both genotypes and it was comparable with literature. We can therefore conclude that the increased binding of the specific ligand in AChE -/- mice striatum is caused by the different receptor levels on the cell surface. We suppose that these changes participate rather on movement regulation than on production of siezures.
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MECHANISMS OF ENDOGENOUS PROTECTION IN ACUTE DIABETIC RAT HEART: ROLE OF THE MITOCHONDRIA AND DUAL INVOLVEMENT OF FREE RADICALS

M. Ferko, J. Mujkošová, T. Holotňáková[1], D. Kincelová[2], O. Pecháňová[3], O. Uličná[4], J. Slezák, I. Waczulíková[2], T. Ravingerová, A. Ziegelhöffer
Inst. Heart Res., Ctr. Excel. Cardiovasc. Res., SAS, [1]Inst. Virol., SAS, [2]Div. Biomed. Phys., Fac. Math.,Phys. Informat., Comenius Univ., [3]Inst. Normal Pathol. Physiol., SAS, [4]Lab. Pharmacobiochem, 3rd Dept. Int. Med., Fac. Med., Comenius Univ., Bratislava, Slovakia

Mitochondria (MIT) from hearts of rats with acute streptozotocin (STZ)-diabetes (DIA) exhibit functional remodeling of membranes (ME) followed by a cascade of changes reflecting a dynamic equilibrium between the pathological processes and those of endogenous protection (EP). The aim was to distinguish and elucidate those changes in MIT ME which are linked with EP in the DIA heart. DIA was induced to male Wistar rats (220±20 g) by STZ (55 mg/kg i.p.). Experiment was terminated on the day 8 after STZ when DIA rats exhibited an incrase (%) in glucose 333.96, triacylglycerols 370.4, cholesterol 153.4 and glycohemoglobin 189.6 in the blood and/or serum. In MIT there were investigated: oxidative phosphorylation (OP), the oxidized Q_{10} , conjugated dienes (CD), total MIT NOS activity, NFκB, eNOS, expression of hypoxic genes and carbonyl anhydrase (CAIX), the fluidity (MF) and trans-ME potential of MIT. DIA heart

exhibited free radicals (FR)-induced damage to MIT indicated by ~17 % ($p<0.05$) increase in oxidized Q_{10} but no considerable rise in CD formation in ME lipids and in expression of MIT eNOS. Further findings: decreased S3 and S4 O_2 consumption, respiratory control index (RCI) and the rate of OP (all $p<0.01$), increased NFκB and MF (both $p<0.01$). MP also decreased ($p<0.001$), but the latter change was accompanied with its increased stability. Linear regression analysis revealed significant association ($r=0.67$; $p<0.05$) between the increase of MF and decrease in MP in DIA MIT. In contrast to healthy controls DIA heart show increased expression of CA IX – a process mediated via the HIF-1α transcription factor. In conclusions, FR are playing dual role: they induce damage mainly to OP, but also activate the processes of EP by alleviating the oxidative damage to ME lipids, increasing MF and upregulating the hypoxic genes.

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ACUTE EFFECTS OF SIGMA RECEPTOR LIGAND HALOPERIDOL ON ELECTROGRAM IN RAT ISOLATED HEART FOLLOWING REPEATED HALOPERIDOL TREATMENT

K. Fialová and M. Nováková
Department of Physiology, Faculty of Medicine, Masaryk University Brno, Czech Republic

The sigma binding sites are considered to be involved in various psychiatric disorders. Treatment with an antipsychotic drug, sigma receptor ligand haloperidol is associated with many cardiovascular side effects (cardiac arrhythmias such as torsade de pointes, ventricular fibrillation or even cardiac arrest). Mechanisms underlying these effects are not fully understood. The aim of the study was to explore the impact of haloperidol on 3-D electrogram in isolated rat hearts after chronic treatment with this compound. Eight adult male rats were injected haloperidol i.p. (2 mg/kg; treated group) and two were given vehicle (control group) once a day, for 21 days. Animals were sacrificed under deep ether anesthesia. The chest was opened and the heart was quickly excised. The hearts were perfused according to Langendorff with Krebs-Henseleit solution ($CaCl_2$, 1.2 mM) at constant pressure (85 mmHg) and 37 °C. The experiment consists of four 30 min periods: control, 10 nM haloperidol, washout, 10 nM haloperidol. Ten consecutive RR intervals were averaged at the end of control (steady state heart rate). This value was used for normalization of heart rate during the rest of experiment. In the same way, QT intervals were measured in order to determine L-QT. Normalized spontaneous heart rate showed a tendency to decrease during all periods of the experiment in both groups. In all hearts, the QT intervals lengthened in the first haloperidol period, partially declined in washout and in the second haloperidol administration QT interval prolongation occurred again. Still, chronic treatment with haloperidol caused much weaker changes than those in control group. To be concluded, repeated administration of haloperidol causes probably down regulation of cardiac sigma receptors. However, these changes are subtle and future experiments are needed to support this hypothesis.

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WHAT CAN A FLY TELL US ABOUT HUMAN PHYSIOLOGY AND PATHOPHYSIOLOGY?

J. Fleischmannová[1], O. Jedličková[1,2], M. Šerý[2,3], M. Žurovec[1,2]
[1]Faculty of Science, University of South Bohemia, České Budějovice, [2]Institute of Entomology, Academy of Sciences of the Czech Republic, České Budějovice, [3]Pedagogical Faculty, University of South Bohemia, České Budějovice, Czech Republic

The majority of human diseases are complex conditions that involve multiple interacting genetic and environmental factors. Even in today's post-genomic era, deciphering the role of single genes in the etiology of diseases requires appropriate animal models. Besides classical mammalian models, the fruit fly (*Drosophila melanogaster*) has become popular model of human diseases and is often described as a little person with wings. Since the origins of genetics, fruit fly has served as an excellent model to understand complex genetic questions. With growing evidence about the genomic similarity between the fruit fly and human (60 % of human disease genes have their homologues in the fruit fly), the effort in genetic manipulations made during the almost hundred years of fruit fly research started to be exploited also in the field of human physiology and pathophysiology including heart function disorders, hypoxia response, metabolic defects, sleep disorders, neurodegeneration, or aging. Despite large morphological and functional differences, there are interesting analogies between the human and insect heart. Several genes involved in human developmental and functional heart disorders also affect heart function in the fly. Moreover, age related functional decline reported in the fly may bring new insight into human heart functional senescence (1). Neurodegeneration belongs to the group of newly emerging diseases connected with prolonged lifespan. So-called trinucleotide repeat diseases – also known as poly-Q diseases – were successfully modeled in the fruit fly. E.g. Huntington disease as one of them can be easily studied by expressing CAG repeat-containing part of human gene under fly eye disc promotor (2). The fruit fly as a model of human diseases and aging will be introduced to the auditory.

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CHANGES OF LOCOMOTOR ACTIVITY IN ANIMAL MODELS OF NEUROPATHIC PAIN

M. Franek, S. Vaculin, A. Yamamotova
Charles University in Prague, 3rd Faculty of Medicine, Dept. of Normal, Pathological and Clinical Physiology, Prague, Czech Republic

Animal models of neuropathic pain are important method for studying of this type of pain. There are several possibilities how to evaluate nociception in these animal models. In our study we combined classical measuring of pain threshold for thermal stimulation with monitoring of locomotion using LABORAS (Laboratory Animal Behaviour Observation, Registration and Analysis System). The aim of the present study was to find the correlations between pain threshold and locomotor activities in two models of peripheral neuropathic pain. We used four groups of adult male Wistar rats – control group, sham operated animals group, partial sciatic nerve injury (PSNI; 1) group and chronic constriction of sciatic nerve (CCI; 2) group. Pain threshold was evaluated by plantar test and locomotion was analyzed by LABORAS. Each animal was tested eight times during one month period after surgical procedure. We observed different pain patterns in CCI and PSNI: in PSNI withdrawal thresholds are lower, the decrease onsets later and lasts longer. Spontaneous motor activity was decreased in all operated animals; the shortest distance was measured in PSNI animals. Compared to sham operated animals, there were no changes in distance travelled in CCI group. There were no significant differences in body weight changes among groups. Both used models of neuropathic pain affect spontaneous motor activity (more in PSNI) and this affection correlates with pain threshold for thermal stimulation. So, the locomotor activity is other parameter applicable for peripheral neuropathy evaluation.

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METFORMIN IN CHEMICALLY-INDUCED MAMMARY CARCINOGENESIS IN RATS

M. Garajová[1], B. Bojková[1], P. Orendáš[1], M. Kassayová[1], P. Kubatka[2], E. Ahlersová[1], I. Ahlers[1]
[1]Department of Animal Physiology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University in Košice, Slovak Republic, [2]Department of Pharmacology, Jessenius Faculty of Medicine in Martin, Comenius University Bratislava, Slovak Republic

The aim of this work was to evaluate the chemopreventive effect of peroral antidiabetic metformin in mammary carcinogenesis in female Sprague-Dawley rats. Mammary carcinogenesis was induced by N-methyl-N-nitrosourea (NMU) administered in two intraperitoneal

doses (each per 50 mg/kg b.w.) between 43rd-55th postnatal days. Metformin was administered in drinking water in two concentrations (50 mg/l and 500 mg/l, respectively) 13 days before the first NMU dose until the end of the experiment. During the experiment the animals were weekly weighed and palpated for the presence of mammary tumours, the incidence, latency, tumour frequency, and tumour volume were recorded. The experiment was terminated 18 weeks after the first NMU dose, basic tumour growth parameters and metabolic and hormonal variables were evaluated. Metformin did not significantly alter the tumour growth although a delay in tumour onset was recorded after higher metformin dose. Metformin altered metabolic and hormonal variables. Insulinemia decreased after both metformin doses in comparison with intact rats without changes in glycemia, triacylglycerols concentration was decreased in liver and increased in serum when compared to intacts. Higher metformin dose attenuated liver lipoperoxidation.

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GLUTAMATE LEVEL ELEVATION IN THE BLOOD AFTER TRANSIENT CEREBRAL ISCHEMIA

M. Gottlieb, P. Kravcukova, V. Danielisova, M. Nemethova, J. Burda
Institute of Neurobiology, Slovak Academy of Sciences in Košice, Slovak Republic

The amino acid L-glutamic acid (Glu), mediates most of the excitatory transactions between neurons in the central nervous system. It is well established that ischemia-induced release of glutamate and the subsequent activation of post-synaptic glutamate receptors are important processes involved in the development of ischemic neuronal damage. In the postischemic interval in brain excitatory amino acids are released and their extracellular concentrations are dramatically increased. Neutralization of these deleterious effects is caused by an increased pumping of glutamate from brain interstitial fluid into blood. The endothelial cells, that form the walls of the brain blood capillaries, harbour glutamate transporters on their antiluminal side (brain side), have the ability to take up glutamate and concentrate it into their cytoplasm at concentrations that exceeds blood glutamate levels (1). In the present work we studied the effect of transient brain ischemia/reperfusion condition on blood glutamate level. For this purpose we used models of transient focal and global ischemia in rat in duration 90 min and 10 min, respectively. Blood samples were collected from *v. jugularis externa* after global ischemia in time intervals 20, 40, 60, 90 and 120 min of reperfusion and in 30 minutes intervals up to 3 hours in model of focal ischemia. Samples were used for fluorimetric measurement of whole blood glutamate concentration. In mode of global ischemia glutamic acid concentration started to increase around 20 min after ischemia and a significant peak was observed at 40 and 60 min of reperfusion, reaching approx. 191 μmol/l, representing a 22.3 % increase in comparison with SHC (150 μmol/l). After a transient decrease of glutamate level at 90 min of reperfusion, its concentration started to rise again around 120 min (18.4 %). In focal ischemia model we have found slight decrease of Glu concentration in total blood at the end of transient 90 min ischemia. During recirculation period Glu level was fluently increased with a peek at 150 min up to three fold. Our observations confirmed that Glu is eluated from brain into blood following cerebral blood supply restoration in different times and concentrations in focal and global ischemia, respectively. Those changes of blood Glu concentration could be used as simple markers of ischemic brain damage range and specify the best reperfusion period for minimizing negative side effects in a model of transient global and focal cerebral ischemia in rats.

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EFFECT OF STREPTOZOTOCIN INDUCED DIABETES ON CLOCK GENE EXPRESSION IN THE LIVER AND PANCREAS OF RAT

I. Herichová, J. Monošíková, A. Veselá, K. Stebelová, M. Zeman
Department of Animal Physiology and Ethology, Comenius University Bratislava, Slovakia

Diabetes is a metabolic disease frequently associated with disturbed circadian rhythms manifestation. The reason for dysfunction of the biological clock under conditions of diabetes is not completely known. Our approach employs analysis of clock gene expression in the liver and pancreas of control and streptozotocin (STZ) treated rats. In addition, daily pattern of activity was measured in all animals and circadian oscillator in the cerebellum was described. Adult Wistar male rats were synchronized to the light:dark cycle 12:12. Diabetes was induced by intraperitoneal injection of STZ (65 mg per kg of body weight) and samples were taken 17 days after administration. Expression of *per2* and *bmal1* was measured in 4 h interval during 24 h cycle by real time PCR. We observed significantly decreased amplitude of *per2* expression in cerebellum and pancreas of STZ-treated rats in comparison with control. Effect of STZ administration was tissue specific when liver, pancreas, cerebellum and the heart were compared. Rhythmic expression of clock controlled genes *dbp* and *rev-erbα* involved in metabolism regulation in the liver was damped after STZ administration. Night time pattern of activity was fractionated and ability to anticipate scotophase was lost in diabetic rats. According expected acrophase of plasma melatonin rhythm in STZ treated rats function of central oscillator was not changed at this stage of diabetes. It is supposed that peripheral tissues can influence each other and eventually feedback to the central oscillator. The reason for the internal desynchronization can relay on relative strength of synchronizing cues changed by the disease.

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INHIBITION OF LEUCOPOIESIS AND ASPIRIN–LIKE EFFECT OF RESVERATROL ON PIGLETS

Z. Holešovská, V. Kotrbáček, J. Doubek
University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

The present study was aimed to determine the effect of resveratrol on hematological parameters in piglets, mainly the total white blood cell (WBC) count and the count and functional activity of platelets. Ten six-week old White Large piglets were administered Resveratrol in alcohol (15 %) solution in a daily dose 3 mg /kg$^{0.75}$ of body weight. Resveratrol was given by a gastric tube for two weeks. Other ten piglets obtained this solution without resveratrol and were used as controls. Both the experimental and the control groups were housed in individual pens and fed ad libitum with a complete food mixture. In the blood samples, collected by puncture of the v. jugularis prior the experiment and after the first and the second week, WBC and platelets were determined using the hematological analyzer CELLTACα (Nihon Kohden, Japan). Neutrophils were counted manually. The platelet aggregation was determined by Bohr´s optic method on two-channel aggregometre by firm Chrono-log using two inductors –ADP (preparation Chrono-PAR ADP by firm Chrono-Log) and Cationic propyl gallate (preparation SPAT by firm Analytical Control Systems, Inc.). Aggregation of platelets was determined at the final concentration10 μM ADP and at the ratio of plasma: SPAT 9:1. The values of aggregation (%) a slope max. (%/min) were automatically deducted from the aggregometric curve. Administration of resveratrol caused a significant decrease in the number of WBC ($P < 0.01$) and of the neutrophils ($P<0.01$) at the end of the first week. The biggest drop was found in young cell forms - bands ($P<0.01$). After two weeks no further reduction in leukocyte numbers was found. In controls a significant ($P<0.01$) decrease of WBC was observed only at the end of the experiment and the number of neutrophils remained unchanged. The count of platelets significantly and gradually decreased in the experimental piglets ($P<0.01$). Aggregation of platelets induced by ADP was significantly lowered ($P<0.01$) after two weeks of resveratrol administration. Cationic propyl gallate used as inductor had also significant effect on this variable ($P<0.01$). Our findings confirm temporary inhibition of leucopoiesis and

an aspirin-like effect of resveratrol administered in relatively high doses to young piglets.

NON-INVASIVE DETERMINATION OF BAROREFLEX SENSITIVITY AND BREATHING

N. Honzíková, B. Fišer, Z. Nováková, E. Závodná
Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Various methods are used for non-invasive determination of baroreflex sensitivity (BRS, ms/mmHg) in various laboratories. In many studies sequential methods or spectral methods with spontaneous breathing are used. We use the spectral method during breathing controlled by metronome at 0.33 Hz (1). The aim of the present study was to evaluate the role of respiration in the spectral method of BRS determination. We recorded blood pressure by Finapres (5 minutes, metronome breathing 0.33 Hz) in 118 healthy subjects (age between 19 and 26 years). We compared a modulus between the cross-spectral density of variation of pulse intervals and systolic blood pressure (ms*mmHg) and the spectral density of variation of systolic blood pressure (mmHg*mmHg) at 0.1 Hz (BRS 0.1 Hz), and a modulus at 0.33 Hz (BRS 0.33 Hz). A statistically significant correlation was found between BRS 0.1 Hz and BRS 0.33 Hz ($r=0.52$, $p<0.001$). The regression equation (BRS 0.33 Hz = 2.63 + 1.14 * BRS 0.1 Hz, difference between slope and identity line was insignificant) indicated the existence of a breathing-dependent BRS-non-related component. The ratio of BRS 0.1 Hz to BRS 0.33 Hz (0.876+-0.419) was significantly lower than 1 ($p<0.01$). Thus BRS evaluated at the breathing frequency overestimates the real baroreflex sensitivity. This is more pronounced at low values of BRS, which are more important for diagnostic purposes. In conclusion, determination of BRS by the spectral method at the breathing frequency overestimates the real BRS. For diagnostic purposes we recommend the evaluation of BRS at the breathing frequency controlled by metronome, which is substantially higher than 0.1 Hz.

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PREDICTION OF RESTING ENERGY EXPENDITURE DURING PREGNANCY BY NEW EQUATION

M. Hronek[1], Z. Zadak[2], J. Tošner[3]
[1]Charles University, Faculty of Pharmacy, [2]Department of Research and Development, University Hospital and Faculty of Medicine, [3]Obstetric and gynecological clinic, University hospital, Hradec Kralove, Czech Republic

There are equations for the calculation of resting energy expenditure (REE) in the normal physiological state, such as the Harris Benedict(1), Schofield(2) or Kleiber(3), but no such equation for the estimation of REE in the pregnant organism is known. A total of 152 healthy pregnant Czech women from the Hradec Kralove region were recruited into this longitudinal prospective study. The subjects have parity 2, and were non-smokers, non-users of chronic medications and non-abusers of alcohol or drugs. These women were, normoglycemic, euthyroid, and not anemic. Pregnant women were divided into two groups at random. In the derivation group (used for determination of the new equation), all 31 pregnant women were observed during whole pregnancy, and examined four times in each assigned period during pregnancy, especially up to the 20th week of pregnancy, between 21st and 29th weeks, between 30th and 36th weeks, between 37th and 39th weeks (P4). In the verification group (for cross-validation test of new formula) 121 volunteers were haphazardly assessed; each woman was examined only once throughout the different periods of pregnancy. REE-IC of the pregnant women in both study groups was examined via indirect calorimetry (Vmax Series, V6200 Autobox, SensorMedics Corporation, California, USA) along with anthropometry after 12 hours of fasting in four periods of pregnancy. A statistical comparison of three basic equations – Harris Benedict, Schofield and Kleiber – was used for the prediction of REE. Through correlation analysis and linear regression, a new equation of REE in kcal during pregnancy P REE = 346.43943 + 13.962564*W + 2.700416*H – 6.826376*A (W-weight, H-height, A-age) with standard deviation 116 kcal was determined. This corresponds with real REE-IC and maternal changes in each phase of pregnancy and can be applied for prediction of REE during gestation. Analysis of other predictive equations of REE with the addition of kcal and a corrected multiplication factor for each stage of pregnancy expressed low concordance.

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DOES „CROSS-FOSTERING“ AFFECT LEARNING AND MEMORY OF ADULT MALE RATS PRENATALLY EXPOSED TO METHAMPHETAMINE?

