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I. Neurophysiology

CHANGES OF THE POSTSYNAPTIC ELEMENT DURING ACUTE KINDLING. *M. Langmeier, J. Mareš,* Institute of Physiology, First Faculty of Medicine, Charles University, Prague.

The cortical sensorimotor area of laboratory rats was repeatedly stimulated at 10 min intervals. This led to progressive lengthening of the self-sustained afterdischarges (SSAD) (2). One hour after termination of the third SSAD, type I synapses according to Gray (1) were examined. A significant increase of the postsynaptic apparatus, an enlargement of the area by 30 %, an increase of perimeter by 13 % and an increase of maximum diameter by 15 % are being reported. No changes in the shape or size were demonstrated in presynaptic structures or in the morphology of presynaptic mitochondria. These findings are discussed in relation to the increased functional readiness of the synapses during acute kindling and persistent hyperexcitability of the tissues one hour after the termination of SSAD as signs of active reconstruction of the synaptic apparatus.

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THE "RESTING LEVEL" OF EXTRACELLULAR POTASSIUM ION CONCENTRATION AND pH IN THE BRAIN OF RATS. *N. Kříž, R. Rokyta,* Department of Physiology, Third Medical Faculty, Charles University, Prague, Czech Republic.

The "resting level" of extracellular potassium ion concentration $[K^+]_e$ was measured in the brain hemispheres of control and deafferented rats using the authors' method previous described (1). The level of anaesthesia was monitored by examining pupillary size, stability of blood pressure, heart rate and body temperature. The resting $[K^+]_e$ level is commonly attained within 5–6 minutes, therefore a standard 10-min interval was used. Different "resting levels of $[K^+]_e$" were found in specific brain structures. Special attention was paid to the $[K^+]_e$ distribution in the thalamus, namely the ventroposteromedial nucleus VPM. The average $[K^+]_e$ resting level calculated from 10 control rats under Equithesin anaesthesia (3.8 mM) was compared with the resting $[K^+]_e$ depth profile in each experiment of control or deafferented rats. The "resting level" of extracellular pH was measured during penetration of the pH ion selective electrode (ISM) from the brain surface in the same manner as the resting $[K^+]_e$. Keeping the pH-ISM tip in a definite depth under the surface, the time course of altered pH in comparison with $[K^+]_e$ is about twice longer. The standard of the 20 min interval was used.

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PHOSPHOINOSITIDES, INOSITOL 1,4,5-TRISPHOSPHATE AND CYTOSOLIC CALCIUM LEVEL IN RESTING PLATELETS OF SCHIZOPHRENIC PATIENTS. *D. Řípová, V. Němcová, A. Strunecká[1], P. Mohr, C. Höschl,* Department of Biochemistry, the Prague Psychiatric Centre and [1]Department of Physiology and Developmental Biology, Faculty of Sciences, Charles University, Prague, Czech Republic.

Disturbances in the regulation of the phosphoinositide signalling system have been proposed as the possible biological markers connected with the etiology of schizophrenia. Earlier studies presented changes and abnormalities in the turnover of inositol lipids and in the formation of inositol phosphates in platelets of schizophrenics. We investigated the $[^{32}P]$ orthophosphate incorporation into phosphatidylinositol 4,5-bisphosphate, phosphatidylinositol 4-phosphate, phosphatidylinositol and into phosphatidic acid (PA), the level of inositol 1,4,5-trisphosphate (IP_3) and the cytosolic calcium concentration ($[Ca^{2+}]_i$ in resting platelets of neuroleptic treated and untreated schizophrenic patients. We found that there are no differences in the turnover of inositol phospholipids between control healthy subjects (n=24) and neuroleptic treated patients (n=26) as well as in the group of untreated schizophrenics (n=10). We observed that the turnover of PA in the group of neuroleptic treated patients was increased. This difference was not found either in the group of untreated patients or in the same group of patients after 1 month neuroleptic therapy. The level of IP_3 was significantly higher in the group of treated patients in comparison with the controls. $[Ca^{2+}]_i$ was increased to 207 % in platelets of drug-naive patients as compared with healthy subjects. This value decreased with the duration of neuroleptic therapy, but remained significantly higher than in the controls.

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THE EFFECT OF QUINOLINIC ACID ON IRON-INDUCED LIPID PEROXIDATION IN THE RAT BRAIN. *S. Štípek, J. Crkovská, T. Zima, F. Šťastný[1],* First Department of Medical Chemistry and Biochemistry, First Medical Faculty, Charles University, Prague and [1]Institute of Physiology, Academy of Sciences, Prague, Czech Republic.

Quinolinic acid (QUIN), a glutamate agonist with a relative selectivity for the N-methyl-D-aspartate (NMDA)-receptor, produces neuronal loss in various regions of the mammalian brain similar to those seen in Huntington's chorea and Alzheimer's disease (1). QUIN has been shown as a potent lipid peroxidant. In the presence of Fe^{2+} (0.5–8.0 μM)/ ascorbate (250 μM) system, QUIN stimulated lipid peroxidation in homogenates of rat cerebral hemispheres in a concentration range from 0.15 mM to 1.5 mM. However, higher concentrations of QUIN (3–15 mM) decreased the formation of thiobarbituric acid reacting substances (TBARS). These results were confirmed by HPLC determination of the complex formed by thiobarbituric acid (TBA) and free malondialdehyde (MDA) which is a particularly sensitive indicator of this process. When endogenous iron was chelated by deferoxamine (10 μM), QUIN failed to induce lipid peroxidation in rat brain homogenates. The results suggest that low concentrations of QUIN promote the Fe^{2+} plus ascorbate-dependent lipid peroxidation, but its concentrations above 1.5 mM depressed this process, perhaps by chelation of Fe^{2+}.

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TOLERANCE TO KETAMINE-INDUCED BLOCKADE OF CORTICAL SPREADING DEPRESSION TRANSFERS TO MK-801. *A. Rashidy-Pour[1], J. Bureš,* [1]Department of Physiology, School of Medical sciences, Tarbiat Modarres University, Tehran and Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

An important role of NMDA receptor gated channels in the initiation and propagation of cortical spreading depression (CSD) is initiated by CSD blockade induced by systemic injection of non-competitive NMDA receptor antagonists such as ketamine (KET) and MK-801. The KET-induced SD blockade declines with repeated KET injections due to the development of the specific tolerance which was examined in 15 rats anaesthetized with pentobarbital. The capillary microelectrodes were stereotaxically inserted 1 mm below the surface of the parietal cortex exposed by a 4 mm trephine opening and connected through calomel halfcells to a DC amplifier input of a computerized polygraph. CSD was evoked by injection of 1 μl of 5 % KCl to a point 3 and 6 mm caudal from the near and far recording sites, respectively. After the control recording, five injections of KET (50 mg/kg, i.p.) were applied at 60–75 min intervals. The first injection of KET blocked CSDs elicited at regular 15 min intervals for 30–45 min at the near and for 60–75 min at the far electrode. CSD blockade induced by subsequent KET injections gradually weakened and was not detectable after the 5th injection. MK-801 (1.5 mg/kg) injected to animals with marked KRET tolerance 30 min after the last KET dose, failed to block CSD. Without KET pretreatment, the same dosage of MK-801 elicited complete CSD blockade lasting more than 2 hours. It is concluded that repeated injections of KET may cause conformational changes of the NMDA receptor at a site shared by both KET and MK-801.

SENSITIVITY CHANGES TO GLOBAL BRAIN ISCHAEMIA DURING POSTNATAL DEVELOPMENT OF RATS. *J. Pokorný, J. Sivenius,* Institute of Physiology, First Faculty of Medicine, Charles University, Czech Republic and Institute of Neurology, Faculty of Medicine, University of Kuopio, Finland.

Clinical observations and experimental models indicate that global cerebral ischaemia can cause functional neuronal changes as well as cause neuronal damage. The destruction is mostly restricted to pyramidal cells of the CA1 hippocampal region (3) and to some types of hilar interneurones (especially somatostatin positive neurones) (2) During ontogeny, the sensitivity to hypoxia seems to increase (1). The question arose whether the sensitivity to global ischaemia is also related to ontogenic development. Cerebral ischaemia was induced by bilateral coagulation of vertebral arteries combined with 15 min common carotid arteries clamping (the 4-vessel-model). Wistar male rats aged 25, 30, 45, 75 and over 90 days (adult) were used. Perfusion fixation and histological processing followed three days after the period of ischaemia. Silver staining of the dying cells and the immunocytochemical procedure visualizing the somatostatin positive cells were used in alternate vibratome sections. In the silver-stained material, the dark (=dying) cells were found not only in the CA1

hippocampal region, but also in the CA3 and CA2 regions, in the dentate hilus and in the cortex. Individual regions differ in the duration of the sensitive period: higher numbers of dark cells were present only in 75-day-old and adult animals; the cortical region and the dentate hilus revealed a high number of dark cells in adult animals only. The number of somatostatin-positive cells in hippocampal regions was lower in adults and 75-day-old rats. No age dependent differences were found in the cortex. Both the lower incidence of dying cells and the higher density of somatostatin cells in the young age groups indicate that the sensitivity to global ischaemia increases during ontogeny.

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TRANSIENT EFFECT OF ACUTE HYPOBARIC HYPOXIA ON CORTICAL EPILEPTIC AFTERDISCHARGES AND THEIR MODULATION BY NOOTROPIC DRUGS. *D. Marešová,* Institute of Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

In our previous experiments we found that acute altitude hypoxia prevents the ability to elicit cortical epileptic afterdischarges (ADs) in rat pups aged 12 days. In 25-day-old rats it influences the length of the postictal depression (PD) and the duration of AD. Animals exposed to altitude hypoxia at the age of 12 days and stimulated on the 18th day, do not differ from control 18-day-old rats (1). Using an interval of 15 min between the end of hypoxia and the cortical stimulation with a short interval between stimulations (1 min), the blocking effect of hypoxia was eliminated in 12-day-old rats and the elicited ADs differed from the controls in their duration. One hour after the end of hypoxia, the results in the experimental group did not differ from those of the controls. In 25-old-rats, when an interval of 15 min between the end of hypoxia and stimulation was used, hypoxia prolonged the duration of the first two ADs. Stimulation one hour after the end of the hypoxic period only prolonged the second AD. Using intervals between stimulations that bypass the influence of the postictal depression (10 min), hypoxia blocks the elicitation of ADs only just after its termination, in 15 min the ADS differ from that of the controls in the duration of the last AD, and in one hour after the end of the acute hypoxia ADs they do not differ from that of the controls. Our experiments showed that hypoxia has an important, though only temporal effect, on the duration of both ADs and PDs. Nootropic drugs (piracetam, aniracetam, sabeluzole) and MK-801 interfere with the mechanisms regulating the epileptogenic phenomena are age-dependent and have no effect on hypoxia changes.