L. Hrubá, B. Schutová, R. Šlamberová, M. Pometlová, R. Rokyta
Charles University in Prague, Third Faculty of Medicine, Department of Normal, Pathological and Clinical Physiology, Prague, Czech Republic

Our previous studies demonstrated that methamphetamine (M) administered during gestation and lactation periods impairs maternal behavior, alters the functional development of rat pups and affects behavior in adulthood. The aim of our study was to investigate the impact of prenatal M exposure and cross-fostering on learning and memory tested in Morris water maze (MWM) in adult male rats. Mothers were daily exposed to injection of M (5 mg/kg) or saline (S): prior to impregnation and throughout gestation and lactation periods. On postnatal day 1, pups were cross-fostered so that each mother received some of her own and some of the pups of mother with opposite treatment. Based on the prenatal and postnatal treatments 4 experimental groups (S/S, S/M, M/S, M/M) were tested in MWM. Three types of tests were used: (1) “Place navigation test” (Learning), (2) “Probe test” (Probe) and (3) “Retention memory test” (Memory). In the test of learning, S/M and M/M groups had longer latencies and greater search errors relative to S/S and M/S groups. Further, S/M groups had longer trajectories than S/S and M/S groups. Rats prenatally exposed to M had lower speed of swimming relative to rats prenatally exposed to saline. Our results showed that neither prenatal nor postnatal M exposure affected the test of memory and the Probe test. The present study demonstrates that cross-fostering may affect learning in adulthood. Significantly, postnatal care of control mothers suppressed the negative effect of prenatal M exposure in test of learning.

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PLANTAR-FLEXOR MUSCLE FORCE PRODUCTION AND H-REFLEX DURING AND EARLY AFTER ACUTE ISCHEMIA OF LOWER LIMB IN MAN

P. Charalampidis[1], K. Fáberová[2,3], P. Kubový[1], J. Otáhal[1,2]
[1]Faculty of Physical Education and Sport, Charles University, Prague, [2]Institute of Physiology of the Academy of Sciences of the Czech Republic v.v.i., Prague, [3]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

Ischemic lower limb is a syndrome that causes neuromuscular dysfunction in both acute and chronic periods. Only a short period of ischemia can produce intracellular metabolic disturbances affecting peripheral and spinal mechanism of human motor control. It affects mainly sensory and motor neuron excitability, neuromuscular transmission and skeletal muscle contractile mechanism. The aim of this study was to assess the influence of short term ischaemia in lower limb on plantar-flexor (PF) muscle force production, eliciting by H reflex and M wave recruitment curves and to determine the main site in the neuromuscular system affected by the pathogenic condition of ischaemia. Seventeen healthy adult volunteers (11 male and 6 female) participated in the study with their Informed consent, experimental procedures were performed in accordance with the Declaration of Helsinki, and the study was the approved by the Ethics Committee. None of the subject had any history of vascular or other medical deficits known to affect neuromuscular function. The subjects lay prone on a table with both legs extended and the right foot attached (with respect to muscle tone) and secured to the force platform. The force platform was

in vertical position. In order to induce ischemia blood pressure cuff was placed around the thigh 15 cm above the knee before the experiment started and inflated to a pressure of 200 mmHg for 10 min after the first series of measurements ended. Blood occlusion was repeatedly checked by an auscultation of the popliteal artery. During the experiment, the plantar-flexor (PF) force, H and M-responses of soleus muscle evoked by tibial nerve stimulation were measured at rest, during 10 minutes of ischaemia, 10 and 20 minutes after the occlusion was released. Background EMG activity of the soleus muscle was not significantly different between the four periods of experiment. However, threshold and recruitment curves were significatnly altered during and 10 minutes after the ischemia respectively. At the post-ischeamic period the PF force fall significantly compare to preischemic values and to ischaemia. In conclusion our data show that short term ischaemia induces post-ischaemic PF muscle force output and not even the restoration of blood flow allowed the muscle to reach the initial values.

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THE ASSOCIATION OF MYOCARDIAL RELAXATION INDEX EM WITH LONG-TERM DIABETES COMPENSATION IN TYPE 2 ASYMPTOMATIC DIABETIC PATIENTS

J. Charvat, J. Chlumsky
2nd Faculty of Medicine of Charles University Prague, Czech Republic

Diabetic patients suffer from diastolic dysfunction of left heart ventricle more frequently than nondiabetic population. Tissue Doppler derived myocardial velocity is a reliable parameter of myocardial relaxation (E_m). The aim of our study was to evaluate the association of E_m with mass of left ventricle, systemic blood pressure examined by 24 hours ambulatory blood pressure measurement (ABPM) and long-term diabetes compensation expressed by level of glycolated hemoblobin (HbA_1C) in type 2 diabetic patients with no history of cardiovascular disease. The index E/E_m was calculated (E is preload dependent transmitral early diastolic velocity) which is suggested for evaluation of left ventricle end diastolic pressure. We analyzed 82 patients, 41 with Em $\geq$ 7,5 cm/sec and 41 with Em < 7,5 cm/sec. Tissue Dopler echocardiography, ABPM and laboratory test (HbA_1C) were examined in each patient the same day. Chi-square and T-test were used for statistical analysis.

	Em≥7,5 cm/sec	Em<7,5 cm/sec	p
Average age (years)	60,5±5,4	61,7±4,7	NS
Number of women	14 (34%)	14(34%)	NS
Mass of LV (g/m^2)	115,3±14,7	113,2±13,1	NS
Mean SBP (mmHg)-ABPM	131±5,8	132,0±5,9	NS
Mean DBP (mmHg) -ABPM	81±4,6	82±3,4	NS
E/Em	0,091±0.0130	0,112±0,018	<0,001
HbA1C (%)	5,6±1,0%	6,4±1,3	0,042

SBP=systolic blood pressure, DBP = diastolic blood pressure

In conclusions, our results show the significant association E_m with long-term type 2 diabetes compensation and E/E_m . No relation was found between E_m and mass of left ventricle, mean systolic and diastolic pressure evaluated by ABPM.

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MITOCHONDRIAL COMPLEXES I AND IV ARE IMPAIRED DUE TO GLOBAL ISCHEMIA/REPERFUSION IN RAT BRAIN

M. Chomová, Z. Tatarková, D. Dobrota, P. Račay
Department of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University, Martin, Slovak Republic

In the mammalian mitochondrial electron transfer chain, the majority of electrons enter at complex I, go through complexes III and IV to be finally delivered to oxygen. Oxidative stress impairs respiration and initiates the activation of the mitochondrial - dependent apoptotic route by directly triggering the release of cytochrome c from the mitochondrial intermembrane space. In this study we have investigated the activities of mitochondrial complexes I and IV isolated from rat hippocampus and cortex after global ischemia/reperfusion. Global brain ischemia was induced by 4-vessel occlusion in duration of 15 min, follows 1, 3, 24 and 72 hours of reperfusion. Our results showed significantly decreased activities of NADH:decylubiquinone oxidoreductase in the hippocampus while activities in the cortex were not changed. Interestingly, spectrophotometric measurement of the NADH:ferricyanide oxidoreductase didn't reveal any statistically significant changes in both hippocampus and cortex. We have also observed inhibition of complex IV activities to 94,2 % and 90,7 % of control in hippocampal and cortex mitochondria after 1 hour of reperfusion, respectively. However, activities of cytochrome c oxidase (complex IV) were returned to the control levels after 24 hours of reperfusion in both hippocampus and cortex. Next, we have examined immunoreactivity of proapoptotic and antiapoptotic members of Bcl-2 family (Bax, Bcl-xL) in connection with p53 protein, where mitochondrial translocation of p53 was observed. Based on our date, impairment of mitochondrial respiratory complexes results in the induction of apoptotic cascade of events culminating in the death of sensitive neuronal cells.

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ROLE OF TRANSMEMBRANE AROMATIC RESIDUES IN P2X RECEPTOR FUNCTIONS

M. Jindrichova[1], V. Vavra[1], T. Obsil[1,2], S.S. Stojilkovic[3], H. Zemkova[1]
[1]Institute of Physiology Academy of Sciences of the Czech Republic, [2]Faculty of Science, Charles University, Prague, Czech Republic, [3]Section on Cellular Signaling, Program in Develop. Neuroscience, NICHD, NIH, Bethesda, USA

Purinergic P2X receptors (P2XRs) are membrane ATP-gated cation channels which play a role in neurotransmission, pain sensation, immune response and modulation of hormone release. In vertebrates, seven genes encode P2XR subunits (termed $P2X_{1-7}$), which are 40-50 % identical in amino acids sequence. Each subunit consists of two transmembrane domains (TM1 and TM2) separated by a large extracellular loop containing ATP binding site, and intracellular N- and C- termini. Both TM1 and TM2 are predicted to adopt α-helix structures that line the pore and move during gating. The TM2 region is dominant for channel assembly, gating, and ion selectivity. The TM1 segment is apparently involved in ATP recognition. For example, it has been shown that replacement of the TM1 domain of the rat P2X subunit with one from $\alpha\beta$meATP-sensitive subunits ($P2X_1$ or $P2X_3$) allowed the resulting chimera to display $\alpha\beta$meATP sensitivity. We examined the functional relevance of aromatic residues of P2X in the upper part of the TM1. Replacement of the conserved tyrosine residue with alanine had a receptor specific effect: $P2X_1R$ was expressed but was nonfunctional, $P2X_2R$ and $P2X_4R$ and to smaller extend $P2X_3R$ exhibited enhanced sensitivity to agonists accompanied with prolonged deactivation, whereas the $P2X_7R$ function was not affected. At $P2X_4R$, replacement of this residue with other amino acids also enhanced the agonist sensitivity and delayed deactivation in order: Gly > Ile > Ala > Cys > Trp > Phe > Tyr; indicating that aromatic residue at conserved TM1 position is needed for the three-dimensional structure of the P2XRs and is required for proper channel function. Mutants of other aromatic residues in the upper TM1 domain of $P2X_2R$, $P2X_3R$, and $P2X_4R$ also altered the sensitivity of receptors for agonists or time-course of their deactivation. In $P2X_4R$-Y42A mutant, the replacement of -Trp^{46} and -Trp^{50} residues restored the receptor functions, whereas mutation of aromatic Phe^{48} residue and non-aromatic Val^{43} and Gly^{29} residues was ineffective. These results indicate that conserved tyrosine and nearby aromatic residues in the upper part of TM1 play important roles in three-dimensional structure of P2XRs required for agonist binding and/or channel gating.

PLASMA CONCENTRATIONS OF FIBROBLAST GROWTH FACTORS 19 AND 21 IN PATIENTS WITH ANOREXIA NERVOSA

P. Kaválková, I. Dostálová, D. Haluzíková[1], Z. Lacinová, M. Mráz, H. Papežová[2] and M. Haluzík
3rd Department of Medicine, [1]Department of Sports Medicine, [2]Department of Psychiatry, 1st Faculty of Medicine, Charles University and General University Hospital, U Nemocnice 1, 128 00 Prague 2, Czech Republic

Fibroblast growth factors 19 and 21 (FGF19 and FGF21) are novel metabolic regulators that improve insulin sensitivity and increase lipolytic rate in mice. Gene expression of FGF21 in the mouse liver is under tight nutritional control being stimulated by fasting and ketogenesis and inhibited by feeding. However, little is still known about the nutritional regulation and bioactivity of FGF19 and FGF21 in humans. We measured plasma FGF19 and FGF21 levels in patients with anorexia nervosa (AN) and explored its relationship with anthropometric and selected endocrine parameters. Seventeen untreated women with a restrictive type of AN (age: 25.0±1.34, body mass index (BMI): 15.9±0.33 kg/m^2) and seventeen healthy women (control group; age: 24.7±0.59, BMI: 22.9±0.41 kg/m^2) were included. Ten patients with AN were examined also after two months of partial realimentation. Blood samples for FGF19, FGF21, leptin, leptin receptor, adiponectin, resistin, insulin, ketone bodies, free fatty acids, thyroid hormones, C-reactive protein, insulin-like growth factor-1 and biochemical parameters evaluation were withdrawn after overnight fast at 8.00 a.m. Plasma FGF19 and FGF21 were measured by commercial ELISA kit (Biovendor, Brno, Czech Republic). Fasting plasma FGF19 levels did not significantly differ between the groups studied (246.8±34.1 pg/ml in AN vs. 205.3±25.9 pg/ml in C), whereas fasting plasma FGF21 levels were significantly reduced in AN relative to control group (112.4±23.4 pg/ml u AN vs. 272.3±40.0 pg/ml u C; $p<0.05$). Plasma FGF19 was not related to any of the parameters studied. In contrast, plasma FGF21 significantly positively correlated with BMI ($r=0.54$; $p<0.001$), serum leptin ($r=0.62$; $p<0.001$) and serum insulin ($r=0.42$; $p<0.05$) and was significantly inversely related to serum adiponectin ($r=-0.48$; $p<0.005$) levels in both groups. Plasma levels of FGF2 in all patients with AN were significantly reduced after 2 months of realimentation. Pre-treatment plasma FGF21 significantly positively correlated with change of BMI during treatment ($r=0.62$; $p<0.05$). We suggest that reduced plasma FGF21 levels could be involved in the pathophysiology or adaptive metabolic response to starvation in patients with AN.

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POLYMORPHISMS IN HOT AUTISM CANDIDATE GENES IN SLOVAK AUTISTIC POPULATION

S. Kelemenová, E. Schmidtová, P. Celec[1], D. Ostatníková
Comenius University, Faculty of Medicine, Department of Physiology, Bratislava, [1]Comenius University, Faculty of Medicine, Department of Pathophysiology, Bratislava, Slovakia.

Autism is the most genetically influenced neuropsychiatric disorder, however the detailed genetic processes of the disorder heritability are not well understood. Results of genetic autism research revealed more than 10 candidate genes for the disorder. These genes are mainly connected with the synaptic processes and neuroendocrine pathways. In our study we focused on oxytocin receptor gene (OXTR) and RELN gene. Receptors for neuropeptide oxytocin are localized especially in the brainstem regions crucial for regulation of social and reproductive behavior. In addition, autistic patients have low blood levels of oxytocin. RELN gene encodes reelin protein crucial for neuronal development and maintaining synaptic plasticity. After signing the consent, 70 autistic boys and 80 healthy controls were included into the study. DNA was extracted from buccal swabs. OXTR gene polymorphism (rs2228485) was analysed using RFLP analysis. 5´UTR (GGC)n STR polymorphism was analysed using fragment analysis. In previous studies higher number of triplets (more than 12) was associated with autism. This polymorphism was found to have also phenotypic consequence of decreased reelin expression. This corresponds with the fact that autistic patients have decreased reelin levels in blood and brain (1, 2). Our preliminary results showed that slightly higher GGC repeats are associated with autism phenotype in Slovak autistic population.

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DISPARATE EFFECT OF DAUNORUBICIN IN NORMAL AND HYPERTROPHIED LEFT VENTRICLE IN RATS

J. Klimas, K. Turcekova, T. Stankovicova, E. Kralova, A. Adameova, Z. Kyselova[1], P. Musil, Z. Bajuszova, M. Snopkova, J. Kmecova, P. Ochodnicky, P. Krenek, J. Kyselovic
Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University, 832 32 Bratislava, Slovak Republic; [1]Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovak Republic. e-mail: klimas@fpharm.uniba.sk

Usage of anthracyclines impairs cardiac function. We tested, whether the daunorubicin-induced reduction of left ventricular performance and/or the different susceptibility to arrhythmias are present in the settings of hypertension and cardiac hypertrophy. Wistar rats and spontaneously hypertensive rats (SHR) were treated for two weeks with daunorubicin (W-D and S-D, resp.; 3mg/kg, i.p., dosage in 48 h interval, n=7-8 per group), control rats (W-C and S-C, resp.) received vehicle. Left ventricular function was studied using left ventricular catheterization, arrhythmias were induced by occlusion/reperfusion of left coronary artery. Expression of the NADPH subunit (gp91phox) was determined in left ventricular homogenates using SDS-PAGE and Western blotting (n=8-10, per group). Daunorubicin medication led to an impairment of left ventricular function in Wistars but not in the SHR (see table; *$p<0.05$ vs. W-C; #$p<0.05$ vs. W-D). In contrast, severity of reperfusion-induced arrhythmias was decreased by daunorubicin in Wistars ($p<0.05$), but not in SHR. We tested whether this effect is associated with changed NADPH expression. SHR showed increased amount of gp91phox as compared to Wistars (by 25%; $p<0.05$) but daunorubicin had no further impact on its expression.

	W-C	W-D	S-C	S-D
LVP (mmHg)	152±5	123±7*	151±6	157±6#
dP/dt_{max} (mmHg/s)	7078±462	4295±449*	6396±823	6386±649#
dP/dt_{min} (mmHg/s)	-5705±377	-3285±477*	-4735±418	-5306±556#

In conclusion, we found depressive effect of daunorubicin on left ventricular hemodynamics and decreased severity of reperfusion-induced arrhythmias in normotensive but not in hypertensive rats.

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SYSTEM FOR SIMULTANEOUS DETECTION OF OPTICAL AND ELECTROPHYSIOLOGICAL SIGNALS

R. Konopková, E. Krajčovičová, M. Martinková, H. Kubová, J. Otáhal
Institute of Physiology of the Academy of Sciences of the Czech Republic v.v.i., Prague, Czech Republic

In present, it is of great interest to detect many physiological parameters in the one experiment. Especially combining optic (image) and electric measurements are very usefull. However, unified simple to use software interface capable to synchronously capture video and data is not easy accessible. Therefore we developed software providing unified user interface for synchronous electrophysiological and video acquisition and analysis enabling external devices and powerful Matlab interoperability. The software consists of two acquisition components electrophysiological data component and video component. National Instruments M series PCI data acquisition boards were chosen for digitalization of voltage signals for their high temporal and bit resolution. For image acquisition we have developed software component enabling to acquire from standard webcams, DV cams and selected high sensitive cameras that use when we detect the activity of

hippocampal in pyramidal cell layer. To test the functionality of the system we have performed experiment on acute hippocampal slice detecting both evekoed potentials and intrinsic optical signals of the CA3 region during stimulation of mossy fibers. Cooled12-bit CCD-camera (RETIGA2000R) connected to the water immersion epifluorescence microscope (Olympus BX51WI) was used for image capturing. Synaptic activity was associated with increase in light transmittance (LT) in CA3 region in stimulation intensity dependent manner. The change in maximal intensity increase of LT during 3T and 4T stimulation was 150.6±16.3 % and 173.9±21.8 % of 2T intensity. The changes in LT were almost completely abolished by TTX (1 uM in ACSF), LT increase was led down to 5.4±2.4 % compared to the control measurement, indicating that the synaptic activation was responsible for the changes of LT. To enable glutamate release but prevent from binding glutamate to ionotropic glutamate receptors APV (50 uM in ACSF) and CNQX (10 uM in ACSF) were used. In this case LT was also decreased to 57.9±4.9 %. The signal was significantly blocked but the percentage of the change was distinctively higher than in the previous measurement with TTX. In conclusion, the system makes possible to detect the both changes of LT and at the same time the electrophysiological signal. In the future we also plan to further define the usage of LT for the activity detection of the nervous tissue and to finally set up the system for the whole animal measurements.