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ANTICONVULSANT ACTION OF NMDA ANTAGONISTS MK-801 AND APH AGAINST CORTICAL AFTERDISCHARGES IN IMMATURE RATS. *R. Šlamberová, P. Mareš*[1], Department of Pathophysiology, Third Medical faculty, Charles University and [1]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Excitatory amino acids are involved in functions of the motor system as well as in epileptogenesis. We therefore studied the action of two antagonists of NMDA receptors: a noncompetitive one MK-801 (dizocilpine) and a competitive antagonist 2-amino-7-phosphonoheptanoic acid on cortical afterdischarges in immature rats. Animals 12, 18 and 25 days old were used; cortical stimulation and recording electrodes were implanted under ether anaesthesia. After a recovery period, the sensorimotor cortical area was stimulated four times at ten (MK-801) or 20 min intervals. Drugs were injected i.p. in the middle of the interval between the first and second stimulation – MK-801 in doses of 0.5 or 1 mg/kg, APH in doses of 30 or 60 mg/kg. Control animals received an injection of the solvent (physiological saline for MK-801 and DMSO for APH). The duration of ADs was measured and the intensity of movements accompanying stimulation and ADs was quantified. MK-801 exhibited a marked dose-dependent anticonvulsant action against AD duration and intensity of clonic seizures. The lower doses were able to block progressive prolongation of ADs with repeated stimulation. The action of APH was less pronounced, but the limited crossing of the blood-brain barier might play a role in this result.

EFFECT OF PHENYTOIN ON THRESHOLDS FOR CORTICAL EPILEPTIC AFTERDISCHARGES IN ADULT AND IMMATURE RATS. *P. Kršek, S. Novák, R. Haugvicová, P. Mareš,* Institute of Physiology, Academy of Sciences of the Czech Republic and Department of Pathophysiology Third Medical Faculty, Charles University, Prague, Czech Republic.

Rhythmic cortical stimulation at different frequencies can result in different patterns of epileptic afterdischarges. In addition to the 8-Hz stimulation series (Haugvicová et al. – this volume), we studied the effects of cortical stimulation at the 50 Hz frequency and of shorter duration. Experiments were performed in adult and 12-day-old rats with implanted electrodes. The stimulation series were repeated at a gradually increasing intensity; an interval between the two stimulations was at least 10 minutes. Stimulation of the sensorimotor cortical area lead to an accentuated tonic component of movements so that the animals could become prostrated. Clonic movements of the forelimbs appeared only during ADs of the spike-and-wave type. The transition to the limbic type of AD was more common than with the low frequency stimulation. In adult animals, an increase in the threshold for stimulation-bound movements did not reach the level of significance after PHT (60 mg/kg i.p. 10 min before the first stimulation); the increase of threshold for S-and-W AD was significant in contrast to the threshold for limbic AD. Changes in 12-day-old rat pups did not attain the level of statistical significance.

ACTION OF PHENYTOIN ON CORTICAL EPILEPTIC FOCI IN IMMATURE RATS. *K. Bernášková, P. Mareš,* Institute of Physiology, Academy of Sciences of the Czech Republic and Department of Pathophysiology Third Medical Faculty, Charles University, Prague, Czech Republic.

The action of phenytoin on cortical epileptic foci in infants and children is still a matter of controversy. Models of epileptic foci in developing animals might help to solve this question. Our experiments were performed in 12-day-old rat pups, i.e. at the developmental stage corresponding to the early postnatal human brain. Foci were elicited by means of bicuculline methiodide applied to the sensorimotor cortical area through an implanted cannula. Simultaneous registration of EEG and behaviour was possible. All control animals (naive and solvent-pretreated) exhibited a clear-cut epileptogenic focus; its discharges were accompanied mostly by jerks of the contralateral limbs. Spontaneous transition into ictal activity was seen in the majority of rats. Phenytoin (PHT, Epanutin Parke Davis) was administered i.p. 30 min before elicitation of the focus in doses of 30 or 60 mg/kg i.p. The latency of the first focal discharge was not changed by PHT, whereas the projection of focal discharges to the nonprimary cortical region as well as motor correlates of interictal discharges appeared significantly later than in control rats. These two effects were dose-dependent. The transition into ictal phases tended to be diminished without relation to the dose of PHT used.

ACTION OF VALPROATE ON CORTICAL EPILEPTIC FOCI IN ADULT RATS: COMPARISON WITH PHENYTOIN. *I. Matějovská, R. Mikolášová, P. Mareš,* Department of Pathophysiology, Third Medical Faculty, Charles University and Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Valproate (VPA) is one of the most common antiepileptic drugs but its possible action against focal epilepsies is still unclear. Therefore, we started a study of its action in a model of neocortical foci elicited by bicuculline methiodide in freely moving rats with implanted electrodes. Phenytoin (PHT) was used as a drug of choice against neocortical foci. Rats were surgically prepared under Nembutal anaesthesia and after one week of recovery the experiments were started. Bicuculline methiodide was applied to the sensorimotor cortical area and EEG as well as the behaviour of animals were registered. The solvent for the PHT did not influence the activity of the cortical focus. PHT (30 or 60 mg/kg i.p. 30 min before bicuculline methiodide) suppressed the generalization of focal discharges in a dose-dependent manner, i.e. their projection to other cortical areas, to the motor system and transition into interictal activity. On the contrary, VPA (100 or 200 mg/kg i.p. 30 min before elicitation of the foci) reliably suppressed only the transition into the ictal phases leaving the generation and spread of interictal discharges unaffected, i.e. it was active only against secondary generalization.

ANTICONVULSANT ACTION OF NBQX AGAINST CORTICAL EPILEPTIC AFTERDISCHARGES IN DEVELOPING RATS. *P. Mareš, M. Pometlová,* Department of Pathophysiology, Third Medical Faculty, Charles University and Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Antagonists of excitatory amino acids exhibit a marked anticonvulsant action. The synthesis of selective AMPA antagonists NBQX and GYKI 52466 led to a shift of attention from NMDA antagonists to AMPA receptors. We started a study of anticonvulsant action of NBQX against cortically induced epileptic afterdischarges (ADs) in immature rats. Animals 12, 18 and 25 days old were implanted with stimulation and recording electrodes and after a recovery period were stimulated four times at 20-min intervals. Ten minutes after the first AD either NBQX freshly dissolved in dimethylsulfoxide in doses of 10, 30, 60 or 90 mg/kg or DMSO in a volume of 1 mg/kg were injected i.p. DMSO did not change either ADs duration or the motor phenomena accompanying stimulation or ADs. NBQX shortened ADs in a dose-dependent manner and decreased the intensity of movements accompanying stimulation as well as of clonic seizures of the head and forelimbs accompanying ADs. The anticonvulsant effects were more marked in the youngest group than in the older animals. The higher dose which was able to abolish ADs disabled the animals and therefore a study of the effects of NBQX on motor performance was started (Mikulecká and Mareš, this volume).

EFFECTS OF ANTICONVULSANTS ON THE THRESHOLD FOR CORTICAL EPILEPTIC AFTERDISCHARGES IN ADULT RATS. *R. Haugvicová, E. Bílková, A. Schenková, P. Mareš,* Institute of Physiology, Academy of Sciences of the Czech Republic and Department of Pathophysiology Third Medical Faculty, Charles University, Prague, Czech Republic.

Rhythmic electrical stimulation of the sensorimotor cortex elicits movements synchronous with individual stimuli. With increasing intensity, epileptic afterdischarges characterized by a spike-and-wave rhythm and clonic forelimb seizures appear. The majority of rats also exhibit a transition into a limbic AD. The thresholds for elicitation of these three phenomena may be used as a measure of excitability of the brain and possibly as a test of anticonvulsant action. Therefore, we studied the action of two antiepileptic drugs, phenytoin (PHT) and ethosiximide (ESI) in this paradigm. Adult rats with chronically implanted electrodes were used. The thresholds were estimated by means of repeated 8-Hz stimulations with increasing intensity; the intervals between two stimulations were at least ten minutes. Neither phenytoin (60 mg/kg i.p.) nor ethosuximide (125 mg/kg i.p.) injected ten minutes before the first stimulation changed the thresholds. For comparison, phenobarbital (20, 40 and 80 mg/kg) was included into this study. An increase of thresholds was observed with the highest dose. Repeated testing with a one week interval demonstrated a decrease of thresholds in the sense of kindling.

INFLUENCE OF NBQX ON MOTOR SKILLS OF IMMATURE RATS. *A. Mikulecká, P. Mareš,* Institute of Physiology, Academy of Sciences of the Czech Republic and Department of Pathophysiology Third Medical Faculty, Charles University, Prague, Czech Republic.

Marked anticonvulsant action of NBQX, a competitive antagonist of AMPA receptors, together with strong side effects (up to the loss of righting reflexes with the 90 mg/kg dose of NBQX) described in the previous presentation (Mareš and Pometlová - this volume) led us to study the action of NBQX on the motor system in immature rats. Five tests were performed in three age groups of rat pups (12, 18 and 25 days old): surface righting, negative geotaxis, bar holding, wire mesh ascending and traversing of a bridge. Control animals received dimethylsulfoxide (DMSO) in a volume of 1 mg/kg i.p., experimental animals NBQX dissolved in DMSO in a basic dose of 30 mg/kg. Additional doses of 10 mg/kg (12 and 18 day old animals) and 60 mg/kg (25 day old rats) were used. The testing started ten minutes after NBQX injection and was terminated by the 50th min after administration. DMSO did not change the motor skills tested. NBQX exhibited an age-dependent effect: the 30 mg/kg dose did not influence the 25-day-old rats, but it worsened the performance of 12 day old pups in all tests. The 10 mg/kg dose did not influence the motor skills in younger pups, the 60 mg/kg dose in 25-day-old animals resulted in decreased score in all the tests. Younger animals are more sensitive to NBQX action than more mature ones.

RHYTHMIC BICUCULLINE-INDUCED ACTIVITY – A MODEL OF ABSENCE SEIZURES. *L. Velíšek, J. Velíšková, P. Mareš,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

In the rat, systemic administration of low doses of a $GABA_A$ receptor antagonist, bicuculline, induces period of rhythmic EEG spike-an-wave activity associated with behavioural arrest. This situation is similar to human absence seizures. To verify the model, we tested the effects of ethosuximide (ESI; 125 and 250 mg/kg i.p.; used in clinical practice against absences) on the rhythmic EEG activity induced 15 min later by 2 mg/kg s.c. of bicuculline in 7 adult rats. We determined the effects of ESI on the latency to onset of rhythmic EEG activity and on the frequency of its occurrence. Both doses of ESI either extremely increased the latency to onset of bicuculline-induced activity or there was a complete blockade. Therefore, the frequency of occurrence was very low after the ESI pretreatment compared to controls which received only s.c. bicuculline. Our EEG and pharmacological data suggest that a new model of human absence seizures is available for testing of putative antiabsence drugs.

ALTERATIONS IN THE SYNAPTOSOMAL PLASMA MEMBRANE DURING OXIDATIVE STRESS *IN VITRO* AND PROTECTIVE EFFECT OF STOBADINE. *M. Matejovičová, P. Kaplán, J. Lehotský, V. Mézešová,* Department of Biochemistry, Jessenius Medical School, Comenius University, Martin, Slovak Republic.

Damage of the plasma membrane plays an important role in the process of ischaemic neuronal injury. In the present study, we examined alterations in the activity of the plasma membrane transport systems, Na,K-ATPase and Na/Ca-exchanger, and alterations in membrane fluidity during oxidative stress, induced by incubation of the synaptosomal fraction with ferrous ions. We examined the protective effect of stobadine under these conditions. The synaptosomal fraction was prepared from the brain homogenates of Mongolian gerbils (1). The activity of Na/K-ATPase was determined by coupled enzyme assay (3) and the activity of Na/Ca-exchanger was estimated by radioisotopic assay (2); total Ca^{2+}-uptake (K^+-dependent) and activity of Na/Ca-exchanges (choline-dependent uptake) were measured. We has found a decrease of Na,K-ATPase activity (49.3 % in comparison with control values), in parallel with a decrease of total Ca^{2+}-uptake (46.7 %) and Na/Ca-exchanger activity (44.0 %). A significant increase of both total Ca^{2+}-uptake (by 31 % in comparison with values in the presence of Fe^{2+}) and activity of Na/Ca-exchanger (by 38 %) was found in the presence of 50 μmol/l stobadine. A complete recovery of total Ca^{2+}-uptake and Na,Ca-exchanger activity was found in the presence of 500 μmol/l stobadine. However, the Na,K-ATPase activity was restored only partially (71.6 % in comparison with the controls), even in the presence of 1 mmol/l stobadine. The effect of oxidative stress on membrane fluidity was evaluated by the fluorescence method using a membrane probe 1,6-diphenyl-1,3,5-hexatriene (DPH). Incubation of synaptosomes significant decreased membrane fluidity, however, 500 μmol/l stobadine completely protected the membranes against this change.

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ALTERATION OF NA^+,CA^{2+}-EXCHANGER AND CHANGES IN BRAIN SYNAPTOSOMAL PHOSPHOLIPID COMPOSITION AFTER ISCHAEMIA AND REPERFUSION. *V. Mézešová, M. Matejovičová, A. Drgová, J. Lehotský,* Department of Biochemistry, Jessenius Medical School, Comenius University, Martin, Slovak Republic.

Tansport systems of the plasma membrane such as Na^+,K^+-ATPase, Ca^{2+}-ATPase and Na^+,Ca^{2+}-exchanger have been shown to play an important role in the maintenance of neuronal ionic homeostasis. Optimal functioning of these systems requires both an appropriate tissue saturation with ATP as well as the presence of anionic PLs in the annular lipid bilayer. In our experiments, we investigated the influence of total forebrain ischaemia (15 min) and ischaemia following reperfusion (60 min) on total (K^+-dependent) Ca^{2+}-uptake as well as on the activity of the Na^+,Ca^{2+}-exchanger (choline-dependent Ca^{2+}-uptake) of the synaptosomal fraction. Mongolian gerbils was chosen as the model and ischaemia was induced by bilateral occusion of the common carotid arteries. S1 and S2 fractions were obtained by subcellular fractionation, which was based on the activity of marker enzymes largely containing presynaptic PM (S1) or synaptosomes (S2). The S1 fraction was used for the study of Na^+,Ca^{2+}-exchanger due to the higher content of this transport system. After ischaemia, we found both a decrease in total Ca^{2+}-uptake (72 % as compared with the controls) as well as decreased activity of the Na^+,Ca^{2+}-exchanger (50 %). After reperfusion we observed no significant changes either in total Ca^{2+}-uptake or in the activity the Na^+,Ca^{2+}-exchanger. By HPTLC analysis of PLs we found that ischaemia had no influence on the composition of individual PLs in fraction S1, while we found a decrease of phosphoinositides (by 45 %) as well as phosphatidylethanolamines (by 14 %) in fraction S2. After reperfusion,, in fraction S1 was found a decrease of

phosphatidylserines (by 20 %) in fraction S2 persists only decrease of phosphatidylethanolamines.

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FATE OF MUSCLE SPINDLES IN RAT SKELETAL MUSCLES GRAFTED DURING THE FIRST FOUR POSTNATAL WEEKS.

T. Soukup, I. Jirmanová, L.-E. Thornell[1], Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic and [1]Department of Anatomy, University of Umea, Umea, Sweden.

Extensor digitorum longus (EDL) muscles from 2 to 28-day-old inbred rats of the AVN strain were grafted into EDL muscles of adult inbred recipients. Three to 12 months after the operation, host muscles containing the grafts were removed and examined for the presence of muscle spindles and histo- and immunochemical reactions of intrafusal fibres. Regenerated muscles grafted during the first week after birth were virtually spindle-less and grafts of muscles transplanted 10 and 15 days postnatally contained 5 – 8 muscle spindles on the average. In contrast to this, the regenerates originating from muscles of 24 and 28-day-old rats were spindle-rich like mature muscle grafts; the number of spindles in these grafts (25.0 ± 2.3; mean ± S.E.M) attained values comparable to free standard autografts of EDL muscles of adult animals. Similarly as in standard autografts, almost all intrafusal fibres in the regenerated spindles exhibited the ATPase reaction and MHC expression similar to extrafusal fibres. Thus, the critical period after grafting involving the loss of both the nerve and vascular supply is considerably longer than the critical period for muscle spindle survival after nerve injury. We assume that the low resistance of immature spindle capsules to ischaemia accounts for their massive degeneration and abortive spindle regeneration in grafts from 10 to 15-day-old rats.

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REGENERATION OF TACTILE LAMELLAR CORPUSCLES FOLLOWING FREEZE INJURY IN POSTNATAL RATS.

I. Jirmanová, P. Dubový[1], *J. Zelená,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and [1]Department of Anatomy, Medical Faculty, Brno, Czech Republic.

Tactile corpuscles localized in glabrous skin consist of sensory axon terminals and lamellar cells derived from Schwann cells; the cells are covered by a basal lamina and embedded in the extracellular matrix. In immature rats, the corpuscles destroyed by freezing have been found to regenerate both in the innervated and denervated skin (1). The question has arisen as to whether the corpuscles could also regenerate during development, when their morphogenesis is nerve-dependent and their regeneration hampered after nerve crush (2). Therefore, we studied rat digital corpuscles by electron microscopy and after staining for non-specific cholinesterase following destruction by freezing at 7, 13, 21 and 34 postnatal days with the aim to find out whether and when the corpuscles would redifferentiate. In the innervated skin, digital corpuscles differentiated well in dermal papillae of all age groups. In denervated toes, however, no regeneration was observed after freezing the toes during the first month after birth. The results indicate that sensory axons are essential for regeneration of digital corpuscles after freeze injury during their development and maturation.

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HOMOTOPIC AND HETEROTOPIC INTERHEMISPHERIC RESPONSES IN THE CORTICAL VISUAL AREA OF RATS.

V. Kuthan, Institute of Physiology, First Medical Faculty, Charles University, Prague, Czech Republic.

The interhemispheric responses to electrical stimulation of the homotopic point of contralateral cortex are well known (e.g. 2.3). The heterotopic response in rat parietal cortex have been described recently (1). In the visual cortex, stimulation of lateral parts of area 17 evokes homotopic responses and heterotopic ones on area 18a. When the central part of area 17 is stimulated, there is only an indistinct response at the homotopic point (relatively acallosal territory), but the heterotopic responses in area 18a may be observed. When the anterior parts of area 17 are stimulated, the clear response in area 18a is present, but only a small positive response at the homotopic point is recorded. From the stimulation of the medial region of area 17 only indistinct responses at the homotopic point and at a heterotopic point situated in area 18a can be obtained. The stimulation of area 18a evokes maximal responses at the homotopic point and somewhat weaker ones in the adherent territory of area 17 in the contralateral hemisphere. When area 18 is stimulated, the interhemispheric response is limited to the homotopic point contralaterally. It is concluded that there exist connections between homotopic and heterotopic areas in the visual cortex, some territories being acallosal. These results correspond closely with morphological studies (4).

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EFFECTS OF ADRENERGIC AGENTS ON INTRAOCULAR PRESSURE IN CONTROL AND PERTUSSIS TOXIN PRETREATED RABBITS AND ON ADENYLYL CYCLASE ACTIVITY OF CILIARY PROCESSES.

J. Čepelík, M. Caicedo, M. Dědina, S. Hynie, Institute of Pharmacology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

Topical applications of p-aminoclonidine (PCLN) (0.5 %) elicited a decrease of intraocular pressure (IOP) both in control and in pertussis toxin (PT)-pretreated rabbits (by a single dose of PT two weeks earlier). However, in PT-pretreated rabbits, the ocular hypotensive effect of PCLN was considerably reduced. In control rabbits, both epinephrine (EPI) (1) and an adenosine agonist R-(-)-PIA (1) increased IOP during the first hour after application which was followed by a decrease of IOP for several hours. In PT-pretreated rabbits, the initial increase of IOP was considerably potentiated. The succeeding ocular hypotensive effect of EPI was markedly reduced in PT-pretreated animals, however, the ocular hypotensive effect of R-(-)-PIA was influenced only marginally by PT-pretreatment. As far as the effects of these agents on the activity of ciliary processes adenyl cyclase are concerned, PCLN led solely to its inhibition, EPI exhibited both stimulatory and inhibitory effects (at higher concentrations) and R-(-)-PIA elicited only marginal inhibition of this enzyme.

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THE DESENSITIZATION OF ADENYLYL CYCLASE OF RABBIT CILIARY PROCESSES BY ADRENERGIC AGONISTS.

M. Dědina, M. Caicedo, J. Čepelík, S. Hynie, Institute of Pharmacology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

The phenomenon of desensitization of adenylyl cyclase (AC) of pigmented rabbits ciliary processes (CP) by adrenergic agonists was studied both *in vitro* and *in vivo*. In *in vitro* experiments, we studied the influence of preincubation of CP for 20 min with 10 μmolar isoproterenol (ISO), clenbuterol (CB) and fenoterol (FT) on basal and drug-stimulated activities of AC in homogenates prepared from these processes. All three adrenergic agonists moderately increased basal AC activity and decreased the enzyme stimulation by ISO and vasoactive intestinal polypeptide (VIP), but not by forskolin (FK). *In vivo* we repeatedly applied FT (1 %) topically into the eyes of rabbits (one dose per day) and followed up its effects on intraocular pressure (IOP) *in vivo* and on AC activity of CP from these eyes *in vitro*. On the first day of application, FT elicited a profound and prolonged decrease of IOP. From the second to fifth day of application, FT elicited a considerable increase of IOP. However, this repeated application of FT did not elicit any observable changes in AC activity of CP, tested in preparations removed from rabbits treated for four days, with the exception of a moderate decrease in the stimulatory effect of FK. These results suggest that in CP the role of AC desensitization to the effects of adrenergic agonists on intraocular pressure is not yet clear and would deserve further study.

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CORTICAL GLYCOGEN PHOSPHORYLASE IN RATS DURING DEVELOPMENT AND ITS CHANGES DURING HOMOCYSTEINE-INDUCED SEIZURES.