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APOLIPOPROTEINS IN SUBJECTS WITH HYPERTRIACYL-GLYCEROLEMIA AND OVERWEIGHT

J. Koprovičová, D. Petrášová
Institute of Experimental Medicine, Medical Faculty of the P. J. Šafarik University, Košice, Slovak Republic

Apolipoproteins (apo) mainly apo B_{100}, apo A-I, apo B/apo A-I ratio and apo C-III are important independent predictors of early aterosclerosis and atherothrombosis. Apo C-III blood serum levels have been proposed to reflect triacylglycerol (TG) metabolism more accurately than the serum level of TG. Triacylglycerols or especially TG-rich lipoproteins (TG-RLPs) with apo C-III may provide additional information to apo B_{100} (1, 2). The aim of study was to estimate lipids and apolipoprotein serum levels in the overweight subjects with hypertriglyceridemia (HTG).The results have been compared with the Control group (C) of the same age and sex. The 27 overweight subjects (Ow) with the HTG and the 21 healthy subjects (C) with normal body weight, lipid and apolipoprotein serum levels have been investigated. The mean level of the BMI of the Ow/C was: 27.1±1.7 $kg.m^{-2}$/24.2±0.7 $kg.m^{-2}$. The mean age for both groups was: 47±9 years of age. Serum levels of the apo A-I, apo C-II, apo C-III have been determined by using of radial immunoassay and apo B_{100} by electroimmunoassay. Serum levels of the triacylglycerols (TG), total-cholesterol (TC) and HDL-cholesterol (HDL-C) have been detected by using commercial biochemical sets. The LDL-cholesterol (LDL-C), non-HDL-cholesterol serum levels, value of apo B/apo A-I and other ratios have been calculated. The data have been tested by using of the ANOVA-1, ANOVA-2 and Pearson´s correlation test. In the Ow in comparison with the C the mean of the apo C-III serum levels has been significantly increased: ($p<0.002$), inclusive of the apo C-II: ($p<0.001$) and apo B_{100}: ($p<0.001$). The mean of the apo A-I serum level has been found significantly decreased: ($p<0$ .001). The mean value of the apoB/apoA-I ratio has been found highly significantly increased: ($p<0.001$). The mean serum levels of the TG, TC, and LDL-C have been significantly increased: ($p<0.001$). The mean serum level of the HDL-C has been significantly decreased: ($p<0.05$). In conclusions, hypertriacyl-glycerolemia in combination with the overweight associates with significantly highly elevated TG, apo C-III and apoB_{100} and significantly increased TCH, LDL-CH and decreased apo A-I and HDL-C serum levels which promote the metabolic syndrome and accelerate development of cardiovascular diseases (3).

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ENZYME ARCHITECTONICS OF THE MICRO-VASCULATURE OF THE RAT AND HUMAN BRAIN

I. Koutná[1], M. Šmídl[1], V. Lisá[2], V. Mareš[2]
[1]*Faculty of Education and Faculty of Natural Science of the University of J. E. Purkinje,* [2]*Joint Laboratory of the Cancer Cell Biology of the Institute of Physiology, Academy of Sciences and the 1st Medical Faculty, Charles University, Prague, Czech Republic*

Data on enzymatic equipment of the microvascular and ventricular apparatus (MVA) of the brain of different species and brain regions are incomplete. Here, we report activity of γ-glutamyltranspeptidase (γ-GT), dipeptidyl peptidase-IV (DPP-IV) and alkaline phosphatase (ALP) in adult rat forebrain and human brain samples represented by peritumoral and amygdalo- hippocamal region tissue from patients operated for tumor or epilepsy at the Neurosurgery Dept., Hospital Na Homolce, Prague. In rats, activity of all 3 enzymes appeared mainly in the forebrain capillary bed and the choroid plexus of the lateral ventricles while only threshold values occurred in the interspaced neuronal parenchyma. Activity of γ-GT and ALP remarkably exceeded DPPIV in all regions studied. The former 2 enzymes occurred also in the ependymal lining of the lateral ventricles. In the choroid plexus, γ-GT and ALP were present both in its stromal and epithelial part, while DPP IV occurred only in the vascular stroma. The total area of the enzyme-positive vasculature in the gray matter was twice as high as in the white matter. All 3 enzymes also occurred in microvascular apparatus of human brain samples, both in the gray and white matter tissue. Similarly to rats, activity of DPPIV was much lower than γ-GT being better expressed in the perivascular cells. Intensity of the histochemical reaction, expressed as density per an unit area of the enzyme- bearing structures, was similar in both species studied. However, the total size of γ-GT and DPPIV positive capillary bed, especially as to DPPIV, was less extensive in human than rat brain. In both species, the total size of γ-GT positive vascular bed was the same as that stained for ALP considered to be an universal capillary marker in different organs. In both species, γ-GT activity remarkably exceeded DPP IV. The data suggest that majority of the forebrain capillaries in both species posses catalytically active γ-GT which may take part in transport of amine acids and detoxification processes at the blood-brain interface. A more restricted expression of DPPIV remains open to speculations, especially on its role in control of the hematoencephalic barrier.

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EMPATHIZING AND SYSTEMIZING IN CHILDREN WITH ASPERGER SYNDROME

P. Krajmer[1], D. Jánošíková[2], D. Ostatníková[1]
[1]*Institute of Physiology, Comenius University Faculty of Medicine, Bratislava,* [2]*Outpatience Department of Clinical Psychology for Children and Youth, Trnava, Slovak Republic*

Empathizing is the drive to identify another person´s emotions and thoughts, and to respond to these with an appropriate emotion. Empathizing allows us to predict a person´s behaviour, and to care how others feel. Systemizing is the drive to analyse the variables in a system, and underlying rules that govern the behaviour of a system (1). Previous studies report male superiority in systemizing and female superiority at empathizing. Children with Asperger syndrome (AS) or high-functioning autism (HFA) are predicted to be either normal or superior at systemizing but impaired at empathizing. We employed adapted self report questionnaires SQ and EQ in group *n=40* with normal or superior IQ Asperger males and in control group *n=99* students of grammar school. Both groups were aged from 8 to 18 years. Their mean scores were compared. AS/HFA probands in comparison with control group scored higher in systemizing quotient and lower in empathizing quotient as predicted. Results reveals strong drive to systemize in AS/HFA. Psychological aspects of this research involving measurements of empathizing and systemizing are directly connected to the neurohormonal and genetic examinations. Results achieved in this interdisciplinary research are valuable in further biological, psychological and social approaches in neurocognitive research and diagnostics of children with AS.

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MYOCARDIAL REMODELING INDUCED BY REPEATED DOSES OF ISOPROTERENOL

E. Kráľová[1], A. Mikušová[2], M. Novotová[2], T. Stankovičová[1]
[1]Comenius University, Faculty of Pharmacy, Department of Pharmacology and Toxicology, [2]Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia

Development of left ventricular hypertrophy increases the risk of sudden death. As a predictor of mortality is usually accepted prolonged QT interval. We focused on the effect of repeated application of isoproterenol (Iso) on the electrical activity of rat heart. Electrocardiograms (ECG) were recorded from animals under general anesthesia (thiopental 45 mg/kg) as well as from the isolated spontaneously beating perfused hearts according to the Langendorff. Myocardial remodeling in rats was induced by repeated injections of isoproterenol (5 mg/kg s.c.) either for 7 days (Iso5/7, n=7) or for 21 days (Iso5/21, n=5). Voltage criteria e.g. Sokolow-Lyon indexes were decreased in the order Iso5/7 > Iso5/21 > baseline criteria found in control (n=5) group as well as the QT interval duration both in the animals and isolated hearts. However, heart and left ventricular wet weight, as well as the Cornell index were increased with longer Iso application compared to controls. Hemodynamic measurements of Iso5 hearts revealed weaker contractile ability (87±12 vs 99±32 mmHg) of spontaneously beating hearts compared to control hearts but the weakest contraction was observed in the Iso5/21 (21±2 mmHg). Mortality of Iso5/7 rats was about 40-50% and increased with longer Iso application to cca 80 %. Tissue examination showed in Iso5/7 focal necrotic loci in the left ventricle but large areas of necrosis in the endocardium in Iso5/21. It could be concluded that repeated doses of isoproterenol induce myocardial remodeling associated with hypertrophy, however, longer application could result into the failing myocardium with increased incidence of sudden death.

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EFFECT OF TRANSIENT FOCAL AND GLOBAL BRAIN ISCHEMIA ON BRAIN-DERIVED NEUROTROPHIC FACTOR LEVEL IN BLOOD

P. Kravcukova, V. Danielisova, M. Nemethova, J. Burda, D. Cizkova, M. Gottlieb
Institute of Neurobiology, Slovak Academy of Sciences in Kosice, Slovak Republic

Brain-derived neurotrophic factor (BDNF) acts on certain neurons of the central nervous system and the peripheral nervous system, helping to support the survival of existing neurons and encourage the growth and differentiation of new neurons and synapses. Various studies have shown possible links between low levels of BDNF and conditions such as depression, schizophrenia, Obsessive-compulsive disorder, Alzheimer's disease, Huntington's disease, Rett syndrome, and dementia, as well as anorexia nervosa and bulimia nervosa. In present work we studied possible connection of BDNF and brain ischemia reperfusion injury. For this purpose we used models of transient focal and global ischemia in rat in duration 90 min and 10 min, respectively. Blood samples were collected from *v. jugularis externa* before and during ischemia and in time intervals 40 and 90 min of reperfusion, than were samples processed and BDNF concentration was measured by a sandwich enzyme immunoassay. Results of our experiment showed changes of BDNF level in total blood, plasma and blood cells. In model of focal ischemia BDNF concentration in total blood, as well as in plasma and blood cells, rapidly decreased during first 40 min of blood supply restriction. In samples of whole blood and blood cells BDNF started to rise at the end of ischemic insult at least on level of control. On the contrary, plasma level of this protein significantly decreased about 55 %. In model of global ischemia we didn't observed some important changes after ischemic insult. During first 90 min of reperfusion in both models BDNF level in total blood and in blood cells continuously decreased. Plasma level of BDNF started to rise at 40 min of reperfusion. At 90 min of recirculation BDNF level in model of focal ischemia reached 92 %, in model of global ischemia was its concentration elevated about 155 %. In conclusion we can state that ischemia-reperfusion leads to changes of BDNF level in total blood, as well as in plasma and blood cells. Brain ischemia causes reduction during ischemia and subsequent elevation of BDNF concentration in blood cells at the end of ischemic insult followed by decreasing in early period of reperfusion. On the other side, plasma level of this protein reduced during ischemia markedly rises during recirculation. Our observation could be helpful for a next investigation of BDNF role in brain ischemia-reperfusion, as well as for an examination of its benefits in pathology of this injury.

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ACE INHIBITION DECREASES LEFT VENTRICULAR MASS, BUT NOT LEVELS OF GROWTH FACTORS IN THE NORMOTENSIVE RAT

P. Krenek, K. Mackovicova, P. Ochodnicky, A Gazova[1], J. Klimas, P. Musil, J. Kyselovic
Department of Pharmacology and Toxicology, Faculty of Pharmacy; [1]Institute of Pharmacology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

Angiotensin II can induce cardiac hypertrophy directly or via induction of growth factors, such as endothelin-1 (ET-1), transforming growth factor beta-1 (TGF-β1) or cardiotrophin-1 (CT-1). Angiotensin converting enzyme inhibitors (ACEi) decrease the production of angiotensin II and left ventricular mass. We tested the hypothesis that decrease of left ventricular mass after ACE inhibition could be due to decreased expression of growth factors in vivo. 16-weeks old Wistar rats (n=7 in each group) were administered enalapril hydrochloride p.o twice daily, in a total dose of 5 mg/kg/d (E5) or 15 mg/kg/d (E15). Control group received the vehicle (0,5 % methylcellulose). Systolic blood pressure and heart rate were measured 10 hours after the last dose. Real-Time PCR with SYBR Green detection was used to determine expression of preproendothelin-1 (pET-1), TGF-β1 and CT-1 in the left ventricle and renin in the kidney. Blood pressure was significantly decreased only after the higher dose of enalapril ($P<0.05$). Enalapril did not affect heart rate. Body weight did not change after enalapril. Relative left ventricular mass was decreased after both doses of enalapril (by 8.7±1.7 % and 9.2±1.5 % in E5 and E10, respectively, both $P<0.05$ vs control). We observed a dose-dependent increase of renin expression in the kidney (5.4±1.3 and 9.6±1.7 fold in E5 and E10, respectively, $P<0.05$). Expression of pET-1, CT-1 or TGF-beta1 in the left ventricle was not altered after enalapril treatment. In conclusions, our results indicate that the decrease of left ventricular mass after ACE inhibitor administration to normotensive rats is not associated with expression of growth factors and may follow a different mechanism.

COGNITIVE LOAD DURING A VISUAL ODDBALL TASK DISPLAYS DIFFERENTIAL IMPACT ON BETA 2-BAND PHASE SYNCHRONY

M. Kukleta[1,3], P. Bob[1,3], M. Brázdil[2], R. Roman[1], I. Rektor[2]
[1]Department of Physiology and [2]First Department of Neurology, Faculty of Medicine, Masaryk University, Brno, [3]Department of Psychiatry, First Faculty of Medicine, Charles University, Prague, Czech Republic

The correlation of filtered EEG records (25-35 Hz) obtained from pairs of depth electrodes during a visual oddball task exhibited irregular rapid oscillations of r-values and a great variability of other indicators derived from the correlation data. The present study investigated the impact of increased cognitive demand during the processing of non-target stimuli on one of these indicators, namely the mean r-value calculated from a definite time epoch of averaged records. The EEG records were obtained in eight epileptic patients with intracerebral electrodes;

correlation data from 180 pairs of frontal and temporal records (all with evoked potential in response to stimulus presentation) were used in the analysis. The effect of stimulus processing was investigated by statistical comparison of mean r-values from the averaged 1 s epochs which preceded and followed the stimulus onset. The number of trials from which these averages were calculated varied from 90 to 165 (in each patient the trials without artifacts were used). The mean prestimulus r-value differed largely in the sample of 180 pairs investigated (highest value +0.78, lowest value -0.37, median +0.20). In single patients, the difference between maximal and minimal r-values was: 0.53 (patient 1); 0.81 (patient 2); 0.84 (patient 3); 1.12 (patient 4); 0.66 (patient 5); 0.98 (patient 6); 0.82 (patient 7); 0.58 (patient 8). Great differences in prestimulus r-values were evident even when they were compared within one pair of loci (mean standard deviations 0.48±0.09; no significant difference in this indicator was found between the patients). Together these data demonstrated that a prestimulus r-value was unstable both in space (when pairs from different locations were investigated) and in time (when consecutive trials from one location pair were investigated). In contrast to this prestimulus instability, the response of the indicator to the increased cognitive demand in the poststimulus epoch showed a high level of determinism. In the subgroup of pairs with the mean prestimulus r-value lower than median, a significant poststimulus increase was found (-0.001±0.15 versus 0.19±0.23; df = 89, $p<0.000001$, t-test for dependent samples). In the subgroup of pairs with the mean prestimulus r-value higher than median, a significant poststimulus decrease was found (0.38±0.13 versus 0.31±0.24; df = 89, $p<0.0023$, t-test for dependent samples). In summary, the results demonstrated a significant modification of inter-regional activity synchronization during the stimulus processing which was differentially dependent on the prestimulus state (a decrease after high initial values, an increase after low initial values).

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EFFECT OF CHRONIC RENAL FAILURE ON NORADRENALINE, ADRENALINE, DOPAMINE, AND NPY LEVELS IN THE RAT HEART AND ARTERIES

J. Kuncová, M. Chottová Dvořáková, J. Švíglerová, M. Štengl, J. Slavíková
Department of Physiology, Faculty of Medicine, Charles University, Plzeň, Czech Republic

Cardiovascular disease is a major cause of morbidity and mortality in chronic renal failure (CRF) and may be explained in part by abnormalities in autonomic regulation (1). Several lines of evidence indicate a presence of the sympathetic overactivity in CRF (2). The aim of our study was to determine whether an increased sympathetic activity is associated with a change in distribution and content of adrenergic neurotransmitters in the heart and arteries. CRF was induced in adult animals by surgical 5/6 nephrectomy and confirmed by increased plasma levels of creatinine and urea. Sham operated rats served as controls. At 10 weeks after operation, sympathetic innervation was analyzed in heart sections by immunohistochemical method and concentrations of noradrenaline (NA), adrenaline (A), dopamine (DA), and neuropeptide Y (NPY), a cotransmitter of NA, were determined in tissue extracts of all heart compartments and large arteries using commercial radioimmunoassay method and expressed in ng/g wet weight. In CRF animals, no change in distribution and density of immunoreactivity for catecholamine-synthesizing enzymes tyrosine hydroxylase and dopamine-β-hydroxylase, and for NPY were revealed. Concentrations of NA were significantly reduced in the left atria, left ventricles, aorta, and femoral artery, whereas no changes were found in the right atria, right ventricles, and mesenteric artery. Dopamine concentrations were significantly decreased in both atria and in the femoral artery; however, no changes were observed in ventricles, aorta, and mesenteric artery. NPY showed significant decrease in concentration in the left ventricle and in all arteries examined. Adrenaline levels were significantly reduced in all heart compartments and also in the femoral artery. In conclusion, since the sympathetic innervation was morphologically intact in CRF, changes in concentrations of neurotransmitters may rather reflect changes in their release or uptake/metabolism.

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ANALYSIS OF SELECTED INFLAMMATORY CYTOKINES IN THE HUMAN ADIPOSE TISSUE: RELATION TO OBESITY, INSULIN RESISTANCE AND SYSTEMIC INFLAMMATION

Z. Lacinová, M. Bártlová, P. Kaválková, M. Mráz, T. Roubíček, D. Michalský[1], M. Kasalický[1], M. Haluzík
3rd Department of Medicine, [1]Department of Surgery, 1st Faculty of Medicine and General University Hospital, Prague, Czech Republic

It is now generally accepted that obesity is associated with subclinical inflammation that might be involved in the etiopathogenesis of insulin resistance. Numerous proinflammatory cytokines such as interleukin-1 (IL-1β), IL-6, IL-8 and tumor necrosis factor-α (TNF-α) are produced also in adipose tissue and in turn directly or indirectly stimulate the production of acute phase proteins such as C-reactive protein (CRP) in the liver. Adipose tissue can also produce IL-10 that inhibits the expression of IL-1 and TNF-α. The aim of our study was to determine mRNA expression of IL-1β, IL-6, IL-8, IL-10 and TNF- α in human adipose tissue (subcutaneous-SAT and visceral-VAT) and to test the hypothesis that subclinical inflammation in adipose tissue could participate in increased circulating CRP levels. In lean women, the mRNA expression for IL-6 and TNF-α were 100times higher than the mRNA expression of IL-1β, 8, 10 in both SAT and VAT. The expression of TNF-α in SAT was significantly higher in obese women compared to controls. The mRNA expression of TNF-α in VAT and IL-6 in both adipose tissue did not differ between the groups. TNF-α mRNA expression in adipose tissue (SAT and VAT) correlated with circulating CRP levels ($p<0.05$). On the contrary, IL-6 mRNA expression in adipose tissue did not correlate with CRP levels. IL-1β and IL-8 mRNA expression in SAT as well as in VAT were 2-3 times higher in morbidly obese women (BMI>40 kg/m^2) compared with the control group, IL-10 mRNA expression in adipose tissue did not differ between the groups. mRNA expression of IL-1β positively correlated with mRNA expression IL-8 in adipose tissue ($p<0,01$) and was not related to IL-10 mRNA expresssion. No significant relationships were found between the circulating levels of CRP and IL-1β, 8 and 10 mRNA expression. Taken together, we did not find a direct relationship between adipose tissue mRNA expression of most of proinflammatory cytokines and CRP concentrations. These results suggest that adipose tissue production of proinflammatory factors is only partially responsible for the development of obesity-associated subclinical inflammation.

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MODULATION OF CALCIUM SIGNALLING IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS BY STEROID HORMONE 1α,25(OH)$_2$D$_3$

I. Lajdova[1], D. Chorvat Jr.[2], A. Chorvatova[3,4], A. Oksa[1], V. Spustova[1]
[1]Slovak Medical University, Bratislava, [2]International Laser Centre, Bratislava, Slovak Republic, [3]Research Centre, CHU Sainte-Justine, University of Montreal, [4]Department of Pediatrics, University of Montreal, Montreal, Canada

The steroid hormone 1α,25(OH)$_2$D$_3$ produces biological responses via both genomic and nongenomic mechanisms. We have examined rapid effects of 1α,25(OH)$_2$D$_3$ on calcium mobilization and calcium entry into resting human peripheral blood mononuclear cells (PBMCs) as well as possible implication of purinergic receptors in this action. Human PBMCs were obtained from heparinized blood samples, obtained of healthy volunteers. Intracellular calcium was measured using Fluo 3-AM fluorescent probe. P2X$_7$ pore function was assessed by ethidium bromide confocal imaging. 1α,25(OH)$_2$D$_3$ induced a time-dependent increase in intracellular calcium concetration ([Ca^{2+}]$_i$). The initial 1α,25(OH)$_2$D$_3$-stimulated calcium increment was sensitive to thapsigargin (Tg), indicating its origin in calcium release from intracellular stores. 2-aminoethyldiphenyl borate (2APB), an inhibitor of capacitative calcium entry, caused significant [Ca^{2+}]$_i$ decrease in human cells treated with 1α,25(OH)$_2$D$_3$. Furthermore, in contrast to

observations in osteoblasts and skeletal muscle cells, nifedipine had no effect on the 1α,25$(OH)_2D_3$-induced calcium entry, suggesting that L-type calcium channels are not implicated in this action. Besides, 1α,25$(OH)_2D_3$ prevented calcium entry induced by 3´-O-(4-benzoylbenzoyl)ATP (BzATP), a specific agonist of $P2X_7$ receptors. The hormone also significantly reduced 4-aminopyridine (4AP) stimulated Ca^{2+} increase via $P2X_7$ channels. This finding was further confirmed by 1α,25$(OH)_2D_3$-induced reduction of BzATP- and of 4AP-stimulated ethidium bromide fluorescence. The presented results demonstrate that 1α,25$(OH)_2D_3$ induces: 1) two-step calcium response through calcium release from internal stores, followed by store refilling via calcium release activated calcium channels, but not L-type calcium channels, 2) nongenomic inhibitory effect on $P2X_7$-induced Ca^{2+} increase, as well as the permeability reduction of large molecules through pore-forming $P2X_7$ receptor.