J. Folbergrová, Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic.

Previously, the total (a+b) glycogen phosphorylase activity was studied in the brain of immature animals. The aim of the present study was: a) to determine the total, as well as active form of glycogen phosphorylase in the rat cerebral cortex during development, b) to assess the response of this enzyme to induced seizures. Experiments were performed on 7, 12 and 18-day-old male rats (Wistar strain). Seizures were induced by i.p. administration of homocysteine. Glycogen phosphorylase was determined in the presence (total activity) and in the absence of 1 mM AMP (phosphorylase a)(1). Activity was expressed in μmol glucose-1-P.g^{-1}.h^{-1} and phosphorylase a also as the percentage of total activity. Total activity increased from 54.76 ± 2.33 to 181.14 ± 5.79 μmol.g^{-1}.h^{-1} and phosphorylase a from 3.45 ± 0.45 to 63.73 ± 1.41 μmol.g^{-1}.h^{-1}, from postnatal day 7 to 18, respectively. In 7-day-old pups phosphorylase a corresponds to 6 % of total activity only. At the onset of seizures in all the age groups, there

was rapid activation of the enzyme. However, in 7-day-old rats, in spite of marked activation, phosphorylase a remains very low (6 μmol.g^{-1}.h^{-1}) and can thus explain the slow onset of glycogenolysis in this age group. Cyclic AMP levels remained unchanged in 7 and 12-day-old pups; only a very mild (+ 25 %) rise could be seen in 18-day-old rats. The present results thus suggest that, at least in 7 and 12-day-old rats, activation of glycogen phosphorylase has occurred by a cAMP-independent mechanism, in which Ca^{2+} most likely play a role.

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ALLOSTERIC CONTROL OF THE BINDING PROPERTIES OF MUSCARINIC RECEPTOR SUBTYPES M1-M5 IN CHO CELL LINES. *J. Jakubík, L. Bačáková, E.E. El-Fakahany[1], S. Tuček,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic and [1]University of Minnesota Medical School, Minneapolis, Minnesota, USA.

It has previously been found that alcuronium has a positive allosteric effect on the binding of (^{3}H)methyl-N-scopolamine ((^{3}H)NMS) to muscarinic receptors in the rat heart atria, ileal smooth muscle and cerebellum and in the chick heart, while it has a negative allosteric effect on the binding of (^{3}H)NMS to receptors in the brain cortex and salivary gland (1). At the same time, alcuronium was observed to diminish the binding of another muscarinic antagonist – (^{3}H)quinuclidinyl benzilate ((^{3}H)QNB) – in all rat tissues that were investigated. The present experiments were performed to identify the subtypes of muscarinic receptors on which alcuronium displays either the positive or the negative allosteric effect on (^{3}H)NMS binding. Chinese hamster ovary (CHO) cell lines stably transfected with one of the genes for the M1 – M5 subtypes of muscarinic receptors were used. Positive cooperativity between the binding of alcuronium and (^{3}H)NMS was found on the membranes containing the M2 or M4 subtypes, while negative cooperativity was present on the membranes containing the M1, M3 and M5 subtypes. Alcuronium had a negative allosteric effect on the binding of (^{3}H)QNB.

INOSITOL TRISPHOSPHATE AND CYTOSOLIC CALCIUM LEVEL IN ACTIVATED PLATELETS OF SCHIZOPHRENICS AND THE EFFECT OF NEUROLEPTIC THERAPY. *A. Strunecká, D. Ripová[1], V. Němcová[1], P. Mohr[1],* Department of Physiology and Developmental Biology, Faculty of Sciences, Charles University and [1]Laboratory of Biochemistry, Psychiatric Center Prague, Czech Republic.

Inositol trisphosphate (IP_3) is a second messenger formed by the hydrolysis of phosphatidylinositol 4.5-bisphosphate in response to many stimuli. IP_3 regulates the cytosolic calcium level ($[Ca^{2+}]_i$). In the search for changes in cellular physiology in schizophrenia we have studied the level of IP_3 and $[Ca^{2+}]_i$ in platelets of schizophrenic patients after activation. We compared the effect of neuroleptic therapy and the effect of chlorpromazine *in vitro*. Psychiatric diagnosis was based on DSM-IIIR criteria. The content of IP_3 was estimated using the IP_3 [^{3}H] assay system (Amersham). $[Ca^{2+}]_i$ was measured with a fluorescent probe Fluo-3. In the platelets, multiple signal-transducing pathways were described which are activated by various extracellular stimuli. The majority of platelet agonists evoke a rise in $[Ca^{2+}]_i$. We have found, that after platelet activation with thrombin, phospholipase C attacks phosphatidylinositol in platelets of schizoprenics, without changes in the generation of IP_3. Cytosolic Ca^{2+} mobilization was observed in all the groups tested (in % of the unstimulated value ± S.E.M.): controls – 154.7 ± 7.9; treated schizophrenics – 155.7 % ± 7; drug-naive patients – 146.6 ± 14. 100 μM chlorpromazine reduces $[Ca^{2+}]_i$ to 31 % of control unstimulated value in healthy subjects, to 23 % in drug-naive patients and to 20 % in neuroleptic-treated group. In agreement with this observation neuroleptic therapy decreases $[Ca^{2+}]_i$ to 123 %. Neuroleptic therapy significantly increases the level of IP_3 in platelets of schizophrenic patients from 55.9 ± 12 to 61.2 ± 8.4 pmol/10^9 cells.

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EMBRYONIC MOTILITY: RELATIONSHIP BETWEEN NMDA- AND NO-ERGIC MECHANISMS. *J. Sedláček,* Institute of Physiology, First Faculty of Medicine, Charles University, Prague.

The relationships between NMDA activation of spontaneous motility and the NO-ergic mechanism, represented by L-arginine (L-ARG) and by NO-nitroarginine methyl ester (L-NAME), the depressor of NO-synthase (cNOS), were studied in 17-day-old chick embryos after the systemic application of the tested solutions. 1. The cocktail (NMDA + L-ARG) composed of subthreshold concentrations of both drugs (10 mg/kg e.w., and 20 mg/kg e.w. respectively in 50 μl physiologic saline) evoked paroxysmal activation of spontaneous motility. This effect was significantly blocked by L-NAME (20 mg/kg e.w.). 2. L-NAME (20 mg/kg e.w.) in 60 % of cases completely blocked the activatory effect of NMDA (20 mg/kg e.w.) alone, whereas in 40 % of the cases it increased, on the contrary, the activatory effect of NMDA by 30 %. The expressivity of the block-effect of L-NAME depended on the order of application of both components: it was potent in the order of NMDA – > L-NAME with the 10-min interval. The results are considered as evidence of the possible participation of NMDA- and NO-ergic systems in the genesis of spontaneous motility and, as an evidence, that the relationship of both systems, function in the neuronal apparatus of the generator of spontaneous motility even during the embryonic period.

CHANGES OF POSTURAL STABILITY DEPEND ON AVIATION EXPERIENCE. *M. Sázel,* Institute of Aviation Medicine, Prague, Czech Republic.

A number of methods are being used for the control and selection tests of pilots and pilot candidates. Therefore, all type of the aviator's reactions are being investigated. Not even in the air force has postural control been used in monitoring the state of health. Postural stability was measured on a stabilometer with a 10 cm layer of foam rubber during 50 s. Fighter jet pilots (n = 36), applicants for jet pilots (79), helicopter pilots (27) and controls (45) were examined. Lower amplitude of sway in two planes was significant in fighter jet pilots ($p < 0.01$), the highest was found in the controls. A higher value of power spectrum density was significant in fighter pilots at 0.25 Hz ($p < 0.05$). The results support the hypothesis that a fighter pilot career has some effect on postural stability. It seems that intensive flying induces a tendency to a better postural stability. We assume that the importance of vestibular afferents spatial orientation is enhanced with aviation practive. This presumption is supported by higher value of PSD in 0.25 Hz in fighter jet pilots (2). It is necessary for further research to find out exactly the possible influence of age, flight training or selection of candidates for pilots (1). Some prospective study observing the same subjects during their flight career would be optimal.

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THE EFFECT OF K^+ AND EXCITATORY AMINO ACIDS ON DIFFUSION PARAMETERS IN ISOLATED RAT SPINAL CORD. *L. Vargová, P. Jendelová[1], C. Nicholson[2], E. Syková[1],* Institute of Physiology, Third Medical Faculty, Charles University, Prague, [1]Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague and [2]Department of Physiology and Biophysics, New York University Medical Center, New York, USA.

Extracellular space (ECS) volume fraction (α), tortuosity (λ) and nonspecific uptake (k) are three parameters affecting the diffusion of substances in the nervous tissue. Many physiological and pathological processes are accompanied by a release of excitatory amino acids and excessive ionic shifts in ECS (2). The effect of excitatory amino acids (EAA) and of increased concentrations of K^+ on diffusion parameters were studied in the developing rat spital cord *in vitro*. Using the real-time iontophoretic method (1,2) the changes in ECS diffusion parameters were measured in 5 to 10-day-old rats (P5 – P10) by quantitative analysis of tetramethylammonium duffusion curves. Superfusion of spinal cords with a solution, in which 50 mM K^+ was substituted for Na^+, resulted in a decrease of α and an increase of λ. At P5, a decreased in 20 – 30 min from 0.26 ± 0.01 to 0.10 ± 0.02 and λ increased from 1.61 ± 0.03 to 1.92 ± 0.36 (n = 4, mean ± S.E.M). On P10, α decreased in 20 – 25 min from 0.21 ± 0.03 to 0.11 ± 0.04, while λ increased from 1.64 ± 0.14 to 1.96 ± 0.19 (n = 5). Although there were no significant differences in the peak values of α and λ achieved by application of 50 mM K^+ in the two age groups, the recovery time in normal Ringer solution was significantly slower in the younger animals. On P5, α and λ returned to the control values in 70 – 120 min, while on P10 in 25 – 35 min. The application of NMDA (5×10^{-5} M) in both age groups resulted in more pronounced and rapid shrinkage of ECS (to $\alpha = 0.04 \pm 0.02$) which was accompanied by only a small increase in tortuosity to $\lambda = 1.67 \pm 0.09$ (n = 4). This effect was blocked by the NMDA inhibitor MK-801 (10^{-5} M). Application of glutamate or AMPA resulted in a smaller shrinkage of ECS than after NMDA. Our results show that both an increase in K^+, and release of EAA, can result in cell swelling and changes in ECS difusion parameters. However, the mechanisms of cellular swelling and ECS shrinkage evoked by K^+ and EAAs might be different.

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DIFFUSION PROPERTIES OF NEONATAL RAT CORTEX AND CORPUS CALLOSUM DURING TERMAL ANOXIA. *I. Voříšek, L. Vargová[1], T. Mazel, C. Nicholson[2], E. Syková,* Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, [1]Third Medical Faculty, Charles University, Prague, Czech Republic and [2]Department of Physiology and Biophysics, New York University Medical Center, New York, USA.

The neonatal rat brain is known to have a large extracellular volume fraction (α) compared to that of the adult rat brain, but the turtuosity (λ) is similar at all postnatal ages (1). Ischaemia brings about a drastic decrease in α in the adult nervous tissue along with an increase in λ (2). This study followed the effect of ischaemia on α and λ in the developing rat brain at postnatal stages P4-6 (4–6 days) and P10-12. The extracellular space diffusion parameters α, k and non-specific uptake k' were determined in the cortex (lamina V.) and the corpus callosum using the real-time iontophoretic method, which uses ion-selective microelectrodes to follow diffusion of tetramethylammonium (1,2). Global ischaemia was induced in anaesthetised rats by intraperitoneal injection of saturated KCl or $MgCl_2$. At P4-6, fell from a control level of 0.46 ± 0.03 to 0.06 ± 0.01, while λ increased from 1.49 ± 0.05 to 2.0 ± 0.1 (n=6, mean ± S.E.M.) At P10-12, fell from 0.27 ± 0.02 to 0.06 ± 0.004 and λ increased from 1.41 ± 0.04 to 1.95 ± 0.09 (n=15). In white matter (corpus callosum), at P4-6 α fell from a control value of 0.50 ± 0.05 to 0.03 ± 0.006, while λ increased from 1.41 ± 0.03 to 1.8 ± 0.1 (n=6). At P10-12, α fell from 0.31 ± 0.01 to 0.04 ± 0.003 and λ increased from 1.59 ± 0.03 to 2.0 ± 0.2 (n=6), i.e. the final α and λ values are not significantly different from those in the gray matter. However, at P4-6 the maximal changes in α and λ were attained at 50 ± 4 min after cardiac arrest, while at P10-12 at 24 ± 3 min. Our results show that, during early postnatal days, the changes in extracellular space diffusion parameters are similar to those in adults, but the time course is significantly slower in the younger animals.

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POTENTIATION OF $GABA_A$ RECEPTOR BY NEUROPEPTIDE MIXTURE FROM CEREBROLYSISN. *H. Zemková, J. Krůšek, F. Vyskočil,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Biologically active peptides in the central nervous system, which are released from petidergic neurones or as contrasmitters with classic neurotransmitters, are known to activate a specific class of receptors or to modulate classic synaptic transmission. Cerebrolysin (EBEWE Austria) is a peptide mixture which is produced by standardized enzymatic hydrolysis of lipid-free pig brain proteins. It is clinically used in the therapy of neurodegenerative, ischaemic and traumatic cerebral disorders. In animal experiments, Cerebrolysin was found to facilitate learning and the memory in old rats but the molecular mechanism of the complex benefical action of Cerebrolysin is not yet known. Using a patch clamp technique in the whole cell configuration we aimed to determine what is the direct effect of Cerebrolysin in cultured hippocampal neurones. Application of Cerebrolysin (0.1 μg per l ml) by a fast microperfusion system induced an inward current of 0.2–1 nA in all neurones from newborn mouse hippocampi held at –30 mV membrane potential. Cerebrolysin induced currents were reduced by a $GABA_A$ antagonist bicuculline (2 μM) by 65 %, by an NMDA antagonist aminophosphovaleric acid (APV, 10 μM) by 27 % and by a non-NMDA antagoonist cyanonitroquinoxalinedione (CNQX, 10 μM) by 20 %. Cerebrolysin dialyzed through a 3.6 kD gut did not induce any transmembrane current but potentiated the response induced by GABA (10 μM) to 135 %. We conclude that in addition to amino acids which activate $GABA_A$, NMDA and non-NMDA receptors, Cerebrolysin also contains a peptide which potentiates the $GABA_A$ receptor responses.

II. Physiology of Blood Circulation

ADENYLYL CYCLASE ACTIVITY IN HUMAN MYOCARDIUM. *M. Caicedo, S. Hynie, V. Klenerová[1], B. Hučín[2], M. Šamánek[2],* Institute of Pharmacology, [1]Psychiatric Clinic, First Faculty of Medicine, Charles University, and [2]Kardiocentrum, University Hospital Motol, Prague, Czech Republic.

Human β-adrenergic receptors (AR) which belong to the super-family of G-protein coupled receptors are very intensively studied. However, until now the data about human myocardial adenylyl cyclase (AC) are very scarse. Since it is known that this system participates in the overall heart efficiency and is changed under various pathophysiological conditions, we decided to study this system in children patients undergoing cardiac surgery. The aim of this study was to provide data on AC which would extend our previous findings on β-AR in patients with Fallot's tetralogy (TOF). In this study we analysed 9 samples of ventricular tissue (3 TOF, 2 trimepranol (TRIM) treated TOF, the remaining being other defects) and 8 atrial tissue (3 TOF, 2 TRIM treated TOF, the rest comprising other defects) by a method using ^{32}P-α-ATP as substrate. The effects of several concentrations of isoprenaline (ISO), glucagon, Gpp/NH/p and forskolin (FSK) were estimated. The limited number of samples in the analysed groups did not allow difinitive conclusions to be drawn on the changes of AC under various pathological conditions. Preliminary analysis disclosed higher AC activity in ventricules than in the atria, roughly equal percentage stimulation by ISO in both tissues and about 5 times higher activity by FSK than by ISO. Three of our four samples from TRIM treated patients showed higher AC activity than samples from untreated groups.

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MYOCARDIAL ADENYLYL CYCLASE ACTIVITY IN TWO INBRED RAT STRAINS WITH ISOPRENALINE INDUCED HEART HYPERTROPHY. *S. Hynie, M. Mráz, M. Šamánek[1],* Institute of Pharmacology, First Faculty of Medicine, Charles University and [1]Kardiocentrum, University Hospital Motol, Prague, Czech Republic.

In two inbred rat strains, differing in their resistance to the induction of myocardial lesions by the administration of isoprenaline (ISO), we followed myocardial adenylyl cyclase (AC) activity under control conditions and in rats exposed for five days to ISO (2 mg/kg b.w.). This ISO treatment was used as a model of heart hypertrophy on which we tested changes in the responses of the receptor-AC complex to various stimulating drugs. Both in ISO resistant (IR) and ISO sensitive (IS) rats the ISO pretreatment significantly increased the heart weight and cardiac glycogen content. ISO pretreatment caused significant desesitization of ISO stimulated AC activity both in IR and IS rats, but no differences were observed in the response between both inbred rat strains. Similar, but not significant, changes were found when Gpp[NH]p and forskolin were used as the stimulating agents. These findings show typical homospecific desensitization of ISO stimulated myocardial AC in chronically ISO treated rats but did not provide a plausible explanation for the strain differences in their sensitivity to ISO induced myocardial lesions. These findings, together with finding of equal ^{125}I-pindolol binding in both strains, indicate that the differences between both strains are due to the receptor-AC complex.

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MODELLING OF THE ACTION POTENTIAL IN MYOCARDIAL CELLS. *V. Rojíková, F. Bartákl, P. Pučelík[1],* Computer Laboratory and [1]Department of Physiology, Faculty of Medicine, Charles University, Pilsen, Czech Republic.

The mathematical modelling of heart electrical activity based on the Hodgkin-Huxley equations leads to a dynamic system which can be described by a system of differential equations. For the purpose of education (making allowance for maximal clearness), the system of eight ordinary differential equations was chosen. This model presents the basis of a computer programme "CHANNEL", which demonstrates the behaviour of Na, K and Ca currents during the action potential. The programme simulates physiological and pathophysiological behaviour of working myocardial fibres: the influence of clamped membrane potential, the membrane conductance for calcium ionts, the time course of the action potential as a function of the stimulating frequency, the influence of the outward current. The programme is employed in physiological courses at the Faculty of Medicine in Pilsen (in Czech and English versions). The model will become the basis of an exact model of electrical activity of the working cardiomyocyte.

A MODEL OF THE 'BEAT-TO-BEAT' CONTROL OF CONTRACTION OF CARDIAC CELL AND ITS INTERPRETATION. *J. Šimurda, M. Šimurdová, P. Bravený, G. Christé[1],* Department of Physiology, Faculty of Medicine, Masaryk University, Brno and [1]INSERM U121, Bron, France.

The beat-to-beat control of cardiac cell contraction is governed by the total amount of calcium crossing the membrane during each cycle. The aim of this study was to interpret the available experimental results concerning Ca^{2+} transport in terms of a quantitative model of force regulation. The basis was the analysis of negative feed-back modulation of the calcium current and the Na/Ca exchange current by Ca^{2+} released during excitation (1). The model is represented by a signal-flow diagram. Each contraction is calculated on the basis of a recurrent formula from a number of foregoing amplitudes of contractions and intervals. In contrast to the previously

described models, the present attempt made it possible to interpret the constants of the formulas in real physiological terms.
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SINGLE BEAT CARDIAC MICROPOTENTIALS (LATE POTENTIALS) ARE ABLE TO DISCRIMINATED HEALTHY SUBJECTS AND SUBJECTS WITH MYOCARDIAL ELECTRICAL INSTABILITY. *Z. Drška, M. Polánková, P. Tilšer[1], D. Valová[2],* Stress Research Centre, Institute of Psychology, Academy of Sciences of the Czech Republic, Prague, [1]Department of Cardiovascular Investigations, University Hospital, Hradec Králové and [2]Biomedical Research Centre, Faculty of Physical Education and Sport, Charles University, Prague, Czech Republic.

Late potentials are considered to be related to electrical instability of the myocardium – pathophysiological basis of ventricular tachyarrhythmias, i.e. life threatening arrhythmias of sudden cardiac death. This instability is closely related to inhomogeneity of functional electric properties of the working myocardium – pathophysiological basis of reentry mechanisms. Clinically this electrical instabillity can be demonstrated by late potential extraction and proved by programmed electrical ventricula stimulation. The non-dipolar residue concept led to the development of the procedure of cardiac micropotentials (late potentials) extraction from single systoles. A simultaneously measured matrix by eighty surface ECGs and three orthogonal vectorcardiographic ECGs were processed by single value decomposition permitting the elimination of white noise without utilization of any standard filtering procedure. To obtain maximum parallelism with standard late potentials, subsequent signal processing and evaluation was adopted to the analogous to the standard late potential extraction procedure. Data from 31 healthy subjects (43.00 ± 13.95) and 19 patients (49.76 ± 15.05) with myocardial electrical instability were verified by programmed ventricular electrical stimulation. Using stepwise discriminant analysis, the sensitivity, specificity and positive predictive value have been found equal to 87.1 %, 73.7 % and 82.0 % respectively. It can thus be concluded that 1. the ability of single beat micropotentials under investigation (i.e. of the non-dipolar residue) to discriminate subjects with or without electrical instability has been verified; 2. in this study the validity of the described procedure of single beat micropotentials extraction and their physiological interpretation have been confirmed.

ELECTROPHYSIOLOGICAL DIFFERENCES BETWEEN SPONGIOUS AND COMPACT MYOCARDIUM OF THE HEART VENTRICLE IN COLD ACCIMATIZED CARPS. *P. Králíček, B. Ošťádal[1], P. Pučelík,* Department of Physiology, Charles University, Plzeň an [1]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

The purpose of our work was to compare some basic electrophysiological parameters of myocytes from spongious and compact layers in the ventricular myocardium of cold acclimatized carps. The measurements were performed using conventional glass microelectrodes. The mean value of action potential (AP) duration of the spongious cells is significantly longer than that in the compact layer. It seems that tetraethylammonium blocks potassium channels in the compact cells and their absence in cells of spongious myocardium is the cause of this difference. In both layers, the amplitude of the maximal diastolic potential, or resting membrane potential, increased at higher concentrations of extracellular Ca^{2+} (8 $mmol.l^{-1}$). This phenomenon may be due to the presence of Ca-activated potassium conductivity. The mean speed of the AP depolarization is slow in both layers. The mechanism of AP depolarization is probably ensured by Isi channels. This conclusion follows from the depolarization insensitivity to TTX. The high extracellular Ca^{2+} concentration causes increase of the mean depolarization velocity of AP, and – in this case – the TTX sensitivity appears. This phenomenon may indicate that fast inward sodium channels may be present latently. The distribution into the spongious and compact myocardium is also known in warmblooded animals during their early prenatal life period, including human embryos. Therefore, the ventricualr myocardium of the carp could serve as a suitable model for studying the ontogeny of the mammalian myocardium.