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EARLY LIFE STRESS INDUCED CELL DYING IN THE RAT ROSTRAL MIGRATORY STREAM

K. Lievajová, E. Račeková, M. Martončíková, J. Orendáčová
Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic

The subventricular zone (SVZ) persists as a germinal zone into adulthood and thus functions as the largest neurogenic region in the adult brain. SVZ cells migrate along the rostral migratory stream (RMS) to the olfactory bulb where they differentiate into local interneurons (1). Along the route of migration a certain number of SVZ-generated cells undergo cell death in the rat brain in physiological conditions (2). Although neurogenesis occurs continuously throughout adulthood, this process may be affected by exogenous factors. In this study, a well-described model of maternal separation (MS) has been used to induce odor deprivation in neonatal rats. Our aim was to investigate the possibility that early life stress of an olfactory nature has an effect on cell dying within the RMS. Rat pups were subjected to MS daily for 3 hours, starting from the first postnatal day (P1) till P14 or P21. Brains were analyzed at the age of P14, P21 and P28, respectively. The controls matched the age of MS animals. For detection of apoptosis we have used a fluorochrome, Fluoro-Jade C, which has been confirmed to detect dying neurons regardless of the cause of cell death (3). Fluorescent microscope analysis revealed the presence of Fluoro-Jade C stained cells both in the RMS of control and MS rats. However, the MS animals showed higher density of labeled cells. Evaluation of the number of Fluoro-Jade C positive cells showed that two weeks of MS did not cause marked changes in dying cells number. Conversely, after three weeks lasting MS (P21 rats) and at P28 animals, which survived one week after three weeks lasting MS, the number of these cells significantly increased in comparison with control rats. These data indicate that an exposure of rats to adverse environmental factors during early postnatal periods may strongly influence survival of newly generated cells within the RMS.

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MOTONEURON'S VULNERABILITY TO SPINAL CORD TRANSECTION

N. Lukáčová, A. Dávidová, A. Schreiberová, Ľ. Capková
Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic

Currently, electrophysiological and anterograde tracing studies have suggested that fibers of ventral medullary gigantocellular reticular nuclei (composed of the gigantocellular ventralis and pars alpha nuclei and the lateral paragigantocellular nucleus (Gi-LPGi) descend in the ventrolateral funiculi and activate inhibitory interneurons in the spinal cord (1). The interneurons may, in turn, exert inhibitory effects onto α-motoneurons, χ-motoneurons and interneurons in transmission of reflex pathways. The aim of this study was to examine the effect of spinal cord transection performed at lower thoracic level on vulnerability of motoneurons in the lumbosacral enlargement of the rat. The fluorescein derivate Fluoro-Jade B was used to detect dying neurons after transection and in control rats. In addition we investigated the neuronal nitric oxide synthase (nNOS) producing nitric oxide and parvalbumin (PV), a member of calcium-binding proteins. After surgical procedure the experimental animals survived two weeks. Fluoro-Jade B, nNOS and PV were assessed by immunohistochemical and fluorescent techniques. The level of nNOS protein was measured by the Western blot analysis. In the control animals, only a few small PV-IR cell bodies were occassionally seen in lamina IX. However, α-motoneurons were PV- and nNOS-immunonegative. In the experimental animals surviving two weeks, both the nNOS-IR and PV-IR were upregulated in neurons localized in lateral part of laminae VII. Fluoro-Jade B positive cells were not visible in the motoneurons. The PV-IR had increased in a number of small neurons in the dorso- and ventrolateral part of the ventral horn. Spinal cord transection followed by 14 days of survival caused the significant increase of the level nNOS protein in lumbosacral segments. The results indicate that PV is not included directly in motor activity, but it may play a role in motor control in the spinal cord.

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POSITIVE ALLOSTERIC MODULATOR OF GABA-B RECEPTORS CGP7930 EXHIBITS AN ANTICONVULSANT ACTION IN IMMATURE RATS

P. Mareš
Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Agonists of GABA-B receptors baclofen and SKF97541 exhibit mixed anti- and proconvulsant action in pentylenetetrazol-induced motor seizure test in immature rats. To modify the influence on these receptors an allosteric modulator CGP7930 (3,5-bis(1,1-dimethylethyl)-4-hydroxy-b,b-dimethyl-benzenepropanol) was studied. Experiments were performed in 7-, 12-, 18-, 25-day-old and adult rats. Animals were pretreated with CGP7930 (freshly solved in dimethylsulfoxide) in doses from 1 to 40 mg/kg i.p. and 15 min later PTZ was administered in a dose of 100 mg/kg s.c. Control animals received dimethylsulfoxide in a volume corresponding to the highest dose of CGP7930. Rats were observed in isolation for 30 min, body temperature of rats in the three youngest groups was maintained by means of a heating pad. Incidence and latency of two types of seizures (minimal clonic and generalized tonic-clonic) were evaluated. CGP7930 suppressed at first the tonic phase of generalized tonic-clonic seizures and higher doses were able to abolish the whole pattern of generalized seizures in all age groups. Minimal clonic seizures that appear under control conditions only in 18-, 25-day-old and adult rats were less affected by than generalized tonic clonic seizures but their latencies were significantly prolonged in all three age groups. Positive allosteric modulator of GABA-B receptors CGP7930 exhibits marked anticonvulsant action at all developmental stages. It is in contrast to GABA-B receptor agonists baclofen and SKF97541 which exhibit mixed anti- and proconvulsant properties. High doses of CGP7930 resulted in clear signs of muscle relaxation (similarly to GABA-B receptor agonists) therefore the action on motor performance should be studied as the next step.

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DAMAGE AND REPAIR IN THE IMMATURE RAT BRAIN AND GLIOMA CELLS IN CULTURES INDUCED BY BORON-NEUTRON-CAPTURE REACTION (BNCR)

V. Mareš[1,4], J. Burian J[2], V. Lisá[1], M. Marek[2], I. Tomandl[4]
[1]Joint Cancer Cell Biology Laboratory of the .Institute of Physiology Academy of Sciences & 1st Medical Faculty, Charles University, Prague, [2]Nuclear Research Institute Řež, plc, [3]Nuclear Physics Institute, Acad. Sci., Řež, [4]University of J. E. Purkinje, Ústí nad Labem, Czech Republic

The BNCR leads to localized lethal damage of cells by alpha particles emitted due to interaction of ^{10}B and thermal neutrons. Concentration of ^{10}B and purity and dose of thermal neutron beam are critical. In this study sodium borocaptate (BSH) or ^{10}B-phenylalanine-fructose complex (BPA-F, both Katchem, Řež) were applied to 1-week-old rats and C6 glioma cells in cultures at different doses and administration routes. Irradiation by epithermal neutrons (LVR-15 reactor in Řež, 8x8x 10^8 n/cm^2, 9 MW, 16.1 cGy/min) followed 90 min later and lasted for 5 min for animals and 30 min for cell cultures. Animals were examined histologically 8 h later. Cell cultures were followed-up intravitally for 196 h. The ^{10}B content was measured by Prompt Gamma Activation. It the brain, the BNCR caused cell death appeared to be proportional to the degree of differentiation of brain regions, being highest in germinative zones of the cerebellum and around the forebrain ventricles. The lethal effect exceeded that of the bare beam 2.5 to 5 times. Damage of cells appeared at critical ^{10}B level 20 μg per g brain weight. This level was *in situ* achieved only by intraventricular administration of BSH or subcutaneous application of BPA-F which was better tolerated at higher doses and more penetrating into the animal brains. Moreover, the latter was found to be enhanced by hyperthermia. In cell cultures treated with BSH or BPA the lethal damage appeared at/since 48 h and was increasing up to196 h. The surviving cells hypertrophied and slowed-down proliferation. The latter was starting earlier (24 h) and lasted longer (196 h). These changes were also several times higher than in cultures irradiated without ^{10}B. In addition, the BNCR inhibited recovery of cell division which re-appeared in cultures irradiated without ^{10}B. Cytochemical examination of surviving cells revealed up-regulation of γ-glutamyltranspeptidase (γ-GT). The study demonstrates biological effectiveness of BNCR provided by LVR-15 reactor epithermal neutron beam as well as conditions for induction of this response via parenteral administration of ^{10}B carrier in our animal model. The up-regulation of γ-GT revealed in cultures may be considered a new defense mechanism which may interfere with therapeutic effects of BNCR.

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EFFECT OF A SINGLE DOSE OF THE NICOTINE ON EVOKED EPILEPTIC SEIZURES IN YOUNG RATS

D. Marešová, V. Riljak
Department of the Physiology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic

Effects of a single dose of the nicotine on excitability of cortical neurones in young rats were studied. Nicotine (1 mg/kg i.p.) or solvent (0.9 % saline solution) were administered 15 min before stimulation of cortical neurones. Stimulation of the right sensorimotor cortex (15 s, bipolar pulses, and intensity 3 – 5 mA) in the freely moving 12 and 25-day-old rats was repeated 5times with interval of 1 min between the end of previous evoked epileptic seizure and the next stimulation. We recorded the duration of evoked epileptic seizures and behaviour of animals. Minimal number of animals for statistical evaluation of results was eight. ANOVA and t-test of GraphPad Software were used. In 12-day-old rats the repeated stimulation of right sensorimotor cortex invoked prolongation of epileptic seizures in comparison with the first (control) seizure ($p<0.001$). After administration of nicotine the prolongation of the first seizure was also observed, however, seizures after the 3rd and 5th stimulation were shorter ($p< 0.001$).In 25-day-old control rats the repeated stimulation of the sensorimotor cortex induced shortening of seizures after the 2nd, 3rd and 4th stimulation ($p< 0.001$). Nicotine administration had only minimal effect on the duration of evoked epileptic seizures. The prolongation only after 3rd stimulation was registered. Maximal behavioural changes (increase of motor activity, body tremor, loss of righting reflex, dyspnoea) lasted up the fifteenth minute. The behaviour was not influenced by the solvent solution. We can conclude that the effect of nicotine administration on evoked epileptic seizure is related to the development of the central nervous system. We registered the decrease of excitation in 12-day-old rats and increase of this process in older rats.

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ONTOGENETIC DEVELOPMENT OF COGNITIVE AND MOTOR FUNCTIONS DURING PROCESS OF OLIVOCEREBELLAR DEGENERATION IN LURCHER MICE

V. Markvartová, J. Cendelín, F. Vožeh
Department of Pathophysiology, Faculty of Medicine in Pilsen, Charles University in Prague, Czech Republic

Lurcher mutant mice represent a natural model of the olivocerebellar degeneration. This degeneration is caused by mutation of the gene for δ2 glutamate receptor. Lurcher mutants suffer from the cerebellar ataxia and cognitive functions deficiency as a consequence of the excitotoxic apoptosis of Purkinje cells in the cerebellar cortex and secondary decrease in granule cells and inferior olivary neurons number. This process is finished until the 90th day of postnatal live, but already in age of 60 days there remains only 1 % of original Purkinje cells number. The aim of our work was to compare the development of motor functions and spatial orientation ability in the course of the ontogenetic development in Lurcher mutant and wild type mice of the B6CBA strain. The mice of age 2, 3, 6, 9, and 22 weeks were used. The examination of their motor skills was performed using the horizontal wire, rotating cylinder, foot bridge and slanting ladder methods. Spatial orientation was tested in the Morris water maze. Lurcher mutant mice reached worse results in both motor and spatial orientation tests than wild type mice. Our research showed that afflicted animals improve their motor ability until the age of 6 weeks. This reality is caused in part by the physiological development of the nervous system and musculature, but undoubtedly also by a certain compensation ability, which is enabled by plasticity of the developing brain. After the 6th week of life, the motor skills of Lurcher mutants decreased rapidly, whereas in wild type mice it stayed constant during the whole observed period. In the development of spatial orientation we observed continuous decrease of this function in Lurcher mutants, while in wild type mice no worsening of this function was detected. It is the question, whether the decrease in these functions, after the end of the degeneration process affecting the cerebellum, is a consequence of the precocious brain aging, or of still unknown secondary CNS changes, which follow the primary degeneration of the cerebellum (1).

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CELL PROLIFERATION IN THE ROSTRAL MIGRATORY STREAM OF RATS AFTER OLFACTORY STIMULATION

M. Martončíková, K. Lievajová, J. Orendáčová, E. Račeková
Institute of Neurobiology Slovak Academy of Sciences, Košice, Slovak Republic

The postnatal central nervous system of mammals retains the potential to generate new neurons. The largest neurogenic region in the postnatal brain is the subventricular zone (SVZ), which lines most of the lateral wall of the lateral ventricles (1). The progenitor cells born in the SVZ migrate along a restricted pathway, called the rostral migratory stream (RMS) to the olfactory bulb (OB), where they differentiate into local interneurons (2,3). The aim of this study was to investigate whether exposure to an odor-enriched environment during the first postnatal weeks affects proliferation of cells in the RMS of young rats. 15 natural or synthetic odorants were used in this experiment. Rats were devided into control and olfactory-stimulated groups in each age category. We created three age categories: P14, P21, P28 (P – postnatal day). Rats in the olfactory-stimulated groups were exposed to different odorants two times per day for one hour for two weeks (P14) or three weeks (P21 and P28). In order to label proliferating cells, the rats in all groups, control and olfactory stimulated, were intraperitonealy injected by single dose of proliferation marker – bromodeoxyuridine (BrdU) in dose 50 mg/kg body weight. Rats were transcardially perfused by fixative solution two hours after BrdU administration.The brain sections from all rats were processed for BrdU immunohistochemistry. To evaluate these sections, images were captured. Proliferating cells were counted in three anatomical parts of the RMS – the vertical arm, elbow and horizontal arm by Disector software (version 2,0) (4) and we compared the number of proliferating cells between control and olfactory-stimulated

groups.We found that olfactory stimulation in the early postnatal period incresed the number of proliferating cells in the RMS of all experimental groups. The highest increase was noticed at P14 animals in the RMS vertical arm, and elbow.

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PROLIFERATION AND APOPTOSIS IN TOOTH GERMS AND RELATED TISSUES DURING PRENATAL ODONTOGENESIS

E. Matalová[1,2], E. Eftimiadisová[1], T. Baňoch[1,2], M. Buchtová[1,2], J. Doubek[2], R. Radlanski[3], I. Míšek[1,2]
[1]Institute of Animal Physiology and Genetics AS CR, v.v.i., Brno, [2]Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, [3]Freie Universität Berlin, Germany

Tooth development is a complex process that involves signalling interplay between the embryonic stomodeal epithelium facing the oral cavity and the underlying neural crest-derived mesenchyme. However, tooth development does not occur as an isolated event and is tightly related to development of other orofacial structures, particularly interdental bone and periodontium. Therefore there is an essential requirement for epithelial-mesenchymal cross-talk for complete craniofacial development. To study physiological synchrony of morphogenetic processes (proliferation and apoptosis) in developing tooth germs and adjacent tissues, paraffin embedded ICR mouse embryonic heads were used. Methods of proliferation (PCNA–immunohistochemistry), apoptosis (TUNEL test), and bone remodelling evaluation (TRAP, AP) were applied to follow development of the first mouse molar from the bud up to late bell stages in context of surrounding ossifying bone tissue. Temporospacial cellular and molecular symphony of prenatal odontogenesis and osteogenesis will be further assembled in 3D models to understand dynamics of tooth osseointegration. The data can be applied with respect to successful surgical procedures of tooth replacement, autologous bone transplantation, and alloplastic bone substitution as well as in emerging molecular dentistry and stem-cell-based tooth replacement followed by tooth-bone integration (1).

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DETECTION OF c-MYB IN DEVELOPING MOLAR TOOTH GERM AND SURROUNDING TISSUES

E. Matalová[1,3], E. Janečková[1,2], P. Vaňhara[3], I. Míšek[1,3], R. Radlanski[4], J. Šmarda[2]
[1]Institute of Animal Physiology and Genetics AS CR, v.v.i., Brno, [2]Institute of Experimental Biology, Masaryk University, Brno, [3]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, [4]Freie Universität Berlin, Germany

The c-Myb transcription factor is well-known as an essential regulator of hematopoiesis but it is also expressed in other tissues, such as smooth muscle and colonic mucosa. The c-*myb* mRNA was reported also in bud staged tooth germs (1). To evaluate temporospatial distribution of the c-Myb protein in embryonic odontogenesis and related osteogenesis, we used the mouse first molar model. Using the Myb-specific monoclonal (hybridoma derived) and polyclonal (Abcam) antibodies, we investigated the *myb* expression in serial frontal sections of paraffin-embedded mouse heads at embryonic days 13.5, 15.5 and 17.5. To visualize c-Myb in histological sections, standard immunohistochemical procedure based on biotinylated secondary antibody/ peroxidase-DAB system and hematoxylin counterstain were used. c-Myb was evaluated in both epithelial and mesenchymal parts of the tooth germ and in the surrounding ossifying tissues. As c-Myb is known not only to inhibit differentiation but also promote proliferation, PCNA (proliferating cell nuclear antigen) was detected at the same stages of molar tooth development and correlated with c-Myb occurrence. Postnatal stages of tooth development and interdental bone ossification are under study and the molecular interplay of c-Myb in signalling pathways during odontogenesis remains to be clarified in functional experiments.

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DIAZOXIDE-INDUCED PROTECTION AGAINST ISCHAEMIA-INDUCED ARRHYTHMIAS IN THE RAT HEART IS NOT DEPENDENT ON PI3K/AKT INHIBITION BY WORTMANNIN

J. Matejíková, D. Pancza, J. Neckář[1], F. Kolář[1], S. Čarnická, T. Ravingerová
Institute for Heart Research & CEKVY, Slovak Academy of Sciences, Bratislava, Slovakia; [1]Institute of Physiology, Academy of Sciences of the Czech Republic and Centre for Cardiovascular Research, Prague, Czech Republic

Mitochondrial K(ATP) channels [mK(ATP)] opener diazoxide (D) was shown to mimick protection against ischaemia/reperfusion (I/R) injury by ischaemic preconditioning (I-PC) and to reduce arrhythmias during ischaemia (1), however, molecular mechanisms of its antiarrhythmic effect are not yet clear. Activation of phosphatidylinositol 3-kinase (PI3K)/Akt occurs during I-PC (2) and is involved in the mechanisms of antiinfarct protection conferred by I-PC (3). PI3K inhibitor wortmannin (W) abrogated both, Akt phosphorylation (2) and infarct size (IS) limitation (3), however, it failed to blunt protection against I-induced arrhythmias in the preconditioned hearts (4). Since both, I-PC and mK(ATP opening share pathways leading to PI3K/Akt activation that further targets mK(ATP) and keeps them in the open state, this study was aimed to investigate the role of PI3K/Akt in the antiarrhythmic protection induced by PC with D (D-PC). Isolated perfused rat hearts exposed to D-PC (15-min perfusion with D prior to I, 50 μM) and non-treated controls (C) were subjected to 30-min LAD coronary artery occlusion with or without 20-min perfusion with W in a concentration (100 nM) that blocked Akt activation during I-PC (2). During I, the total number of premature ventricular complexes (PVC) was significantly lower in the D-PC group than in C (158±20 vs. 551±61 in C; $P<0.05$). The number of episodes of ventricular tachycardia (VTE) in D-PC hearts was also reduced to 2±0.6 as compared with 11±2 in C ($P<0.05$) and VT duration (VTD) was shorter (4.6±1.8 s vs. 41.6±8.1 s in C; $P<0.05$). W did not reverse lower arrhythmogenesis in the D-PC group (PVC 202±41; VTE 1.1±0.5; VTD 6.3±3.7 s; $P>0.05$ vs. D-PC). In contrast, in the protocol of I followed by 2-h R, bracketing of D-PC with W increased IS (TTC staining) by 24.5 %. In conclusion, PI3K/Akt inhibition did not abolish reduced arrhythmogenesis during I in the D-treated hearts indicating that different from its positive role in the irreversible myocardial injury, PI3K/Akt activity is not required for protection against ischaemic arrhythmias induced by mK(ATP) opening in the rat heart.