THE INFLUENCE OF DIFFERENT EXTRACELLULAR CALCIUM CONCENTRATIONS (Ca_e) ON EXCITATION-CONTRACTION COUPLING (ECC) IN WORKING VENTRICULAR MYOCARDIUM OF NEWBORN AND ADULT CATS. *P. Pučelík, M. Štengl, J. Slavíková, F. Barták,* Department of Physiology, Faculty of Medicine, Charles University, Plzeň, Czech Republic.

ECC in the immature (newborn or foetal) mammalian myocardium exhibits profound differences in the regulation of contraction and relaxation processes compared with the adults. The underdeveloped sarcoplasmic reticulum (SR) in neonatal hearts (especially in the case of dog, rabbit and cat) causes that the contraction cycle is more directly regulated via transsarcolemmal fluxes of Ca (sarcolemmal type of ECC). The main goal of this work was to measure the effect of different values of Ca_e (mmol/l: 0.67, 2, 6) on the action potential (AP) and the force of isometric contraction (MG) of the right ventricular papillary muscles from the hearts of newborn and adult cats. The increase of Ca_e causes symmetrical shortening of adult APs. In the case of newborn APs higher Ca_e shortens the plateau phase, while the terminal repolarization is prolonged. The changes in Ca_e tend to more pronounced inotropic responses in the newborn myocardium than in the adult one. During the early postnatal ontogeny, the ability of SR to accummulate and to release Ca is developed and therefore the calcium handling between SR and contractile apparatus rapidly increases (reticular type of ECC). During early stages of the postnatal life immature type of ECC (sarcolemmal type of ECC) is converted into a reticular (adult) one. This process causes the internal cycle of calcium turnover to become more effective.

STRONTIUM AND EXCITATION-CONTRACTION COUPLING IN WORKING VENTRICULAR MYOCARDIUM OF ADULT RABBITS. *M. Štengl, P. Pučelík, F. Barták, J. Slavíková,* Department of Physiology, Faculty of Medicine, Charles University, Plzeň, Czech Republic.

The aim of our study was to describe the sequence of individual steps of excitation-contraction coupling (ECC) in the adult mammalian ventricualr myocardium under the influence of strontium. Strontium is the only element able to substitute the natural activator of contraction, calcium ions. The substitution of Sr^{2+} for Ca^{2+} slows down the kinetics of ECC and so makes the differentiation of separate components of ECC possible. The experiments were carried out on papillary muscle preparations from the right ventricle of adult rabbits. Action potentials (AP) and isometric contractions were recorded simultaneously. The measurements were carried out in solutions with 2 mmol Ca^{2+}/l (control) and in solutions in which Sr^{2+} was substituted for Ca^{2+} (partial and complete substitution). In Sr-solutions, considerable prolongation of AP occurred at low frequencies and with lengthening of AP the contraction became biphasic. These phenomena were entirely suppressed by nifedipine (10^{-6} mmol/l). In the presence of ryanodine (10^{-6} mmol/l), the first (phasic) component of contraction disappeared. Sr-dependent prolongation of AP and contraction remained. In the case, when the preparation did not show rest-dependent shortening of AP (caused obviously by the lower intensity of It_o), triphasic contractions occured. We conclude that the prolongation of AP is caused by the well known effect of Sr^{2+} on I_{CaL}, I_K and I_{to}. The contraction under the influence of Sr^{2+} is divided into two components: the first caused by the release of Sr^{2+} from the sarcoplasmic reticulum (SR), the second is the response to the direct activation of the contractile apparatus by Sr^{2+} flowing into cells *via* I_{CaL}-channels during prolonged AP. In preparations with triphasic contractions, the third component responding probably to Na/Ca (or Na/Sr) exchange is inserted between the first and second components. Ca^{2+} or Sr^{2+} entering into the cardiac cell *via* exchanger causes the inserted component through SR-release or through direct activation of the contractile apparatus, which is otherwise overlapped by the phasic first component.

DISTRIBUTION OF TYROSINE HYDROXYLASE, CGRP AND GAP-43 IMMUNOREACTIVE NERVES IN THE DEVELOPING RAT HEART ATRIUM. *J. Slavíková, A. Dahlström[1],* Department of Physiology, Faculty of Medicine, Charles University, Plzeň, Czech Republic and [1]Institute of Neurobiology, University of Göteborg, Göteborg, Sweden.

The developmental pattern and distribution of tyrosine hydroxylase (TH), calcitonin gene-related peptide (CGRP), and growth associated protein (GAP-43) immunoreactivities (LI) in the rat heart have been studied by indirect immunofluorescence. Anti-TH, -CGRP, -GAP-43 and monoclonal antibodies against synaptic vesicle antigens SV2 and p38 of classical autonomic innervation were applied to whole-mount stretch preparations of the right atria from hearts of newborn to 40-day-old animals. Immunofluorescence was studied by conventinal and confocal laser scanning microscopy. Nerve fibres with all antigens-LI were present throughout the atria already at birth, with the highest density arround the sino-atrial node. A gradual increase in the density of innervation was observed up to the age of 40 days. TH-LI colocalized in most nerve terminal arborizations with SV2 in contrast to CGRP-LI, which was not present in the nerve terminals with either SV2 or p38 synaptic vesicle antigens. The results show that TH-positive, possibly adrenergic, as well as noradrenergic noncholinegic nerve fibres are already present at birth, and that the pattern of innervation is qualitatively similar to that observed in adults. Furthermore, GAP-43-LI was present in nerve terminals at birth, but its density has not changed substantially from newborn to 10-day-old animals.

COMPARISON OF HEART ELECTRICAL FIELD IN HUMANS OF DIFFERENT AGES. *J. Slavíček, O. Kittnar,* Institute of Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

The body surface isointegral and isoarea maps are sensible markers of local electrical repolarization both in controls (1), and in ischaemia (2). In the present contribution, the absolute values of maximum and minumum (extreme values in ECG body surface potential maps BSPM) have been compared in 24 healthy younger persons (20–36 years) with 9 older ones (54–70 years) of both sexes, non-smokers and without cardiovascular illness in their medical history. Twenty-nine parameters of the heart electrical field were registered by 96 electrodes placed regularly on the thorax and analyzed by the system Cardiag (3). The results were statistically evaluated by the t-test and by the Mann-Whitney test. A lower heart rate and a longer QT interval were found in older persons. The maximum of isointegral and isoarea maps was less positive and the minimum was less negative in older than in younger subjects ($p<0.01$). The results confirmed the decrease of QRS and T wave potentials in the elderly.

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POSTNATAL DEVELOPMENT OF THE RAT MYOCARDIUM AND ITS TOLERANCE TO ISCHAEMIA. *N. Tribulová, L. Okruhlicová, B. Ziegelhöferová, J. Slezák,* Institute of Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Republic.

The structural and metabolic changes of the developing heart are related to its functional response during physiological and/or pathological conditions. In the present report the ultrastructure as well as the enzyme activities of 5'nucleotidase (5 NC) and glucose-6-phosphatase (G-6-P) in cardiomyocytes from newborn, 4, 8, 22 and 90-day-old rats were investigated cytochemically. In addition, hearts subjected to 20 min of global ischaemia were also examined. The myocytes during the first and second postnatal week were characterized by a high number of glycogen granules and ribosomes. Moreover, intensive myofibrilogenesis, the development of T-tubules and longitudinal sarcoplasmic reticulum (SR) were observed. The activity of membrane-bound 5 NC was observed predominantly in the capillary endothelium and on the sarcolemma of some myocytes. G-6-P positive sites of the SR were sporadically observed in the first two postnatal weeks. By the 22nd day of maturation, cardiomyocytes were fully differentiated and resembled adult ones; including the distribution of activities of both enzymes. Twenty-minute global ischaemia did not induce any structural or cytochemical alterations in the newborn hearts. Mild ischaemia-related fine structural but not cytochemical alterations were observed in the hearts of 4-day-old rats. The ischaemic injury was more pronounced in the second postnatal week and progressed with age. Thus, the hearts from juvenile and adult rats consisted of moderately or severely damaged myocytes. Decreased but persisting activities of the investigated enzymes were present. The results suggest that the ischaemic tolerance of the myocardium decreases shortly after birth. This has been related to the actual structural and metabolical state of the developing rat heart during the first three weeks of postnatal development.

THE INFLUENCE OF TREATMENT ON HEART RATE VARIABILITY AND BAROREFLEX SENSITIVITY IN POST-INFARCTION PATIENTS. *N. Honzíková, B. Fišer, B. Semrád[1], R. Lábrová[1],* Department of Physiology and [1]First Internal Clinic, Masaryk University, Brno, Czech Republic.

Decreased heart rate variability (HRV) and baroreflex sensitivity (BRS) are associated with sudden cardiac death (SCD) after myocardial infarction (MI). The aim of this study was to assess the influence of treatment by thrombolysis, betalytics, cardiotonics and antidiabetics on BRS and HRV in patients after MI. In 90 patients 8–18 days after MI, HRV was derived from 24-h Holter recordings as SDANN (standard deviation of 5-minute mean RR intervals) and SD (mean of 5-minute standard deviations of RRs) and as RSA (respiratory sinus arrhythmia determined by spectral analysis of 3-minute recordings of pulse intervals). BRS was determined by spectral analysis of blood pressure (recorded for 3 min by the Peňáz method) and pulse intervals. Patients treated with each of the therapeutical procedures were compared with the remaining patients. In those treated by thrombolysis, RSA was significantly higher ($p<0.01$), the increase of SD and SDANN was not significant. The therapy with betalytics influenced neither BRS nor HRV. The diabetics had lower SD ($p<0.01$) and RSA ($p<0.05$). Patients treated with cardiotonics had lower SD, BRS ($p<0.01$) and RSA (0.05) corresponding to the decreased HRV and BRS during heart failure. Whereas treated heart failure, diabetes as well as treatment by thrombolysis modulated the prognostic indexes of SCD in the expected direction, the effect of betalytics was not confirmed.

ALTERED PHOSPHOLIPID COMPOSITION IN PRESSURE-OVERLOAD HYPERTROPHIED RAT HEARTS DURING THE EARLY POSTNATAL PERIOD. *L. Mrnka, O. Nováková, V. Pelouch[1], F. Novák[2],* Department of Animal Physiology, [2]Department of Biochemistry, Faculty of Natural Sciences, Charles University and [1]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

A pressure-overload was induced in male Wistar rats by abdominal aortic constriction two days after delivery. The abdominal aorta was surgically isolated above the renal vessels and constricted by use of the ligature-needle (0.25 mm) technique. Sixty days after surgery, aortic-constricted (banded) and sham-operated rats were killed and the left (LV) and right (RV) ventricles were excised. The phospholipid concentration was estimated in both ventricles by means of the TLC technique. The body weights of banded rats were decreased by 15 % and LV weights were elevated by 70 % in comparison to the sham-operated controls. The increased ventricular to body weight indicated a significant degree of LV hypertrophy and slight RV hypertrophy. The concentration of total phospholipids decreased in both ventricles of banded rats. Individual phospholipids, phosphatidylcholine, phosphatidylethanolamine (PE) and phosphatidylinositol decreased in both LV and RV. The concentration of sphingomyelin and PE plasmalogen increased only in LV. We assume that remodelling in phospholipid composition contributes to the changes of functional performance of pressure-overloaded myocardium in the period of postnatal development.