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TWO MODELS OF CORTICAL ISCHEMIC LESIONS IN LABORATORY RATS

I. Matějovská, K. Bernášková, J. Mareš, O. Raška
Department of Normal, Pathological and Clinical Physiology, Third Faculty of Medicine, Charles University, Prague, Czech Republic

Several models of ischemic lesions of central nervous system exist; in our department two types were established: Rose Bengal technique of photothrombotic occlusion of blood vessels, based on the sensitization of endothelial cells and the subsequent activation of blood coagulation, and cerebral vessels occlusion induced by local application of endothelin, potent vasoconstrictor. Both methods are closely related to the formation of reactive oxygen species (ROS). ROS are also involved in the general mechanisms of neural injury and hypoxic/ischemic preconditioning. In this study, we tested the sensitivity of the photothrombotic and of the endothelin models of focal ischemia to the pre-treatment with the ROS scavenger melatonin. Experiments were performed on adult male Wistar rats. Photothrombotic occlusion was induced by i.v. application of Rose Bengal and subsequent exposition of rats´ scull to a green laser beam (532 nm) for 9 min. Endothelin in the second model (ET-1, 120 pmol solution, 3x1 μl in 6 min) was applied locally on denuded vessels supplying the cerebral cortex. In both models one group served as control and other group was pre-treated by i.p. melatonin 1 hour before exposition to ischemia. All animals were sacrificed 72 hours after the irradiation, the brains were perfused, and 1 mm coronary slices were obtained and stained with TTC (2, 3, 5-triphenyltetrazolium chloride). The developed ischemic lesions were divided into 3 types according to their severity. Results were statistically evaluated by Chi square test. In the control group, the most animals developed deep damage involving the striatum. In the group of animals pre-treated with melatonin mild lesion was observed in most of animals. Chi square test has shown highly significant differences between these groups. Pre-treatment with melatonin significantly reduced the size of brain tissue damage in both two models of focal ischemia. We suppose that the scavenger effect of melatonin influenced primarily the pathological changes in the vascular endothelium and the subsequent blood coagulation during and after ischemia.

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SERUM CONCENTRATIONS AND TISSUE EXPRESSION OF A NOVEL ENDOCRINE REGULATOR FIBROBLAST GROWTH FACTOR-21 IN PATIENTS WITH TYPE 2 DIABETES AND OBESITY

M. Mraz[1], M. Bartlova[1], Z. Lacinova[1], D. Michalsky[2], M. Kasalicky[2], D. Haluzikova[3], M. Matoulek, I. Dostalova[1], V. Humenanska[4] and M. Haluzik[1]
[1]3rd Department of Medicine, [2]Department of Surgery, [3]Department of Sports Medicine, 1st Faculty of Medicine and General University Hospital, Prague and [4]BioVendor, Brno, Czech Republic

Fibroblast growth factor-21 (FGF21) is a novel endocrine and paracrine regulator of metabolic homeostasis. The aim of our study was to measure its serum concentrations in patients with obesity (OB), obesity and type 2 diabetes mellitus (T2DM) and healthy subjects (C) and to assess the changes of its circulating levels and mRNA expression after dietary and pharmacological interventions. We measured biochemical parameters, serum FGF21, adiponectin, leptin and insulin levels by commercial ELISA and RIA kits and mRNA expression in the liver, subcutaneous and visceral fat by RT PCR in 26 OB patients, 11 T2DM patients and 32 control subjects. The interventions were acute hyperinsulinemia during isoglycemic-hyperinsulinemic clamp, very low calorie diet (VLCD) and 3 months treatment with PPAR-α agonist fenofibrate. Baseline serum FGF21 levels were significantly higher in both OB and T2DM patients relative to healthy controls. FGF21 levels in OB did not significantly differ from T2DM group. Both 3 weeks of VLCD and 3 months of fenofibrate treatment significantly increased FGF21 levels. FGF21 mRNA expression in visceral fat was 2-fold higher in OB relative to C group, while it did not differ in subcutaneous fat. VLCD significantly increased FGF21 mRNA expression in subcutaneous fat of OB. 3-hour hyperinsulinemia during the clamp increased FGF21 levels in T2DM but not in C group. We conclude that an increase in FGF21 levels after VLCD and fenofibrate treatment may contribute to positive metabolic effect of these interventions and suggests the possibility of direct positive metabolic effects of FGF21 in humans.

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THE EFFECT OF CAPTOPRIL ON PARAMETERS OF OXIDATIVE PHOSPHORYLATION AND MEMBRANE FLUIDITY IN MITOCHONDRIA FROM HEARTS AND KIDNEYS OF SPONTANEOUSLY HYPERTENSIVE RATS

J. Mujkošová, M. Ferko, O. Uličná[1], J. Fischerová[2], I. Waczulíková[2], J. Slezák, B. Ziegelhőffer[3], A. Ziegelhőffer
Institute for Heart Research., SAS, [1]Pharmacobiochemical Laboratory of Medical Faculty, Comen. Uni., [2]Division of Biomedical Physics, FMPhI Comen Uni, all Bratislava, Slovak Republic, [3]Heart Center of Leipzig, Uni. Leipzig, Germany

Primary hypertension (HYP) leads to alterations in function of the heart, kidney, brain etc. and influences the function of their subcellular organels including the mitochondria (MIT). However, since yet there are still little data available about the HYP-induced changes in function of heart and kidney MIT, particularly when the disease is treated with ACE inhibitor captopril (CAP). Our aims were to monitor the efficacy of CAP in lowering blood pressure (BP) in spontaneously hypertensive rats (SHR), and to investigate the effect of CAP treatment on parameters of oxidative phosphorylation (OXP) and membrane fluidity (MF) of isolated heart and kidney MIT. Animals (16-weeks-old males) were randomly divided into 3 groups: 1) normotensive Wistar rats; 2) untreated SHR rats; 3) SHR rats treated daily with captopril (20 mg/250 g per os, for 4 weeks). Non-invasive measurement of BP on the 1st, 14th and 28th day; Isolation of MIT by means of differential centrifugation combined with protease treatment (Sigma P 6141; 2.5 mg/g of minced tissue); Detection of MF in the MIT by utilizing the fluorescent probe 1,6-difenyl-1,3,5-hexatriene; Estimation of parameters of OXP by means of the Clarc oxygen electrode. Heart: HYP heart MIT exhibited elevated O_2 consumption in the presence of added ADP (S3) and also in absence of added ADP (S4), the respiratory control index (RCI) i.e., S3/S4 and also the rate of oxidative phosphorylation (OPR) in the presence of glutamate and succinate (all $p<0.01$-0.05). Treatment with CAP normalized most deviations. The efficacy of OXP, termed as the ADP:O ratio, remained unchanged in all groups of animals. Kidney: In contrary to heart, O_2 consumption was increased more in S3 than in S4, consequently RCI and OPR decreased significantly ($p<0.01$ and 0.05) for both substrates. CAP was capable to normalize RCI, but failed to prevent further decrease in OPR. None of changes in ADP:O was significant. In conclusions, HYP increased the RCI and OPR in the heart MIT only. In kidney MIT CAP normalized RCI but failed in preventing the depression of OPR. Treatment with CAP allways normalizes the BP effectively and trends to increase MF of MIT in both in heart as well as in kidney.

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PHARMACOKINETIC OF THE FLAVONOID OSAJIN AFTER ORAL ADMINISTRATION

J. Nečas[1], L. Bartošíková[1], T. Bartošík[2], P. Fráňa[3], M. Pavlík[2]
[1]Department of Physiology, Faculty of Medicine and Dentistry, Palacký University Olomouc; [2]Department of Anaesthesiology and Intensive Care, [3]II[nd] Internal Clinic, St. Anne´s University Hospital Brno, Czech Republic

Flavonoid osajin was isolated from the infructences of *Maclura pomifera*, Moraceae. The aim of the study was to determine some pharmacokinetic parameters after single oral administration of osajin. After sample preparation the HPLC system consisted of gradient HPLC pump Knauer 64 equipped with a LCD 2084 UV detector was used. The wavelength was set on 273 nm for osajin determination. The mobile phase consisted of water:acetonitrile, 15:85 (v.v). Isocratic flow was maintained at 1.3 ml/min. Data from analysis were collected and analyzed with the CSW software. The quantification of the flavonoid was achieved from areas of its peaks by comparison with calibration curves obtained using standard solution of individual flavonoids in blank serum. For determination of pharmacokinetic parameters program Kinetica 4.0 was used. The maximum plasma osajin concentration was 76±1.0 μg/L; time to maximum plasma concentration was 40 min; $AUC_{0\rightarrow last}$ was 5776 μg/min/l; elimination half-life was 189±30 min; constant of elimination was 0.003757 min^{-1}; MRT was 249±36 min.

IMMUNOHISTOCHEMICAL STUDY OF ANTIOXIDANT ENZYMES AND APOPTOTIC PROTEINS INCIDENCE IN THE POSTISCHEMIC RAT HIPPOCAMPUS AFTER POSTCONDITIONING

M. Némethová, V. Danielisová, M. Gottlieb, P. Kravčuková, J. Burda
Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovakia

Aim of this study was to evaluate effect of postconditioning on immunoreactivity of antioxidant enzymes (CuZnSOD and MnSOD) and apoptotic proteins (Bcl-2 and Bax) ensuing after ischemic attack and 3-day reperfusion and to compare changes in the selectively vulnerable CA1 region and relatively resistant dentate gyrus of rat hippocampus. Based on our previous studies devoted to protective effects of ischemic tolerance (1, 2, 3, 4), we used delayed, protein synthesis dependent, postconditioning, which means that a short sublethal ischemia was applied 2 days after previous severe ischemic insult as a repeated stress. Transient cerebral ischemia triggers oxidative stress and cells are equipped with effective antioxidant defense mechanisms. Delayed cell death that occurs over several days after transient global ischemia has many features of apoptosis. In the nervous system, Bcl-2 family members are important determinants of neuronal survival during development as well as in response to brain injury and they belong to major molecular signaling pathways regulating apoptosis. In our study we observed increase of both SODs-immunoreactivity and postconditioning caused distinctly higher immunoreactivity in CA1 than in dentate gyrus. A Bcl-2 immunopositive cells number was increased after ischemia and after using postconditioning it was decreased mainly in CA1. In dentate gyrus there were different situation, Bcl-2-immunoreactivity increase was not so abrupt after ischemia and remain approximately equal values after using postconditioning. Bax-immunoreactivity in CA1 and dentate gyrus was increased after ischemia and the postconditioning decreased values to the control level. So, we can conclude that in our experiment effect of postconditioning consisted in suppression of apoptosis triggering Bax protein expression. Moreover postconditioning could increase the immunoreactivity of both antioxidant enzymes in comparison to ischemia/reperfusion only.

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THE EFFECTS OF COLD STRESS ON THE HEART: CAN BETA3 ADRENOCEPTORS SUBSTITUTE THE ROLE OF M_2 MUSCARINIC RECEPTOR?

M. Novakova[1], R. Kvetnansky[2], J. Myslivecek[1]
[1]Inst. of Physiology, 1st Fac. Med., Charles University, Prague, Czech Republic, [2]Inst. of Exp. Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia

Beta3-adrenoceptors (β_3-AR) was identified to produce a negative inotropic effect in ventricles. We investigated the role of β_3-AR in cold stress (1 or 7 days in cold, average temperature 7.82 °C, light/dark 12/12 h) in male mice (weighting 20-25 g, 11-13 weeks old) lacking main cardioinhibititve receptors – M_2 muscarinic receptors (M_2KO). Radioligand binding studies were performed to assess the properties of β_1-, β_2-, β_3-AR, α_1-AR and MR. The gene expression of catecholamine synthesizing enzyme (TH, tyrosine hydroxylase, DBH, dopamine β-hydroxylase, PNMT, phenyletanolamine N-methyl transferase) were investigated in adrenal medulla using RT-PCR. We have found that there was no difference in enzyme gene expression in WT in comparison to KO animals sugesting similar reaction to stress in the WT and KO adrenal medulla. The receptor densities were not changed in the right ventricles. In the left ventricles, there was decrease in all cardiostimulative receptors and increase in cardiodepressive receptors in intact KO animals in comparison to WT: while cardiostimulative β_1-, β_2-AR were decreased (to 52 % and to 55 %), and cardiostimulative α_1-AR were decreased to 64 %, the cardioinhibitive β_3-AR were increased to 205 % of control. Moreover, the binding to MR in M_2 KO animals was not completely abolished confirming our previous data about the presence of minor muscarinic population in the heart (1). The cold stress in WT animals resulted in decrease in β_1- and β_2-AR (to 37 %/35 % after one day in cold and to 27 %/28 % after 7 days in cold). On the other hand, β_3-AR were increased (to 216 % of control) when 7 days cold was applied. MR were reduced to 46 % and to 58 %, respectively. The effects of cold stress on the heart receptors in KO animals were as follows: the reaction of cardiostimulative β_1- and β_2-AR to cold was similar in KO animals to that in WT animals. In contrast to cardioinhibitive receptors in wild types, β_3-AR in KO animals did not changed in reaction to cold. Our results suggest compensative role of β_3-AR in KO animals.

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RELIABILITY OF STROKE VOLUME DETERMINATION BY THE BIOIMPEDANCE METHOD

Z. Nováková, J. Moudr, B. Fišer, N. Honzíková
Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Stroke volume (SV) can be non-invasively determined by the bioimpedance method. Also, the pulse pressure (PP) non-invasively measured by Finapres is related to SV. A positive correlation is expected between SV and the preceding cardiac interval (CI_{i-1}) because of the filling period and the Starling mechanism and because of the time course of restitution of myocardial contractility. To estimate the reliability of the bioimpedance method studied, the relationship between CI_{i-1} and SV and the relationship between CI_{i-1} and PP was the aim of the present study. We examined 9 healthy subjects (age: 19-21 years, body mass index: 20.1±1.4 kg/m^2) lying in supine position in rest condition, metronome-controlled breathing at a frequency of 20/min. We recorded continuously beat-to-beat systolic and diastolic blood pressures (BP) by the Peňáz method (Finapres, Ohmeda, USA). We used an electrical bioimpedance amplifier EBI 100C (BIOPAC Systems, Inc., USA) for cardiac output measurement by the thoracic electrical bioimpedance method. Systolic and diastolic BP, bioimpedance, and the magnitude of the largest impedance change during systole (dZ/dt) were recorded. This data was processed by computer in the MATLAB system; PP and SV were assessed. In eight subjects we found a significant positive correlation between SV and CI_{i-1} (correlation coefficient r=0.32-0.56, $p<0.01$; r=0.17-0.21, $p<0.05$), in four subjects a positive significant correlation was detected between CI_{i-1} and PP (r=0.31-0.43, $p<0.01$; r=0.16-0.24, $p<0.05$). We proposed to determine the relationship between CI_{i-1} and SV as a test for reliability of SV bioimpedance method measurement. To estimate the reliability of stroke volume measured by means of the bioimpedance method the relationship between the preceding cardiac interval and stroke volume should be determined.

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STEREOLOGICAL QUANTIFICATION OF EPENDYMAL PROGENITORS AFTER SPINAL CORD INJURY OR ENHANCED PHYSICAL ACTIVITY

I. Novotna[1], L. Slovinska[1], M. Cizek[2], S. Flesarova[2], M. Nagyova[1], J. Rosocha[3], J. Radonak[4], J. Hlucilova[5], S. Juhas[5], I. Vanicky[1], D. Cizkova[1]
[1]Institute of Neurobiology, Slovak Academy of Sciences, Kosice, [2]University of Veterinary Medicine, Kosice, [3]Associated Tissue Culture Bank, Medical Faculty, University of PJ Safarik, Kosice, [4]II Surgical Clinic, University of PJ Safarik, Kosice, Slovakia, [5]Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Libechov, Czech Republic

Ependymal cells (EC) in the spinal cord central canal (CC) are believed to be responsible for the postnatal neurogenesis following pathological or stimulatory conditions (1). In present study we have analyzed the proliferation of the CC ependymal progenitors in adult rats processed to compression SCI (2) or enhanced physical activity. To label dividing

cells, a single daily injection of Bromo-deoxyuridine (BrdU) was administered over a 14 days survival period (3). Systematic quantification of BrdU positive ependymal progenitors was performed by using stereological principles of systematic random sampling and optical Dissector software (4). The number of proliferating BrdU labeled EC increased gradually with the time of survival after both paradigms, spinal cord injury or increased physical activity. In the spinal cord injury group we have found 8.2-fold (7 days) and 11.3-fold (14 days) increase of proliferating EC in the rostro-caudal regions, 3 mm away from the epicenter. Furthermore, the distant cervical spinal cord segments revealed 2 to 3-fold increase of EC for both time-points analyzed. In the second group subjected to enhanced physical activity by running wheel, we have observed 1.8 fold increase of dividing EC in the lumbar and 3.2 fold increase in the cervical spinal cord segments at 7 days, but no significant progression at 14 days. This data demonstrates that SCI or enhanced physical activity in adult rats induces an endogenous ependymal cell response leading to increased proliferation of neural progenitors, which may be beneficial for recovery of motor function.

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POSTNATAL NEUROGENESIS OF RATS INFLUENCED BY ELECTROSTRESS

J. Orendáčová, M. Orendáč[1], J. Labun[2], E. Račeková, K. Saganová, M. Mojžiš[1], M. Martončíková, K. Lievajová, H. Abdiová[3]
Institute of Neurobiology, CE, SAS, Šoltésovej 4, Košice, [1]Faculty of Electrical Engineering and Informatics, TU Košice, [2]Faculty of Aeronautics, TU Košice, [3]Secondary School for Medical Assistants, Košice, Slovakia

One of the most studied region of postnatal neurogenesis is the subventricular zone (SVZ), which harbours steadily dividing stem cells and progenitors, which migrate along a distinct pathway the rostral migratory stream (RMS), to populate the olfactory bulb and establish connections with their neuronal targets (1). Possible risk of the EMF for nervous tissue is regularly published from the middle of 20th century (2). The current discussion is centered on whether low-level exposure to the EMF evokes neuropathological responses. The pulsed EMF at frequency 2.45 GHz and average power density between 2 and 6.7 mW/cm^2 represented electrostress for neurogenesis. Proliferating cells marker, BrdU, was used to map dose-related immunohistochemical changes within the rostral migratory stream (RMS) after whole-body exposure of newborn (P8) rats. Two dose-related exposure patterns were performed to clarify a cumulative effect of the EMF: the short-term exposure dose, 2 days irradiation (4 h/day) versus long-term exposure dose, 3 days irradiation (8 h/day), both followed by acute (24 h) post-irradiation survival. We found that the EMF, representative of electrostress, induces significant dose- dependent significant changes of proliferating cells number within the RMS. Three sets of the mentioned EMR doses induced significant decrease of proliferating cell number within the RMS. On the other hand the same lower doses of the EMR applied in two sets induced increase of proliferating cells numbers. Our results indicate that concerns about the possible risk of the EMF, generated in connections with production, transmission, distribution and use of electric energy equipment and communication sets, are on the spot at least in relation with early postnatal neurogenesis.

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EFFECT OF SELENIUM ON THE IMMATURE RAT CARDIAC MUSCLE

I. Ošťádalová, Z. Charvátová, *J. Wilhelm, B. Ošťádal
*Centre for Cardiovascular Research, Institute of Physiology, Academy of Sciences of the Czech Republic, *Department of Medical Chemistry and Biochemistry, Charles University Second Medical School, Prague, Czech Republic*

Selenium (Se) is an essential trace micronutrient with antioxidant function. Its effect is dose- and age-dependent and may be significantly modulated by other factors, like chemical form and tissue content of heavy metals. Reactive oxygen species (ROS) contribute to ischemia/reperfusion (I/R) injury in the adult myocardium, their role in the developing heart is, however, not satisfactorily clarified. The aim of the present study was, therefore, to find out whether administration of Se will protect immature heart against I/R. In chronic experiments, control pregnant rats were fed standard laboratory diet (0.237 mg Se/kg diet) and water ad libitum; experimental females were supplemented with 2 ppm Na_2SeO_3 in the drinking water from the first day of pregnancy until day 10 *post partum*. The isolated hearts of 10-day-old offsprings were perfused (Langendorff) with Krebs-Henseleit solution at constant pressure, temperature and heart rate. Recovery of developed force (DF) was measured by an isometric force transducer after 40 min of global ischemia. In acute experiments, 10-day-old hearts were perfused with selenium (Se concentration in Krebs-Henseleit solution was 75 nmol/l) before or after global ischemia. ROS status was evaluated by lipofuscin like pigments (LFP) accumulation. Chronic supplementation of Se decreased significantly LFP concentration in the heart and simultaneously increased tolerance of the immature heart to global ischemia (expressed as the recovery of DF). Similar results were obtained after acute administration of Se during postischemic reperfusion. On the other hand, the preischemic treatment with Se attenuated significantly the recovery. It may be concluded that Se protects the already highly tolerant immature heart against ischemia, both after chronic and acute administration, most likely by its antioxidative action. This suggests that ROS produced in the immature heart may affect its function of the neonatal heart.

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THE ROLE OF TNF-α AND TRPV1 RECEPTORS IN MODULATION OF NOCICEPTIVE TRANSMISSION AT THE SPINAL CORD LEVEL

J. Paleček, M. Růžičková and D. Špicarová
Department of Functional Morphology, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague

Tumor necrosis factor-α (TNF-α) is one of cytokines that has been long identified to be an important factor in number of pathological processes including pain. The role of TNF-α on the periphery was studied especially in neuropathic pain syndromes. However, cytokines were recently also identified as possible neuromodulators of synaptic transmission in the CNS. It was shown that its application can lead to increased number of surface AMPA receptors and increased synaptic strength. Transient receptor potential vanilloid (TRPV1) receptors are considered to be integrators of noxious chemical and physical stimuli. The TRPV1 receptors are expressed on both the peripheral and central branches of a subpopulation of dorsal root ganglion (DRG) neurons that convey nociceptive information from the periphery to the dorsal horn (DH) of the spinal cord. We have recently demonstrated, that spinal cord TRPV1 receptors have an important role on nociceptive transmission especially when phosphorylated under pathological conditions. In our experiments we have used behavioral experiments and patch clamp recordings from identified dorsal horn neurons in acute spinal cord slices to study possible cooperativity between these receptors in modulation of nociceptive transmission. Intrathecal TNF-α application induced robust thermal hyperlagesia but did not affect responsiveness to mechanical stimuli. The thermal hyperlagesia was attenuated by TRPV1 antagonist application (BCTC). In the patch clamp recordings, slice incubation with TNF-α increased amplitude of the recorded AMPA mEPSCs and increased sensitivity to endogenous TRPV1 agonist *N*-oleoyldopamine (OLDA). Our results demonstrate

that TNF-α has an important role in modulation of nociceptive synaptic transmission a the spinal cord level and suggest that it can also alter the function of presynaptic TRPV1 receptors. Spinal cord TNF-α and its appropriate receptors together with TRPV1 receptors represent a clear potential target for analgesic therapy.

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RELATIONSHIP BETWEEN RENAL FUNCTION IN TYPE 2 DIABETIC PATIENTS WITH DIABETIC NEPHROPATHY AND ADIPONECTIN

D. Petrášová[1], A. Galovičová[2], I. Bertková[1] , M. Tajtáková[2]
[1]Institute of Experimental Medicine and [2]1st Internal clinic Faculty of Medicine University P.J.Šafarik, Košice, Slovak Republic

Adiponectin is insulin sensiting hormone with antiatherogenic and antiinflammatory effects. Recent clinical studies have reported that plasma adiponectin levels are lower in type 2 diabetic patients and hypoadiponectinemia is a risk factor for macrovascular disease (1). The role of adiponectin in the pathogenesis of diabetic nephropathy remains unclear. The aim of the study was to explore possibly relationship between serum levels of adiponectin and clinical markers of renal disease in patients with diabetic nephropathy. In our project we have examined 50 patients (30 men, 20 women) with DM type 2 of the mean age 57,04 years with different stages of diabetic nephropathy. Patients were divided into two groups with normoalbuminuria (n=31) was defined as urinary albumin <30 mg/24 hours and microalbuminuria as urinary albumin excretion 30-299 mg/24 hours (n=19). We determined serum adiponectin levels and selected biochemical and functional parameters /urea, creatinine, lipid profile, GFR, HbA1c, C peptid/. Sampling was performed at the same time intervals, fasting. The serum was stored at the temperature of -20°C until its analysing. Adiponectin concentrations in the serum of normoalbuminuria group ranged from 7,53 (4,27–12,89) ug/ml and adiponectin levels in microalbuminuria group were 10,37 (3,23–20,364) ug/ml. Results of recent clinical studies indicate that renal insufficiency possibly play some role in increases in serum and urinary adiponectin levels in overt diabetic nephropathy as a microvascular disease compared to early stages of diabetic nephropathy or to those without nephropathy (2). There is a similar report showing that plasma adiponectin levels are markedly increased in patients with nephrotic syndrome relative to patients with chronic nephropathies but without nephrotic syndrome and healthly subjets (3). Increase in circulating adiponectin in overt diabetic nephropathy might be a physiological response to mitigate renal tubular injury and to prevent the further progression of diabetic nephropathy through its antiinflammatory and anti-atherogenic effects.

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PHASING OF CIRCADIAN RHYTHMS ALONG THE RAT GUT

L. Polidarová[1], M. Soták[2], M. Sládek[1], J. Pácha[2] and A. Sumová[1]
Departments of [1]Neurohumoral Regulations and [2]Epithelial Physiology, Institute of Physiology v.v.i., Academy of Sciences of the Czech Republic, Prague

In mammals, circadian rhythms are controlled by central oscillator located in the suprachiasmatic nuclei of the anterior hypothalamus and numerous oscillators located in peripheral organs. The basic molecular core clock mechanism responsible for generating circadian rhythms in the central and peripheral clocks is composed of transcriptional/translational feedback loops between the clock genes and their protein products. Recently, circadian clocks in the rat and mice digestive system were discovered. The aim of the study was to determine daily rhythms in expression of clock genes within the rat duodenum, jejunum, ileum and distal colon to ascertain whether circadian clocks within the individual parts of the gut are synchronized, or whether there is a difference in their circadian phases. Next aim was to analyze the circadian phases of putative clock-driven rhythms in the expression of the cell cycle regulator *Wee1* in the duodenum and the distal colon. Adult rats were maintained under regime with 12 h of light and 12 h of darkness (LD 12:12). On the day of the experiment, rats were released into constant darkness and sampled every 4 h throughout the whole circadian cycle. In the separate experiment, rats were sampled every 1 h during the light phase of the LD cycle. Daily expression profiles of clock genes *Per1*, *Per2*, *Bmal1*, *Rev-erb-α*, *Clock*, *Cry1* and clock-controlled gene *Wee1* were examined by real-time RT-PCR quantification within epithelium of the duodenum, jejunum, ileum and distal colon. *Per1*, *Per2*, *Bmal1*, *Rev-erb-α* genes were expressed rhythmically in all studied parts of the gut. The rhythms in gene expression exhibited differences in their phases, such that the rhythm in duodenum was phase-advanced to the colon. Also, daily profile of *Wee1* expression was rhythmic and showed similar differences in phasing of its expression between the upper and lower part of the gut. Our data demonstrate that individual parts of the gastrointestinal tract have their own circadian clock and these clocks are synchronized with a phase-delay along the cranio-caudal axis. Moreover, they support the view that the individual circadian clocks may control the timing of cell cycle within different regions of the gut.

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INFLUENCE OF ENVIRONMENTAL CUES ON POSTNATAL NEUROGENESIS IN THE RAT OLFACTORY SYSTEM

E. Račeková, M. Martončíková, K. Lievajová, S. Flešárová[1], A. Raček[1], J. Orendáčová
Institute of Neurobiology, Slovak Academy of Sciences, Košice, [1]University of Veterinary Medicine, Košice, Slovak Republic

The olfactory bulb (OB) is the first relay on the olfactory sensory pathway and the target for neuronal progenitors generated in the subventricular zone (SVZ) of adult rodents. Cells born in the SVZ proceed towards the OB along a concise pathway, called the rostral migratory stream (RMS). Within the OB they mature into two main types of interneurons, which are integrated into functional brain networks (1). It is well established that survival of newborn neurons in the OB depends on sensory input (2). However, little is known about environmental cues that may influence the cells in the RMS, which are destined to become OB interneurons. In our experiments, we have investigated the effect of different exogenous factors such as ionizing radiation, odor deprivation or odor-enriched environment on neurogenesis in the RMS of adult and neonatal rats. Irradiation of adult rats with a single dose of 3 Gy of gamma rays as well as daily odor deprivation of pups during the first three weeks of life negatively influenced cell proliferation within the RMS. Radiation induced alteration of proliferation activity showed a biphasic course characteristic by an early inhibition (1st day after the exposure) and later marked stimulation (5th and 10th days after the exposure) of cell proliferation in the RMS. Odor deprivation caused acute site-specific decrease of dividing cells number at three weeks old rats. With reduction of proliferating cell in odor deprived animals, the number of dying cells significantly increased. Contrary, enriched environment in neonatal rats increased the number of proliferating cells and concurrently decreased the number of dying cells within the RMS. These data suggest that the process of neurogenesis in the neurogenic pathway of the neonatal and adult rat olfactory system may be significantly influenced by both stressful experiences and enriched environment.

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LIMINAL OLFACTORY PERCEPTION

T. Radil
Institute of Physiology, Academy of Science of the Czech Republic, Prague

There exists a certain range of weak olfactory stimuli, which could be labeled „liminal" that can be correctly identified by guessing, although the subjects are not aware of them. The experiments were performed in the following way: First the corresponding thresholds were estimated using ascending binary forced choice methods. The threshold concentration of stimuli and a certain number of higher and lower concentrations steps were presented with a blank, each ten times in random order. The task of the subject was to detect which of two sequential "sniffing bottles" contained the odor (amyl acetate, butyric acid, butanol), relative to blank. In each experiment, after every trial, subjects marked on a continuous scale whether they were certain or guessing. The results revealed that at threshold, or closely below threshold concentrations, the subjects chose the stimuli correctly, while their statements were mostly based on guessing and not on conscious perception. Similar results have been obtained in experiments with odor discrimination. The findings point to the significance of unconscious processing of odorants at below-threshold concentrations. These processes might play role in distant chemical communication and in processing chemical signals.

CARDIOPROTECTIVE EFFECTS OF OSMOTIC PRECONDITIONING IN THE RAT HEART: RELEVANCE TO ENHANCED RESISTANCE TO ISHAEMIC INJURY IN THE DIABETIC MYOCARDIUM

T. Ravingerová, J. Galčíková, J. Matejíková, D. Pancza, J. Čarnická, V. Štrbák[1]
Institute for Heart Research, CEKVY; [1]Institute of Experimental Endocrinology, CENDO, Slovak Academy of Sciences, Bratislava, Slovakia

Hyperglycaemia (HG) represents one of the factors that determine the outcome of ischaemia-reperfusion (I/R) injury, and both, detrimental (1) and beneficial (2) effects of HG on the response to I/R in the diabetic heart have been reported. Hyperosmotic stress induced by HG has been also shown to mimick diabetic state (3) and to exert protective effects in the non-diabetic cardiomyocytes. Changes in cell volume (shrinkage and swelling) underlie both, cell death and survival mechanisms related to I/R, therefore, our study was aimed to investigate the effects of short-term elevation of osmolarity by HG (osmotic preconditioning, O-PC) on susceptibility to I/R injury in the rat heart. Since PI3K/Akt cascade plays a role in cell survival, and its activation by HG accounts for an enhanced ischaemic tolerance in the diabetic heart (4), our further goal was to clarify the role of PI3K/Akt in the mechanisms of O-PC. Langendorff-perfused rat hearts were subjected to 30-min global ischaemia and 2-h reperfusion without or with prior O-PC induced by 5-min HG challenge (glucose 22 mM, osmolarity 325 mOsm/l) followed by 10-min isosmotic perfusion (glucose 5.5 mM, osmolarity 290 mOsm/l). A specific PI3K inhibitor wortmannin (100 nM) was applied 20 min prior to I/R in both protocols. Short osmotic load induced by HG and subsequent relative hyposmotic stress improved postischaemic functional recovery (LVDP 44±7 % of preischaemic values vs. 23±3 % in controls; $P<0.05$). Moreover, the size of myocardial infarction (IS; TTC staining) expressed in % of left ventricle (LV) area was significantly reduced from 42±3 % in controls to 21±2 % ($P<0.05$). Bracketing of O-PC with W completely reversed improved LVDP recovery (24±6 %; $P>0.05$ vs. C) and increased the size of infarction (IS/LV 29±2 %; $P<0.05$ vs. non-treated hearts). In conclusion, changes in cellular osmotic load caused by HG may trigger protection against subsequent I/R. PI3K/Akt activation appears to be a potential mechanism mediating cardioprotective effects of O-PC. The results may contribute to understanding of the mechanisms of reduced sensitivity to I/R injury in the diabetic heart.

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CHARACTERIZATION OF MYELIN AND NEURONAL CELLS CHANGES AFTER BRAIN EDEMA INDUCTION USING BLACK GOLD AND FLUORO-JADE B STAINING

V. Riljak, *P. Kozler, J. Pokorný
*Institute of Physiology, First Faculty of Medicine, Charles University in Prague and *Department of Neurosurgery, First Faculty of Medicine, Central Military Hospital, Charles University in Prague*

The aim of our study was to test the hypothesis, whether brain edema affects myelin structure and/or leads to the extinction of neuronal cells. Fifteen experimental rats were equally divided into three groups. First group received 2 ml of 20 % mannitol (1098 mosmol/l). Manitol was administered (under deep thiopental anesthesia) trough the catheter into the right internal carotid artery (1). Second group was hyperhydrated with distilled water i.p. in quantities equal to 15 % of animal's body weight. The total amount of water was divided into three doses given in intervals of 8 h. Third group was the control group, without any treatment. One week after the induction of edema, animals were fixed by transcardial perfusion and Vibratome sections were stained using Fluoro-Jade B to reveal neuronal degeneration and Black Gold for detection of myelin changes. Analysis of myelin staining confirmed the hypothesis, that brain edema significantly disturbs myelin structure. Fibers in several brain regions exhibited edematous swellings, fragmentation and pallor characteristic. Fluoro-Jade B staining revealed in some cases neuronal degeneration mainly in the hippocampus. These data lead us to the conclusion that regardless the method of induction, brain edema results in selective neuronal death and unevenly intensive impairment of axonal integrity.

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THE ASSESSMENT OF MINOCYCLINE NEUROPROTECTION FOLLOWING EXPERIMENTAL SPINAL CORD INJURY IN THE RAT

K. Saganová, J. Orendáčová, D. Čížková, I. Vanický
Institute of Neurobiology, Center of Excellence, Slovak Academy of Sciences, Košice, Slovak Republic.

Minocycline (MC), a second-generation tetracycline, is anti-apoptic and anti-inflammatory agent that reportedly provides neuroprotection following CNS injury (1). Minocycline penetrates blood brain barrier (BBB) and its action can target the multiple processes involved in secondary tissue injury. MC affects proinflammatory cytokine expression, inhibits a caspase-1 and caspase-3 gene expression and cytochrome-c release from mitochondria. Inhibition of activation of inflammatory microglia, microglia/macrophages and neutrophils was observed as well. The objective of this study was to examine the neuroprotective effects of short and long-term MC administration following balloon-compression spinal cord injury (SCI) (2). Rats subjected to SCI were treated with MC for 1 day (1DMC group) or 5 days (5DMC group), or placebo. The effects of MC treatment on locomotor recovery (Basso-Bettie-Bresnahan scale) and spinal cord white and gray matter sparing were evaluated within the thoracic spinal cord for up to 28 days following SCI. Our results show, that while MC treatment spared spinal cord white and gray matter rostral to the lesion epicenter in both, 1DMC and 5DMC groups, significant sparing of white and gray matter areas was not observed caudal to the traumatic lesion. In addition, MC treatment had no effect on final locomotor recovery. Minocycline has a high oral bioavailability, superior BBB penetration and is well tolerated by humans (1); examination of its efficiency is critical in relation to CNS injury. MC can protect neurons, oligodendrocytes and whitte matter structures, neuroprotection was approved by improving of behavioral and histopathological outcome in different models of CNS injury. However, limited improvement of spinal cord post-compression consequences in our study raises questions

about the neuroprotection efficiency of MC treatment following compression SCI in the rat.

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POLYMORPHISMS IN GENES PARTICIPATING IN TESTOSTERONE METABOLISM IN AUTISTIC PATIENTS

E. Schmidtová, S. Kelemenová, P. Celec[1], D. Ostatníková
Comenius University, Faculty of Medicine, Institute of Physiology, Bratislava, [1]Comenius University, Faculty of Medicine, Institute of Pathophysiology, Bratislava, Slovakia

Autism is concerned when a child or adult has abnormalities in a triad of behavioural domains: social development, communication, and repetitive behaviours. This can be explained by Baron-Cohen's (1) theory which proposed the hyper masculine brain in autistic individuals. The higher testosterone levels in prenatal development lead to formation of hyper androgenic brain. The aim of our study was to observe free salivary testosterone levels in 70 autistic boys in comparison with control group of children and to investigate genetic factors participating in metabolism of testosterone. Androgen receptor (AR) polymorphism could indicate its sensitivity to testosterone in the tissues with expressed AR. Decreased number of $(CAG)_n$ repeats in androgen receptor is associated with stronger androgen effect. We suppose that the effect of testosterone could be influenced also by other types of polymorphisms in genes, which products participate on steroid hormone metabolism, e.g.: aromatase gene (CYP19), 5α-reductase gene (SRD5A2), estrogen receptor gene (ESR1). DNA samples were isolated from buccal cells in saliva and subsequently DNA was amplified by PCR. The *CYP19* C^{1558}-T, *SRD5A2* A49T and ESR1 polymorphisms were determined by RFLP analysis and the *AR* $(CAG)_n$ polymorphism was determined by fragment analysis. Salivary testosterone levels were measured with ELISA analysis. We have found higher testosterone levels in autistic children in comparison with children from general population in the pubertal age. Autistic children have lower number of CAG repeats but the differences between autistic and control groups of children were non significant. Our findings of higher testosterone levels in individuals with autism point to the involvement of sex hormones in autism. Testosterone could contribute to long-lasting changes in the brain architecture and modulate brain activity during development.