QUALITATIVE AND QUANTITATIVE PROTEIN CHANGES IN PRESSURE OVERLOADED MYOCARDIUM. *J. Černohorský, V. Pelouch, B. Ošťádal, M. Milerová,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

We have previously shown (2) that a gradual pressure overload induced in either 2-day (P_2) or 6-day-old rats (P_6) by banding the abdominal aorta (to 0.25 and 0.45 mm respectively) provoked left ventricular hypertrophy in P_2 and P_6 within a relatively short time, however, right ventricular hypertrophy occured in the P_2 group only. The content of total collagenous proteins and non-collagenous proteins in the left ventricle of both P_2 and P_6 was already elevated after 30 days. Whereas the concentration of soluble collagenous cardiac proteins was elevated 30 days after surgery, higher concentrations of both soluble and insoluble collagenous proteins were found 60 days after aorta banding; the changes were less pronounced in P_6. The protein remodelling of the right ventricle in both P_2 and P_6 was characterized by lower concentrations of soluble collagenous proteins. Qualitative changes of collagenous proteins were estimated by using either SDS-PAGE or UV spectrophotometry (220–240 nm). The experimental procedure affected the proportion of major collagen types (higher amount of collagen III and lower amount of collagen type I). Furthermore, qualitative remodelling of myosin light chains was observed in hypertrophied left ventricles in both P_2 and P_6 groups. The above results show that the transition from compensated to decompensated cardiomegaly induced by aotric banding in neonatal rats (1) is accompanied by remodelling of both collagenous and non-collagenous proteins.

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TROPONIN T IN NEONATES AFTER ACUTE AND LONG-TERM TOCOLYSIS. *M. Adamcová, Z. Kokštein[1], V. Palička[2], M. Podholová[3], M. Košťál[3],* Department of Physiology, Faculty of Medicine, [1]Department Paediatrics Faculty of Medicine, [2]Institute for Clinical Biochemistry and Diagnosis and [3]Department of Obstetrics and Gynaecology, Technical Hospital, Hradec Králové, Czech Republic.

The study was designed to evaluate the diagnostic efficiency of cardiac troponin T (cTnT) in neonates exposed *in utero* to β-sympathomimetic therapy. The cardiac troponin T concentration was measured by means of commercial kits (Enzymun-Test System, Boehringer, Mannheim) in the cord blood of 51 neonates. The first group (32 neonates) after acute tocolysis was divided into 5 subgroups determined by the duration of treatment. Troponin T levels were in the physiological range in all of the neonates after 0.5 day of the therapy (0.02 μg/l).

cTnT was elevated in 50 % of neonates after 1 or 2 days of therapy (0.25 and 0.26 μg/l respectively). All of the newborns had cTnT concentrations in the pathological range after 3 days of treatment (0.54 μg/l). When the treatment lasted between 4 and 8 days, the elevated cTnT was found only in 62.5 % (0.23 μg/l). The second group (19 neonates) was divided into 3 subgroups determined by the interval between the end of therapy and delivery. When the interval was 7 days, cTnT was elevated in 40 % (0.11 μg/l). When the interval was 7–14 days or more, cTnT was increased in 33.3 % (0.13 μg/l) and in 18.2 % (0.06 μg/l), respectively. The results of this study seem to show that the employed tocolytic therapy may have side effects on the foetal myocardium. The finding of elevated cTnT concentration in the cord blood corresponds with the kinetics of cTnT and peaks round about the 3rd day of treatment.

LONG-TERM INHIBITION OF NITRIC OXIDE (NO) SYNTHESIS: PATHOPHYSIOLOGICAL IMPLICATIONS. *O. Pecháňová, P. Babál[1], I. Bernátová, A. Holécyová,* Institute of Normal and Pathological Physiology, Slovak Academy of Sciences and [1]Institute of Pathology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic.

L-arginine analogues have been shown to induce NO-deficient hypertension (1). In the present study, changes of cardiovascular parameters were investigated in rats during 4 weeks of oral administration of the NO-synthase inhibitor: N^G-nitro-L-arginine methyl ester (L-NAME). Systolic blood pressure (SBP) and heart rate were measured by tail-cuff plethysmography. A dose of 20 mg/kg/day increased SBP by 31 % after the first week. The increase persisted during the next three weeks and the increase at the end of the fourth week was by 34 % higher as compared with the controls. A dose of 40 mg/kg/day of L-NAME elevated SBP by 35 % after the first week. The increase persisted during the next three weeks and there was no significant difference between the two groups of animals in the SBP increase. The heart rate decreased in both groups of rats as compared with control animals. Histological investigations of rat hearts after 4 weeks of L-NAME administration, especially after the dose 40 mg/kg/day, documented a significant increase in myocardial fibrosis as compared with control hearts. These findings imply that the inhibition of NO-synthase leads to hypertension connected with bradycardia and significant morphological changes in the myocardium.

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LONG-TERM INHIBITION OF NITRIC OXIDE (NO) SYNTHESIS: VASCULAR RESPONSES. *A. Holécyová. O. Pecháňová. I. Bernátová, J. Török,* Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

In the model of hypertension induced in rats by long-term oral administration of L-NAME (competitive inhibitor of NO synthase), the relative contribution of NO to modulation of vascular responses to different vasoactive agents was investigated. After 4 weeks of L-NAME treatment, isolated segments of *truncus a. pulmonalis,* the abdominal aorta and renal artery were mounted for isometric tension recording. Concentration-dependent contractions to noradrenaline (10^{-9}–10^{-5} mol/l) were significantly potentiated in vessels treated with L-NAME as compared with control vessels. The sensitivity to the agonist was also enhanced in the treated vessels. In noradrenaline precontracted vessels, endothelium-dependent relaxations to acetylcholine (10^{-8}–10^{-5} mol/l) were attenuated by about 30 % in the treated vessels. L-arginine improved the reduced relaxation. The endothelium independent relaxations to sodium nitroprusside (10^{-9}–$3x10^{-6}$ mol/l) were unaffected by L-NAME treatment. Isoprenaline concentration-relaxation curves (10^{-8}–10^{-5} mol/l) were also not influenced by L-NAME. The results suggest that NO significantly modulates the vascular responses to noradrenaline and acetylcholine. Decreased NO release as well as exaggerated contraction to noradrenaline may participate in hypertension after L-NAME treatment.

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LONG-TERM INHIBITION OF NITRIC OXIDE (NO) SYNTHESIS. THE CHANGES OF PROTEOSYNTHESIS. *I. Bernátová, O. Pecháňová, A. Holécyová,* Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Garg and Hassid demonstrated in 1989 an inhibitory effect of vasodilator drugs generating NO on mitogenesis and proliferation of vascular smooth muscle cells in culture (2). This finding was confirmed by Curran *et al.* (1) in cultured hepatocyts. The aim of the study was to investigate the involvement of NO in the modulation of preteosynthesis in the myocardium, aotra and brain of rats. NO-deficient hypertension was induced by long-term oral administration of nitro-L-arginine methyl ester (L-NAME) in the doses 20 mg/kg/day and 40 mg/kg/day. After 4 weeks of L-NAME treatment of 20 mg/kg/day, the systolic blood pressure increased by 34 %, while the heart rate decreased by 15 % as compared with the controls. The same dose of L-NAME caused an elevation of the total RNA and DNA content by 10 % and 214 % in the myocardium, by 155 % and 56 % in the aorta and by 47 % and 398 % in brain, respectively. Administration of L-NAME in the dose 40 mg/kg/day increased systolic blood pressure by 30 %, but decreased the heart rate by 20 %. The total RNA and DNA content was enhanced by 15 % and 238 % in the myocardium, by 254 % and 200 % in the aotra and 85 % and 350 % in the brain, respectively. The [^{14}C]leucine incorporation into protein of the myocardium, brain and aorta showed significant increased proteosynthesis.

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CALCIUM PARADOX IN HEARTS OF DIABETIC RATS. *A. Ziegelhöffer, J. Styk, T. Ravingerová, K. Volkovová, A. Breier[1], J. Šeboková, A. Džurba, T. Ziegelhöffer,* Institute for Heart Research and [1]Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic.

The data available in the literature about the tolerance of the diabetic heart to calcium are contradictionary. This may be due, on the one hand, to differences in the species and experimental models used, on the other hand, to the fact that findings may refer to different phases in the development of the disease. In our experiments, we investigated the haemodynamic variables as well as the sarcolemmal ATPases in isolated perfused hearts from normoglycaemic rats and animals in the acute stages of diabetes mellitus subjected to normoxic perfusion and to the calcium paradox. Adult male Wistar rats were made diabetic by a single i.v. injection (45 mg/kg b.w) of streptozotocin (STT). From the second day after STT administration, rats were treated daily with insulin (Interdep, 6 U/kg subcutaneously). After eight days, the hearts were rapidly removed and perfused according to Langendorff with Krebs-Henselelt (K-H) solution containing 1.6 mM calcium and gassed withi 95 % O_2 and CO_2. The calcium paradox was induced by deprivation of calcium (3 min perfusion with calcium free K-H solution) followed by 10 min of calcium readmission in the original Ca-containing perfusate. The hearts of diabetic rats showed a significantly higher resistance against the calcium overload. This was manifested by restoration of heart function after the Ca paradox (heart rate to 86 %, coronary flow to 61 % of the initial values) in comparison to the complete loss of heart function in the normoglycaemic controls. The improved preserved activities of sarcolemmal Na/K-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase in diabetic hearts after the calcium paradox were in agreement with the findings in haemodynamic variables. The results indicate that higher activities of sarcolemmal transport ATPases may be involved in the increased tolerance to calcium in the hearts of diabetic rats.

BIOCHEMICAL ANALYSIS OF HUMAN CARDIAC COLLAGEN. *V. Pelouch, V. Rychterová[1], M. Milerová, R. Jirmář[2],* Institute of Physiology, Academy of Sciences of the Czech Republic, [1]Department of Pathology and [2]Second Internal Clinic of the Third Medical School, Prague, Czech Republic.

Our aim was to evaluate the qualitative and quantitative biochemical characteristics of cardiac collagenous proteins in: a) different parts of the myocardium, b) at different stages of cardiac necrosis. The material was collected from autopsy cases; the interval between death and autopsy did not exceeded 24 h. The excisions were taken from the anterior (ANT) and the posterior (POS) wall of the left (LV) and right (RV) ventricle of both normal (N) structure without macroscopic and microscopic damage and scars (S) and healing (H) of necroses in LV. By using stepwise extraction with 0.5 M acetic acid (a 20-fold multiple of the original amount with 1 mg of pepsin/ml), fractions of pepsin-soluble (S) and -insoluble (IS) collagenous proteins were recovered; the concentration of hydroxyproline (HYP and collagen types in S and HYP in IS and total HYP have been analysed. We have shown there are no differences in HYP of ANT and POS of RV. The concentration of HYP in POS of LV did not differ from HYP of RV, however, the elevated concentration of HYP in ANT of LV was due to higher amounts of HYP in IS. The concentration of HYP in scars was 4–5 times higher; on the other hand, the samples of cardiac tissue with H contained two-fold higher HYP concentrations as compared with N. There was no significant difference in the proportion of HYP in S of all samples (8–11 % from total HYP). Collagen of N is composed mainly as collagen type I with a small contamination of collagen type III; the extracellular matrix structure of scars is formed only by collagen type I.