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EFFECT OF VIBRATION ON THE DISTRIBUTION OF bNOS AND PARVALBUMIN IN THE LUMBOSACRAL DORSAL ROOT GANGLIA IN RAT

A. Schreiberová, D. Kolesár[1], A. Dávidová, N. Lukáčová, S. Jergová
Institute of Neurobiology, Slovak Academy of Sciences, Košice, [1]Institute of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovak Republic

Excessive exposure of occupational vibration may cause the neuromuscular dysfunction (1), usually show in hand-arm vibration syndrome (HAVS), such as Raynaud´s phenomenon and carpal tunnel syndrome (2). Immunohistochemical and histochemical methods were used to map the distribution and morphological patterns of brain nitric oxide synthase-immunoreactive (bNOS-IR), nicotinamide adenine dinucleotide phosphate diaphorase positive (NADPHd+) and parvalbumin- immunoreactive (PV-IR) structures in the lumbosacral spinal cord segments especially in the dorsal funiculi and in the corresponding dorsal root ganglia (DRG) in rat under physiological conditions and after short-term vibrations. The vibration effect was induced by using the specially adjust grinder (frequency in the range 50-60 Hz) with attach platform. The hind-limb and tail of the animal were laying on platform and they were vibrated. The duration of the repeated short-term vibrations was 5 days for 30 min. We found many PV-IR pericarya of different sizes in all of the lumbosacral DRG in the control group. On the other hand, bNOS-IR and NADPHd+ neurons constituted very little population of ganglionic cells. In the spinal cord gray matter bNOS-IR, NADPHd+ and PV-IR neurons and/or dots were observed predominantly in the dorsal horn (lamine I-II) and intermediate zone (lamina X). Many thick PV-IR axons ran through massive medial bundle of the dorsal root and proceed in the dorsal funiculi preferentially near the dorsomedial border of the dorsal horn. There was no significant change in the bNOS-IR, NADPHd+ and PV-IR, we detected only moderate decrease of bNOS-IR in the spinal cord structures after short-term vibrations. We assume that processing and conduction of mechanoreceptive and proprioceptive impulses from the peripheral target tissues is particularly modulated by PV in contrast to nitric oxide. Our findings point out that repeated short-term vibrations (5 days for 30 min) partly influence the retrograde axoplasmic transport of above mentioned proteins in the lumbosacral spinal cord structures and corresponding DRG.

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DOES PRENATAL METHAMPHETAMINE EXPOSURE AFFECT BEHAVIOR OF ADULT MALE RATS AFTER ACUTE APPLICATION OF THE SAME DRUG?

B. Schutová, L. Hrubá, R. Šlamberová, M. Pometlová, R. Rokyta
Charles University in Prague, Third Faculty of Medicine, Department of Normal, Pathological and Clinical Physiology, Prague, Czech Republic

Methamphetamine (MA) is a drug causing potent psychomotor activation. Moreover, intermittent administration of MA and other psychostimulants was shown to induce behavioral sensitization. Other studies demonstrated that psychostimulants alter behavior in both rats and humans; specifically they induce aggressive behaviors and impair social interaction. Our previous study showed anxiogenic effect of acute MA 1 mg/kg in the test of social interaction in adult male rats. The aim of the present study was: (1) to assess the effect of prenatal and acute MA administration on behavior in adult male rats and (2) to find out if the prenatal exposure to MA increases sensitivity to acute MA application in adulthood. Behavior of adult male rats prenatally exposed to MA (5 mg/kg) or saline was tested in Open field (OF) and Elevated plus maze (EPM). Half of the animals were injected with MA (1 mg/kg) subcutaneously 30 minutes before the testing. Locomotion, exploratory, comforting behaviors and anxiety were evaluated in OF while anxiety and exploratory behavior were assessed in EPM. We found that acute MA increased locomotion and exploratory behavior but decreased comforting behavior in OF. However, while the locomotion was increased to the similar extent in both prenatally treated groups, acute MA application increased exploration in rats prenatally exposed to MA, only. Further, acute MA application decreased anxiety in OF as well as in EPM. Our present study demonstrates that acute MA increases overall psychomotor activity, as was shown by increased locomotion and exploration. In addition, unlike the previous study which showed increased anxiety to foreign animal after acute MA application this study shows that the same dose of acute MA decreases anxiety to novel environment. Finally, to further support our hypothesis that prenatal MA exposure increases sensitivity to drugs in adulthood, we plan studies investigating the levels of dopamine in the brain in rats after prenatal MA exposure.

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ROLE OF GENOMIC BIOMARKERS IN PROSTATE CANCER PROGNOSIS

M. Sivoňová[1], I. Waczulíková[2], T. Matáková[1], D. Dobrota[1], J. Kliment jr.[3], M. Tajtáková[4], A. Pidaničová[4] and J. Kliment[3]
[1]Department of Medical Biochemistry, Comenius University, Jessenius School of Medicine, Martin, [2]Department of Nuclear Physics and Biophysics, Division of Biomedical Physics, Comenius University, Faculty of Mathematics, Physics, and Informatics, Bratislava,

[3]Department of Urology, Comenius University, Jessenius School of Medicine and University Hospital, Martin, [4]1st Department of Internal Medicine, Safarikiensis University, Košice, Slovakia

Variation in prostate cancer incidence between different racial groups has been well documented, for which genetic polymorphisms are hypothesized to be an explanation. Genes involved in the androgen metabolism cascade have been identified as possible candidates for genetic influences in prostate cancer. One of them is prostate-specific antigen (*PSA*) gene. A single nucleotide polymorphism with guanine (G) to adenine (A) substitution is identified at position -158 in the ARE-I of the *PSA* gene. Previous studies suggest that this polymorphism may be associated with higher PSA levels and increase prostate cancer risk. It has been suggested that polymorphisms in glutathione-*S*-transferases (GSTT1, GSTM1 and GSTP1) could predispose to prostate cancer through a heritable deficiency in detoxification pathways for environmental carcinogens. We evaluated the association between polymorphisms in the PSA, GSTT1, GSTM1, GSTP1 genes and genetic susceptibility to prostate cancer in Slovak men. GSTs and PSA polymorphisms were determined by the PCR and PCR-RFLP analysis using DNA from peripheral blood samples. Our findings suggest that *GSTP1* (Val/Val) genotype may affect the risk of prostate cancer and tumor aggressiveness. Further, *GSTM1* null and *GSTT1* null genotypes did not appear to influence the susceptibility to prostate cancer. The *PSA* polymorphisms may be associated with the risk of prostate cancer and disease progression, and may also be related to the serum PSA level with prostate cancer.

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PREVENTION OF CIVILIZATION DISEASES BY LIFESTYLE

J. Slavíček, O. Kittnar, G. E. Fraser[1], E. Medová, R. Žižka[2], J. Konečná[2], A. Dohnalová
Institute of Physiology, First Medical Faculty, Charles University, Prague, Czech Republic, [1]Center for Health Research, School of Public Health, Loma Linda University, California, U.S.A., [2]Country Life International, Nenačovice, Czech Republic

The morbidity and mortality of civilization diseases is high in developed countries. The lifestyle is capable to decrease it to 50 per cent (1). The aim of the present study was to measure the parameters of some risk factors before and after a one week NEW START rehabilitative retreat. 1 855 volunteers, 420 men and 1 435 women mean age 51±14.5 (SD) years participated in 44 rehabilitative retreats from 1999-2008 in the Czech Republic, using a low-fat, low-energy, lacto-ovo vegetarian diet and exercise, in a stress-free environment. Body weight, height, BMI, blood pressure, heart rate, serum cholesterol and blood glucose were measured. Body weight decreased in 1 703 measured persons from 71.8±14.77 (SD) to 71.0±14.45 kg ($p<0.0001$), BMI (1 355 measured persons) from 25.3±4.75 to 25.1±4.61 kg/m^2 ($p<0.0001$), systolic blood pressure (1 688 persons) from 130.0±22.78 to 124.4±21.35 mmHg ($p<0.0001$), diastolic blood pressure (1 680 persons) from 79.7±12.16 to 78.0±11.03 mmHg ($p<0.0001$), heart rate (1 582) from 72.6±11.74 to 71.5±11.71. min^{-1} ($p<0.0002$), serum cholesterol (1 446 persons) from 4.87±0.93 to 4.35±0.74 mM ($p<0.0001$), blood glucose (604 persons) from 4.33±1.61 to 3.87±1.30 mM ($p<0.0001$). The parameters were nonsignificantly changed one year after finishing the retreat in the sample of 68 persons showing the positive effect of retreats. Our results showed, that the intake of a low-fat, low-energy diet, over the course of one week in a stress-free environment, decreased the risk factors of diseases.

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MAGNETIC SEPARATION OF OLIGODENDROGLIAL PROGENITORS ISOLATED FROM RAT SPINAL CORD

L. Slovinska[1], M. Cizek[2], S. Flesarova[2] M. Nagyova[1], I. Novotna[1], J. Rosocha[3], J. Radonak[4], J. Hlucilova[5], S. Juhas[5], I. Vanicky[1], D. Cizkova[1]
[1]Institute of Neurobiology, Slovak Academy of Sciences, Kosice, [2]University of Veterinary Medicine, Kosice, [3]Associated Tissue Culture Bank, Medical Faculty, University of PJ Safarik, Kosice, [4]II Surgical Clinic, University of PJ Safarik, Kosice, Slovakia, [5]Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Libechov, Czech Republic

The fetal, neonatal as well as adult rat spinal cord harbors a pool of neural progenitors, which may be easily isolated and used to replace neuronal cell loss, or remyelinate damaged axons following various neurodegenerative disorders (1, 2, 3). In present study we have used magnetic cell sorting (MACS) technology for the isolation of oligodendroglial cell population from fetal (F16) rat spinal cord. Target cells were separated by positive selection, using specific A2B5 MicroBeads labeled antibody, achieving optimal recovery and high purity of pro-oligodendroglial cells. Based on immunocytochemical analyses for oligodendroglial developmental markers (A2B5, NG2, RIP, and MBP) we were able to characterize and quantify oligodendroglial progenitors (OGPs) and mature oligodendroglial cells in: i) unseparated (whole) population of neural progenitor (NP), or in ii) separated MACS A2B5 MicroBeads labeled neural progenitors. Our results showed that MACS technology purified OGPs from heterogeneous population of spinal NP, by selection of A2B5 positive population resulted in a 58-61 % of mature oligodendrocytes content (MBP, RIP positive) in comparison to 6-12 % of oligodendroglial cells isolated from un-separated spinal progenitors. In addition, the separated OGPs, could be cultured in vitro for several < 9 passages, remaining high number of newly formed spheres, as well as high expansion potential. These experiments indicate that MACS technology could be used as an efficient tool for isolation and purification of an abundant pool of oligodendroglial lineage from fetal neural tissue, which may be used in future trials for spinal cord injury therapy, by producing oligodendroglial implants providing a growth-permissive substrate for regenerating axons, or supporting the remyelinization and bridging the lesion cavity.

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MODEL OF DORSAL HORN NEURONS SENSITIZATION USING CALCIUM IMAGING IN RAT SPINAL CORD SLICES *IN VITRO*

D. Sojka and J. Palecek
Department of Functional Morphology, Insitute. of Physiology, Academy of Scences. of the Czech Republic, Prague, Czech Republic

Chronic pain is often characterized by increased sensitivity to noxious (hyperalgesia) and innocuous (allodynia) stimuli. It is thought, that one of the underlying mechanisms is a long-term modulation of synaptic transmission at the spinal cord level - central sensitization of spinal dorsal horn neurons. The aim of our study was to develop an *in vitro* model of the central sensitization. Acute spinal cord slices (360μm) with spinal roots attached, prepared from rats 12-15 days old were used in this study. The slices were incubated with Fluo-4 AM dye. Intracellular calcium concentration changes were recorded with Leica LSM confocal microscope. Activity of the neurons was evaluated as relative fluorescence change evoked by electrical stimulation of the dorsal rootlet. Control stimulation (10 Hz, 1 s duration, 0.5 ms pulse length) was applied with 3 minute interval between repetition. The sensitization protocol consisted of 100 Hz pulse train (1 s duration, 0.5 ms pulse length) or capsaicin (0.2 μM, 3 min) application. Control stimulation resulted in increased calcium concentration in number of dorsal horn neurons. The cells were confirmed as neurons by using *in vivo* glial cells specific marker sulforhodamine 101. Repeated control stimulations induced consistent changes of the intracellular calcium concentration. Delay of the calcium response, sensitivity to AMPA and NMDA receptor antagonists (CNQX, MK801) and to Ca2+ store depletor caffeine allowed to distinguish two types of responses. The first type was potentiated by caffeine, suppressed by CNQX and MK801 coapplication and its calcium peak onset followed immediately after the stimulation. The second type of calcium response was highly reduced by caffeine application and the somatic calcium peak onset was delayed 3 to 8 s after the stimulation. Our preliminary data show that

both sensitization protocols induced a significant increase of the maximal calcium concentration change in a population of neurons, when compared to the responses evoked by the control stimulation before the sensitization stimulation. These results suggest that this in vitro model could be a useful tool to study molecular mechanisms of central sensitization, also with relation to the role of internal calcium stores in this process and to test possible new pharmacological approaches for chronic pain therapy.

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ELECTRODERMAL DIMENSIONAL COMPLEXITY - NON-LINEAR AND STATISTICAL ANALYSIS-BASED EVIDENCE

M. Svetlak, P. Bob, M. Cernik, M. Kukleta
Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

The extent to which a neural system is dynamically segregated to small subsets of the system that tend to behave independently is expressed by neural complexity. EEG studies reveal that the neural complexity measure could discriminate between different mental states. The main question of current investigation was if neural complexity calculated from electrodermal activity (EDA), which typically reflects the activity within the sympathetic axis of the autonomic nervous system and is closely linked to emotional arousal and stress, could discriminate between different mental states as well. In the present study we have carried out electrophysiological measurement of the bilateral electrodermal activity (EDA) performed during three experimental conditions (resting state, non-conflict Stroop and conflict Stroop tasks) in 106 university students (68 males and 38 females; mean age 23.4, SD=1.4; BMI 22.9, SD=2.73). Description of neural complexity was performed using a pointwise correlation dimension (PD2)-calculated EDA record. Statistical analysis revealed that EDA in the non-conflict and conflict Stroop conditions on both hands is significantly higher than the values in the rest conditions (Wilcoxon pair test, $p<0.000001$) on the one hand and that there is no significant difference between PD2 values of non-conflict and conflict Stroop conditions and rest conditions bilaterally (Wilcoxon pair test, $p>0.05$) on the other hand. The results show that mean EDA values increased under the Stroop task condition in each participant bilaterally. Contrary to this, PD2 values under the Stroop task condition increased only in 74 participants on the left hand (24 decreases and 7 did not react) and 71 participants on the right hand (27 decreases and 8 did not react). Asymmetries in electrodermal dimensional complexity PD2 was observed in 21 students. The analysis showed that there is a significant difference in the value of PD2 in the rest conditions on both hands (Mann-Whitney *U* test; $p<0.001$) between the groups of participants whose PD2 value decreased under the experimental conditions compared to participants with increasing PD2. The results support the hypothesis that neural complexity calculated from (EDA) discriminates between different mental states. Regression of PD2 values to the mean under the experimental conditions supports the notion of the existence of a state of optimal complexity, and the idea that normal information processing requires intermediate levels of dynamical complexity. This study represents further evidence that the application of non-linear analysis to physiological signals can supply valuable information beyond that provided by traditional investigation.

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EFFECTS OF PENTYLENETETRAZOL ON CORTICAL EXCITABILITY IN IMMATURE RATS

E. Szczurowska, D. Chepurnova, J. Otáhal, H. Kubová, P. Mareš
Institute of Physiology, Academy of Sciences of the Czech Republic, Prague

Our previous experiments demonstrated that GABA-A receptor competitive antagonist bicuculline does not significantly influence cortical epileptic afterdischarges (ADs) in immature rats (Tabashidze and Mareš – in preparation). In the present series of experiments we used pentylenetetrazol (PTZ), convulsant drug acting at other binding sites of GABA-A receptor, to study further a role of GABA-A receptors in cortical ADs. Experiments were performed in 12-, 18- and 25-day-old rats with implanted cortical stimulation and recording electrodes. ADs were repeatedly (six times with the 10-min intervals between subsequent stimulations) elicited by suprathreshold rhythmic electrical stimulation of sensorimotor cortical area, PTZ (in doses of 10 or 20 mg/kg i.p.) was administered after the first AD and duration of ADs and motor phenomena were evaluated. To have comparison with the effect of PTZ on nonepileptic phenomena, interhemispheric responses elicited by single and paired pulses were also studied under the influence of PTZ (10, 20 or 40 mg/kg i.p.). Each animal was its own control in evoked potential experiments – responses were evoked under control conditions, then PTZ was injected and the whole stimulation procedure was repeated. Amplitude of the first two components of interhemispheric responses (i.e. monosynaptic transcallosal responses) was measured and input-output curves (intensity of stimuli-amplitude of evoked potentials) for single responses and excitability cycles for paired-pulse responses were constructed. PTZ prolonged ADs in all three age groups studied, the effects were most pronounced in 12-day-old rats where both doses of PTZ led to significant changes. Only the higher dose of PTZ resulted in significant increase of ADs duration in 18-day-old rats of all five postdrug ADs. Significant effects in 25-day-old rats were only sparse. Evoked potentials demonstrated marked effect of PTZ on amplitude of single interhemispheric responses in all three age groups – input-output curves were steeper than in controls. Age differences were not so marked as with ADs. Paired-pulse responses were also affected by PTZ in all age groups. Effects of PTZ on cortical epileptic ADs substantially differ from those of bicuculline. It might be explained either by an importance of the site of action of individual drugs or by an additional action of PTZ not connected with GABA-A receptor complex.

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CHANGES IN BEHAVIOR AND IN HIPPOCAMPAL NEUROGENESIS IN PRENATALY IRRADIATED RATS

B. Šmajda, L. Tomášová
P.J.Šafárik University, Faculty of Science, Institute of Biology and Ecology, Department of Animal Physiology, Košice, Slovak Republic

For the effects of external noxes the embryonal development represents the most sensitive period of the ontogenesis. It has been established, that already very low doses of ionizing radiation, applied during the sensitive period of the embryonal development of the CNS can markedly alter the normal development of the nervous system (1). The aim of this work was to assess the effects of irradiation with a very low dose during the intrauterinal development on the behavioral parameters of adult rats and to study the possible correlations of these effects with adult neurogenesis in hippocampus of these animals. Female Wistar rats were irradiated on the 16th day of pregnancy with a 1 Gy dose of gamma-rays. The offspring of both sexes of irradiated, as well as non-irradiated mothers aged 3 months was tested in Morris' water maze (spatial memory test), in open field test and in elevated plus-maze. The behavior of the animals was recorded using a digital video camera and evaluated by a computer software (Panlab, Barcelona). The prenatal irradiation caused statistically significant short-term memory impairment (in the test repeated after one hour), expressed as prolonged time and longer swimming track for finding the hidden platform, whereas the long-term memory (in test repeated after 24 hours) was not affected. The open field test was performed during 5 consecutive days. The basal locomotor activity and the duration of the comfortable behavior (cleaning) were higher in irradiated animals of both sexes in comparison with controls. In elevated plus maze a shorter stay of irradiated animals in the open arms of the maze was observed. The level of neurogenesis in hippocampus was determined immuno-histochemically after administration of bromdeoxyuridine (BrdU) using the method of two-monoclonal antibodies (2). The BrdU incorporation in new cells was not changed in irradiated animals. Our results suggest a possible impairment of short-term memory in adult rats as a consequence of their irradiation during the embryonal development. These findings did not correlate with changes in the intensity of adult neurogenesis in hippocampus.

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THE ROLE OF ENDOGENOUS TRPV1 AGONISTS IN SYNAPTIC TRANSMISSION IN THE SPINAL CORD DORSAL HORN

D. Špicarová[1], E. Uchytilová[1,2], J. Paleček[1]
[1]Department of Functional Morphology, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, [2]Institute of Clinical and Experimental Medicine, Prague, Czech Republic

Transient receptor potential vanilloid (TRPV1) receptors are considered to be integrators of noxious chemical and physical stimuli. The TRPV1 receptors are expressed on both the peripheral and central branches of a subpopulation of dorsal root ganglion neurons that convey nociceptive information from the periphery to the spinal cord dorsal horn (DH) neurons. In our experiments the modulation of synaptic transmission by endogenous TRPV1 agonist *N*-oleoyldopamine (OLDA) in the spinal cord dorsal horn (DH) was evaluated by recording the miniature excitatory postsynaptic currents (mEPSCs) in acute rat spinal cord slices. Intrathecal application of TRPV1 antagonist was used in behavioral experiments. High concentration OLDA (10 μM) solution was needed to increase the mEPSC frequency, while low concentration OLDA (0.2 μM) did not evoke any change under control conditions. The increase was blocked by TRPV1 antagonists SB366791 or BCTC. Application of the low concentration of OLDA evoked an increase in mEPSCs frequency after activation of PKC, bradykinin application or in slices from animals with peripheral inflammation. Increasing the bath temperature from 24 ºC to 34 ºC enhanced the basal mEPSC frequency, but the magnitude of changes in the mEPSCs frequency induced by OLDA administration was similar at both temperatures. Intrathecal SB366791 application prevented thermal hyperalgesia in the model of surgical pain. Our results suggest that endogenous agonists of TRPV1 receptors could have a considerable impact on synaptic transmission at DH of the spinal cord, especially when TRPV1 receptors are sensitized.

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THE HEART RATE CHANGES ON LIGHT-DARK CYCLE DURING LONG-TERM HYPOVENTILATION-INDUCED ASPHYXIA AFTER PRECONDITIONING IN WISTAR RATS

P. Švorc, R. Benačka[1], R. Galanová, I. Bačová, P. Švorc Jr., I. Bračoková
Department of Physiology, [1]Department of Patophysiology, Medical Faculty Safarik University, Kosice, Slovak Republic

The effect of hypoxic preconditioning is relatively less studied as the ischemic preconditioning. Most of available information on hypoxic preconditioning has come especially from the *in vitro* studies but we do not have any informations about the effect of hypoventilation-induced asphyxia in *in vivo* conditions and in the circadian dependence. We hypothesized that circadian changes in the autonomous nervous system activity can play also the important role at preconditioning-induced cardioprotection. Thus, the aim of our study was to obtain information concerning chronophysiological aspect of heart rate changes during hypoventilation-induced asphyxia after preconditioning by the brief asphyxic cycles in *in vivo* experiments. The preconditioning was induced by 1 – 3 cycles (1PC–3PC) of asphyxia (5 min. of artificial hypoventilation V_T=0,5ml/100g, 20breaths/min.) and reoxygenation (5min. of artificial normoventilation V_T=1ml/100g, 40breaths/min.) on heart rate (HR) in the anesthetized Wistar rats (ketamine/xylazine 100mg/15mg/kg, i.m., open chest experiments) after the adaptation on light-dark cycle (LD cycle) 12 : 12h. The HR was decreased in the 1. minute of the long-term hypoventilation in all experimental groups and in the both light parts of the day vs. the last HR value from the single cycles of preconditioning. In the light part of the day, the HR increased gradually with the duration of hypoventilation until the 10.–11. minute in all experimental groups and with the followed stabilization in the control, 1PC and 2PC groups to the end of asphyxic period. In the dark part of the day, the HR was stabilized in the course of the whole period of asphyxia in all experimental groups. From the start to the 10. min. of hypoventilation, the preservation of the statistically significant LD differences was found in control group ($p<0,001$), 1PC group ($p<0,01$), 2PC group ($p<0,002$) and 3PC group ($p<0,03$). The next HR increase and the lost of the LD dependence were seen only in the 3PC group. Our results show to 1. HR changes, depends on the number of the preconditioned cycles and also on the LD cycle during long-term hypoventilation-induced systemic asphyxia, 2. preconditioning by one or two brief asphyxic cycles probably does not any influence on LD fluctuation in the HR, 3. only in the light part of the rat regime day and only after 3 cycle of asphyxia, the HR changes can be probably in the link with the formation of the cardioprotection.

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CHRONOPHYSIOLOGICAL ASPECTS OF THE CHANGES OF HEART-RATE VARIABILITY IN THE REOXYGENATION PROCESS IN WISTAR RATS

P. Švorc Jr, I. Bačová, S. Grešová, E. Švorcová[1]
Department of Physiology, Medical Faculty, Šafarik University, [1]Department of Hematology and Oncohematology Faculty Hospital L. Pasteur, Košice, Slovak Republic

The aim of this study was to evaluate the effect of reoxygenation after an apnoic episode on some basic heart-rate variability parameters in dependence on the light-dark cycle (LD cycle). The experiments were performed in ketamine/xylazine anaesthesia in female Wistar rats (10mg/kg + 15mg/kg, i.m., open chest experiments). The experiments were performed in female Wistar rats after adaptation to the LD cycle of 12:12h with the dark part from 18.00 to 06.00h. The animals were artificial ventilated by respiratory pump at ventilatory parameters: 1 ml/100g of b.w. and respiratory rate 40-50 breaths/min. The apnoic episodes was simulated by the swich off respirator for 2 minutes. RR interval and HF, LF and VLF components were evaluated in the intact animal during spontaneous breathing and before the surgical interventions and ventilatory manoeuvres, after surgical interventions and 5 min. of normal artificial ventilation and in the 5., 10., 15., 20. min. of reoxygenation after apnoic episode. In the intact animals, the statistically significant LD differences were detected in all followed parameters (RR interval duration, $p<0,001$; HF component, $p<0,001$; LF component, $p<0,001$ as well as VLF component, $p<0,02$) with the higher values in the light part of the day. Surgery interventions and 5 min. of stabilization preserved LD differences only in the RR interval duration ($p<0,001$) and in LF component ($p<0,003$). LD differences in RR interval duration were not found during the whole period of reoxygenation. The RR interval increased gradually in the dependence on the duration of reoxygenation in the both lighted phases. HF component showed similar behavior than RR interval but with the preservation of LD differences. Changes in the LF and VLF components did not correspond with the RR interval changes. Their courses were different in the light part compared to the dark part of the rat regime day. It is concluded that ketamine/xylazine anaesthesia increases the parasympathetic and decreases sympathetic tone probably by the same manner in the both lighted phases. The changes in RR interval refer to dominant role of the parasympathetic division of the autonomous nervous system with the modulation influences of the baroreflex activity and sympathetic division in the dependence on LD cycle during long-term reoxygenation after the apnoic episode.

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HEART MITOCHONDRIA AND LONG-TERM OXYGEN THERAPY IN GUINEA PIGS

Z. Tatarkova, A. Calkovska[1], I. Engler[2], A. Drgova, M. Chomova, P. Kaplan, J. Lehotsky, D. Dobrota
Department of Medical Biochemistry, Jessenius Faculty of Medicine, Martin, [1]Department of Physiology, Jessenius Faculty of Medicine, Martin, [2]Department of Physiology, Medical Faculty, Kosice, Slovakia

Molecular oxygen (O2mol) is biological electron carrier, and basic material for its reactive forms. The oxygen cascade relies on the decrease in its tension that occurs from the inhaled air to the

intracellular tension found in cell mitochondria. These are one of major sources of free radicals and also an important target for their damaging effects. The mechanisms, leading to differences in the response of the biological system to different oxygen forms, are not known. Therefore, in the present study we examined the effect of long-term (17 h and 60 h) inhalation of three different oxygen forms on heart functions. Lipid peroxidation in mitochondrial membranes, measured by conjugated dienes and free MDA formation was increased progressively after inhalation of partially negatively ionized oxygen (O2neg). NADH-producing enzyme, ketoglutarate dehydrogenase was sensitive to both, O2mol and O2neg. The activity of complex I of respiratory chain was decreased after exposure to all three oxygen forms. In comparison with this enzyme, O2mol had positive effect on complex IV activity. The same results we observed with partially positively ionized oxygen (O2pos), but protein level for subunit 1 of this enzyme complex was higher in O2neg group against O2mol and O2pos groups. Compared to control (air group) there was negative effect of O2neg in protein dityrosines, lysine conjugates formation and protein sulfhydryl group content, too. Our results suggest that O2neg is strong radical with negative effects on heart mitochondria and on the other side O2pos was protective. Function of mitochondria was impaired after 60 h inhalation of O2mol, accompanied with higher level of oxidative stress. Finally, prolonged inhalation of O2mol, according to our study, is not safe for normal heart functioning.

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EFFECT OF ATP ON THE SENSITIVITY OF THE CARDIAC RYANODINE RECEPTOR AND ITS IMPACT ON KINETICS WITH DIFFERNET LUMINAL Ca^{2+} AND CYTOSOLIC Ca^{2+}

B. Tencerová, A. Zahradníková, M. Gaburjáková
Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia

Ryanodine receptor (RyR2) is a calcium-activated, calcium-permeable channel of the sarcoplasmic reticulum (SR) that mediates excitation-contraction coupling in cardiac muscle cells. There is growing evidence that Ca^{2+} in the lumen of the SR can be effectively involved in different aspects of RYR2 channel regulation (1, 2). To contribute to a deeper understanding of the mechanism by which luminal Ca^{2+} regulates the RyR2 channel it was interesting to investigate its effect on the response of RyR2 channel to different activators (3). In this study, we tested whether luminal Ca^{2+} exerts any effect on the sensitivity of the RyR2 channel to adenosine triphosphate (ATP), a well-known activator of the RyR2 channel with potential physiological importance. We used the method of reconstitution of RyR2 channels from rat cardiac microsomes into planar lipid membranes. Single-channel currents were recorded at 0 mV potential and under voltage-clamp conditions in asymmetric *trans*/*cis* environment. We found out that elevation of the luminal Ca^{2+} concentration enhanced both, the potency (EC50) and the efficacy (Pmax) of ATP as activator of the RyR2 channel. Effects on EC50 were already saturated at 1 mM luminal Ca^{2+}, which means that a physiological concentration of luminal Ca^{2+} (1 mM) was able to induce similar changes in the EC50 as higher concentrations of luminal Ca^{2+}. Importantly, elevation of cytosolic Ca^{2+} at 1 mM luminal Ca^{2+} was significant and similarly, like luminal Ca^{2+}, enhanced Pmax. The changes on kinetic parameters in the presence of luminal Ca^{2+} were not significant, but in the absence of luminal Ca^{2+} the average dwell times were short and the frequency of openings was significantly increased. The changes of RyR2 P_O by ATP in 1 mM luminal Ca^{2+}, as well as the changes of RyR2 P_O by luminal Ca^{2+} in the presence of 1 mM ATP were significant and may contribute to regulation of diastolic Ca^{2+} spark frequency. In summary, we can note that the effect of ATP on RyR2 channel is dependent on experimental conditions and ATP is an important co-activator.

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CHANGES OF NOCICEPTIVE SOMATOTOPY DURING ONTOGENESIS IN RATS

Š. Vaculín, M. Franěk, A. Yamamotová
Charles University in Prague, 3rd Faculty of Medicine, Dpt. of Normal, Pathological and Clinical Physiology, Prague, Czech Republic

The effect of age on pain thresholds has not been fully explained in both human and animal research. Several studies described pain threshold increase in older adults compared to young adults in men, however, reduction of noxious inhibitory system with opposite effect was revealed during aging too. The aim of the present study was to determine nociceptive threshold in rats during their lifetime. Nociceptive thresholds were evaluated using paw withdrawal latency to thermal and mechanical stimulation, and tail withdrawal latency to thermal stimulation. The thresholds were evaluated on postnatal day 7, 16, 23 (weaning), and at the age of 1, 2, 3, 6, 9, 12, 15 and 18 months in forelimbs, hinds and tail. As far as thermal stimulation is concerned, we could observe oscillation of withdrawal latencies. Nociceptive thresholds were lowest at PD 7 and then increased to maximum at weaning period. Since the age of 1 month the thresholds remained unchanged but with two peaks of decrease – at the age of 2 and 9 months. The oscillations were expressed most in tail withdrawal latency and least in forelimb withdrawal latency. Regarding nociceptive somatotopy (different withdrawal latency of fore, hind limb and tail), three types were found as follows: (1) in infants the longest latency was in forepaw and shortest in tail, (2) in weaned animals the longest latency was in tail and shortest in forepaw, and (3) in adults the longest latency was in hind limb and shortest in forepaw. No increase of nociceptive threshold was seen during aging. Concerning mechanical stimulation, withdrawal pressure increased until weaning period and remained unchanged afterwards with decrease in the oldest groups (at the age of 15 and 18 months). It is suggested that nociceptive threshold depends on age until the age of 1 month, while in adult animals the withdrawal latency to thermal stimulation depends rather on external factors like season, air temperature and/or humidity. In the oldest groups moderate decrease of withdrawal pressure might suggest relative importance of inhibitory pain system deficit.

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EFFECT OF SIMULATED SHIFT WORK SCHEDULE ON CARDIOVASCULAR PARAMETERS AND CIRCADIAN PROFILE OF MELATONIN IN PLASMA AND DIFFERENT ORGANS OF RATS

M. Zeman, A. Veselá, L. Molčan, M. Beňová, I. Herichová
Department of Animal Physiology and Ethology, Faculty of Sciences, Comenius University Bratislava, Slovakia

Night shift work has been suggested as one of risk factors for development of civilization diseases, including cardiovascular diseases and cancer. In our study we exposed young male Wistar rats (6 weeks at the beginning of experiment) to a light:dark (LD) schedule simulating shift work on short rotation program (2 days light from 6.00–8.00, 2 days from 14.00–02.00 and 3 days from 20.00–4.00). Control rats were kept under a stabile LD 12.12, lights on 06.00–18.00, food and water *ad libitum*. Body weight, blood pressure (BP) and heart rate (HR) were recorded weekly, on day, when both groups were exposed to light from 06.00 till 18.00. After 10 weeks, both groups of rats (36 animals/group) were killed over 24 hour in 4 hour intervals. Sampling was performed when both groups were exposed to light from 06.00 till 18.00. Samples of different organs (the heart, kidney and pineal gland) were frozen and stored at -80 ^{o}C until assayed for melatonin by radioimmunoassay. We observed higher variability in HR in experimental rats as compared to controls while BP was not increased. Melatonin concentrations in the pineal gland and plasma differed in comparison with controls and a remarkable decrease in melatonin content was determined in hearts of rats exposed to night time shifts. Results suggest that 10 weeks of exposure to a night shift program is not sufficient for inducing hypertension in young rats but the depressed melatonin content in the heart of treated rats indicates that night shift work may negatively influence the cardiovascular system.

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GNRH-II INDUCED CALCIUM SIGNALING AND HORMONE SECRETION MEDIATED BY TYPE-I GnRH RECEPTOR IN NEONATAL RAT GONADOTROPHS

H. Zemkova, A. Balík, A. Bhattacharya, D. Bina, M. Jindrichova
Department of Cellular and Molecular Neuroendocrinology, Institute of Physiology Academy of Sciences of the Czech Republic, Prague

Reproductive functions in vertebrates are controlled by decapeptide GnRH (gonadotropin-releasing hormone), also known as LHRH (luteinizing hormone-releasing hormone). Two forms of this hormone, GnRH-I and GnRH-II, are commonly present in mammals. The main hormone controlling reproduction is GnRH-I acting through its receptor GnRHR-I. GnRH-I is synthesized by hypothalamic GnRH neurons of all mammalian species, and is secreted in a pulsatile manner into the hypophyseal portal system. It governs reproductive functions by regulating the synthesis, glycosylation, and release of LH (luteinizing hormone) and FSH (follicle stimulating hormone) in anterior pituitary gonadotrophs, leading to stimulation of gonadal steroidogenesis and gametogenesis. The function of GnRH-II is unknown. In primates, it has been suggested that GnRH-II is a specific agonist for the structurally distinct GnRHR-II, however, this receptor has not yet been found in other species. In this study we compared effects of GnRH-I and GnRH-II, on intracellular calcium and gonadotropin hormone release in neonatal rat gonadotrophs *in vitro* and the dependence of agonist actions on cyclic nucleotide levels. Both agonists elevated intracellular calcium and stimulated LH and FSH secretion in a concentration dependent manner, with comparable peak amplitudes but GnRH-I was 3-times more potent than GnRH-II. Antide, a specific GnRHR-I antagonist, completely blocked the action of both agonists on gonadotropin release. Inhibition of adenylyl cyclase activity by melatonin and MDL significantly attenuated GnRH-I and GnRH-II-induced calcium signaling and gonadotropin release, whereas inhibition of soluble guanylyl cyclase activity was ineffective. GnRH-II also generated calcium oscillations in a fraction of gonadotrophs not expressing melatonin receptors. These results indicate that GnRH-II can activate GnRH-I receptor in neonatal rat pituitary, and is less effective stimulator of both LH and FSH secretion than was GnRH-I. This finding supports the idea that GnRH-II may have main biological effects in nonreproductive functions in the rat.

WHICH CONFORMATION OF THE MM-CK MOLECULE IS RESPONSIBLE FOR A STRONG BOND IN THE M-LINE OF SARCOMERE?

J. Žurmanová[1,4], D. Horníková, R. Fišer [2], P. Arnoštová [1], J. Mejsnar[3]
[1]Dep. of Physiol., Faculty of Science, [2]Dep. of Microbiol., Faculty of Science, [3]3rd Faculty of Medicine, Charles University, Prague, [4]Inst. of Physiol., Academy of Sciences, Prague, Czech Republic

It is well known that MM-CK located in the M-line of sarcomere maintains high ATP/ADP ratio in the nearest vicinity of myosin ATPase (1). Delivery of substrates and products between both enzymes is realized by „substrate channelling" (SCH). In the previous study we showed maximum of SCH in moderate acid pH 6.95 (2) and subsequently we found a strong pH dependence of CK bond in the M-line within the physiological range pH 6.8-7.2 (3). It could be induced by conformational changes of the CK molecule (4,5). In order to observe underlying conformational changes, we measured emission spectra of excited intrinsic tryptophan residues (Trp) within the pH range of 6.80–7.50. We did not obtain any dramatic differences in the emission spectra with respect to the pH changes. In spite of this data hydrophobic fluorescent probe 1,8-ANS (1-anilinonaphthalene-8-sulfonic acid), which is used for partially mapping of unfolded states of proteins, showed slow but significant unfolding during increasing pH. The results of this study show that the strong bond of MM-CK in the M-line of myofibrils is evoked by more tight conformation of the CK molecule under acid pH.

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THE PRESENCE OF 2b AND 2x/d MYOSIN HEAVY CHAIN mRNAs IN THE RAT SLOW SOLEUS MUSCLE CONFIRMED BY REAL TIME RT-PCR WITH A NEWLY DESIGNED SET OF PRIMERS

J. Žurmanová[1,2], F. Půta[2], R. Stopková[2], J. Říčný[1], T. Soukup[1]
[1]Institute of Physiology, AS ČR, Prague 4, [2]Faculty of Science, Charles University in Prague, Prague 2, Czech Republic

In order to re-evaluate the presence and relative quantity of 2b and 2x/d myosin heavy chain (MyHC) transcripts in the rat slow soleus muscle (1, 2) by using real time RT-PCR we compared the available relevant *cDNA* sequences and designed a new set of primers having similar melting temperatures, matching separate MyHC exons in the regions of maximal differences in MyHC coding sequences, and containing G or C at their 3´-ends. These also yielded PCR products of corresponding length, which is an important requirement for real time RT-PCR quantification. The experiments were performed on 8-month-old inbred female Lewis strain rats used in our current study of regenerating transplanted muscles. The primers used in our study were as follows:
MyHC1_F, AGAGGAAGACAGGAAGAACCTAC;
MyHC1_R, GGCTTCACAGGCATCCTTAG;
MyHC 2a_F, TCCTCAGGCTTCAAGATTTG;
MyHC 2a_R, TTAAATAGAATCACATGGGGAC;
MyHC 2x/d_F, AAGACCGCAAGAACGTTCTC;
MyHC 2x/d_R, TCGTAAGTACAAAATGGAGTGAC;
MyHC 2b_F, GAGGACCGCAAGAACGTG;
MyHC 2b_R, TGTGTGATTTCTTCTGTCACC;
GAPDH_F, GCTGAGTATGTCGTGGAGTC;
GAPDH_R, GTCAGATCCACAACGGATAC.
The real time RT-PCR measurement confirmed the expression of all four MyHC *mRNAs* (type 1, 2a, 2x/d and 2b) in both fast extensor digitorum longus (EDL) and slow soleus (SOL) muscles, the relative proportion of fast 2a : 2x/d : 2b MyHC isoform transcripts in the SOL muscle were, however, by two to three orders of magnitude lower than that of slow MyHC 1 isoform. This result is in agreement with the dominant content of MyHC 1 protein isoform supplemented by a small amount of MyHC 2a isoform usually found in the adult rat soleus muscle (3, 4).

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