GEOMETRY OF CORONARY ARTERY AND GROWTH OF COMPONENTS OF ITS WALL IN ONTOGENESIS. *J. Bodorová, F. Kristek, M. Gerová,* Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

The fact that there is a substantial difference in the structure of the human coronary artery (CA) and the Ca of animals is not well recognized. The wall/diameter ratio (W/D) in the adult human CA 1:7.4 (1) differs significantly from the value 1:23 which we have found in adult dogs. Moreover, the relation between the intima and media thickness changes in the human CA, the tunuca intima exceeds the media thickness very early. The basic parameters of CA and components of its wall were studied in canine CA in three developmental periods: in 8 foetuses one week before birth (F), in 8 newborns (N), and 8 adults (A). The vessels were fixed under different pressures. The inner diameter measured in F 361.85 ± 17.57 μm, in N 570.19 ± 41.9 μm, in A 1.171.13 ± 33.29 μm. The wall thickness was 8.7 ± 0.75 μm in F, 21.16 ± 1.38 μm in FN, 50.39 ± 2.34 μm in A. The calculated W/D ratio represented 41.59 ± in F, 26.94 in FN and 23.57 in A. The values of cross-sectional area of tunica intima in N and A were $2.49 \pm 0.20 \times 10^3$ μm^2, $7.84 \pm 0.76 \times 10^3$ μm^2 and $15.042 \pm 1.50 \times 10^3$ μm^2 respectively. The values of tunuca media were $7.52 \pm 0.55 \times 10^3$ μm^2, $32.32 \pm 4.45 \times 10^3$ μm^2 and $179.98 \pm 10.93 \times 10^3$ μm^2 respectively. Contrary to the human CA, tunica intima did not overwhelm the tunica media during the whole development. The factors which induce the growth of tunica intima in normal CAs are urgently needed.

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SEX DIFFERENCES IN THE GROWTH OF CULTURED A SMOOTH MUSCLE CELLS FROM SUBCUTANEOUSLY HYPERTENSIVE RATS. *L. Bačáková, G. Mazzini[1], J. Kuneš,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and [1]Centro di Studio per l'Istochimica, C.N.R., Pavia, Italy.

Sex differences in the growth ability of vascular smooth muscle cells (VSMC) were described in normotensive Wistar rats (1). In the present study, the growth capacity of cultured VSMC obtained from the thoracic aorta of 8-week-old male and female spontaneously hypertensive rats (SHR) is compared. Explants from the intima-media complex were cultured in collagen-coated NUNC flasks in Dulbecco minimum essential medium supplemented with foetal calf serum (10 %) and gentamicin (40 μg/ml). The migration of VSMC out of the explants started on day 2 in both sexes but the number of explants with VSMC migration rose significantly more rapidly in males (on day 18, it reached 100 ± 32 explants/flask in males but only 24 ± 5 explants/flask in females). In the second passage at the early exponential phase of growth, the doubling time of VSMC from males was shorter (13.5 ± 0.5 h) and the ^{3}H-thymidine labelling index higher (34.0 ± 2.3 %) than in females (19.9 ± 0.6 h and 23.9 ± 1.9 h respectively). The difference in the doubling time became even more apparent in the late exponential phase (51.8 ± 2.0 h in males vs. 91.5 ± 5.8 h in females). In this phase, the cell cycle measured by flow-cytometry was shorter in males (18.0 h) than in females (30.6 h). At the end of exponential growth phase, the VSMC from males reached a higher maximum population density (155 600 ± 9 200 cells/cm^2) than the VSMC from females (123 700 ± 4 800 cells/cm^2). These results show significantly better growth of VSMC from males in cultures prepared from the aorta of SHR.

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NON-INVASIVE MEASUREMENT OF LARGE ARTERIES COMPLIANCE IN HYPERTENSIVE PATIENTS. *B. Fišer, J. Siegelová[1], J. Dušek[1], M. Al-Kubati, E. Savin[2], J.P. Martineaud[2],* Department of Physiology, [1]Department of Pathophysiology, Masaryk University Brno, Czech Republic and [2]Department of Physiology, Hopital Lariboisiere, Paris, France.

The aim of this study was to compare the arterial blood pressure (BP) and aortic compliance (C) in treated and non-treated hypertensives. The measurements were performed in 3 groups of men of comparable age, 10 normotensives (NS), 8 untreated patients with essential WHO hypertension II (EH) and in 8 hypertensives treated for 3 months with verapamil (Isoptin SR240), (EH I). The applied method is based on the occlusion of both lower extremities by cuffs (180 mmHg, 5 min). The abrupt decrease of occluding pressure to 60 mm Hg elicited reactive hyperaemia with an increase of femoral flow (F) and a decrease of BP. The decrease in mean BP during the first cardiac interval after release of occlusion (dP) is caused by the additional outflow of blood (dV) into femoral arteries. C is calculated as C=dV/dP. Pulsed Doppler measurements of F and Penaz non-invasive record of BP were used 6 times in every subject. The following results were obtained:

	Systolic BP	Diastolic BP (mm Hg)	C (ml/mm Hg)
NS	126 ± 11	75 ± 7	1.2 ± 0.3
EH	153 ± 21	87 ± 10	1.0 ± 0.4
EH I	124 ± 15	72 ± 10	1.0 ± 0.4 (mean ± S.D.)

The differences between NS and EH and between EH and EH I in BP are significant (Wilcoxon, $p<0.05$), but not in C. It is concluded that verapamil treatment of 3 months' duration decreased blood pressure but the aortic compliance remained unchanged.

CIRCADIAN VARIATIONS OF BLOOD PRESSURE IN PATIENTS WITH SLEEP APNOEA SYNDROME. *J. Siegelová, M. Moráň[1], B. Fišer[2], Z. Kadaňka[1], G. Cornellisen[3], F. Halberg[3],* Department of Pathophysiology, [1]Department of Neurology, [2]Department of Physiology, Masaryk University, Brno, Czech Republic and [3]Chronobiological Laboratory, University of Minnesota, USA.

The aim of the present study was to analyse 24-hours blood pressure monitoring (Accutracker II, auscultatory, using R wave gating) in 9 patients with the sleep apnoea syndrome (SAS), confirmed by the apnoea + hypopnea index. The results were compared with 16 non-treated patients with essential hypertension (EH) and 11 normotensives (C) by means of Halberg cosinor analysis. The values of MESOR (M), which approximately correspond to 24-hour mean value, and amplitude (A), which correspond s to a half of the night-day difference of systolic and diastolic pressures (SBP, DBP) were calculated. M of SBP in SAS corresponded to EH group (SAS: 143.5 ± 12.7 mm Hg; EH: 142.2 ± 14.2 mm Hg, C: 122.3 ± 8.2 mm Hg). M of DBP in SAS corresponded to C (SAS: 74.7 ± 7.1 mm Hg, EH: 85.6 ± 6.1 mm Hg and C: 77.0 ± 4.8 mm Hg). Despite the fact that M of SBP in EH and SAS was equal, A (SAS: 9.1 ± 5.1 mm Hg, EH: 13.9 ± 5.5 mm Hg, C: 10.8 ± 4.5 mm Hg) was lower in SAS (t-test, $p<0.05$). M of DBP was equal in EH and C but A (SAS: 5.1 ± 1.8 mm Hg, EH: 10.6 ± 3.5 mm Hg, C: 9.9 ± 2.4 mm Hg) in SAS was again lower ($p<0.0001$). It is concluded that circadian variations of SBP and DBP in SAS are decreased. Higher night values of arterial pressure probably contribute to the organ damage in SAS patients.

BLOCKADE OF NO-SYNTHASE IN THE POSTERIOR HYPOTHALAMUS INCREASES BLOOD PRESSURE (BP) IN RATS. *C. Mašanová, M. Gerová, J. Pavlásek,* Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Prevention of NO production in endothelial cells by NO-synthase blockade (NOS) induces contraction of vascular smooth muscle and/or a BP increase (2). NOS was revealed in neurones and fibres of the central and peripheral nervous system (3). Question arose whether NOS blockade in central nervous structures relevant to cardiovascular control, and namely in the posterior hypothalamus (PH), influences vasomotor tone and/or BP. In 7 adult Wistar rats under anaesthesia, BP was monitored in the tail artery. A micropipette filled with nitro-L-arginine-methyl ester (L-NAME) was positioned in the PH using a stereotaxis apparatus: AP-3.8 mm, L-0.4 mm, V-8 mm(1). L-NAME administered to PH in a dose 3 mg/kg in 3 μl of ACSF increased BP in all 7 rats from 96.5 ± 0.8 mm Hg to 124.4 ± 7.5 mm Hg ($P<0.001$) and was maintained for about 30 min. Repeating the same dose after 30 min, BP increased to 142.5 ± 8.3 mm Hg ($p<0.05$). An ACSF microinjection (3 μl) into the PH did not alter BP. The mechanisms affecting the increase of smooth muscle tone and-or BP are open for further study.

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HYDROGEN PEROXIDE VASOCONSTRICTION IN ISOLATED RAT LUNGS. *J. Herget, J. Wilhelm,* Department of Physiology and Department of Chemistry and Biochemistry, Second Medical School Charles University, Prague, Czech Republic..

The release of H_2O_2 in the lung tissue may participate in the regulation of pulmonary vascular smooth muscle tone. Experiments were performed on preparations of isolated ventilated ($F_{iO2}=0.21$ and $F_{iCO2}=0.05$) male rat lungs. The lungs were perfused with a recirculating volume of 25 – 30 ml physiological saline solution with albumine (4 %) and meclophenamate at a constant flow of 6 ml/min/100 g b.w. After 15 min stabilization, the lungs were primed by two challenges with AII (0.2 μg) and hypoxia ($F_{iO2}=0$, $F_{iCO2}=0.05$).

Arterial injection of H_2O_2 produced dose-dependent (range of doses 0.25–5 mM) transient vasoconstriction associated with a transient increase of lung weight. The vasoconstriction induced by H_2O_2 was potentiated by ouabain (7.10^{-7}M) and tetrodotoxin (1 μg/ml of perfusate). This was influenced by the concentration of K^+ in the perfusate (potentiated up to 25 mM). K^+ ionophore (valinomycine) inhibited the H_2O_2 vasoconstriction. A combination of Na^+ ionophore (monensin) with ouabain lead to substantial potentiation. We hypothesize that H_2O_2 increases the Na^+ influx in the pulmonary vascular smooth muscle which results in an elevation of intracelular concentration of Ca^{2+} and subsequent vasoconstriction.
Supported by grants of GACR 305/93/2395 and of Charles University No. 214.

RESPONSES OF RAT PULMONARY ARTERIES TO VASOACTIVE DRUGS IN HYPERTENSION INDUCED BY LONG-TERM NITRIC OXIDE SYNTHASE BLOCKADE. *J. Török, I. Bernátová, O. Pecháňová, A. Holécyová,* Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

The aim of this study was to analyze the effect of acetylcholine, histamine and noradrenaline in isolated pulmonary arteries in hypertension induced by inhibition of nitric oxide synthase with N^G-nitro-L-arginine methyl ester (L-NAME) for 4 weeks. Acetylcholine or histamine produced concentration-related relaxation in phenylephrine precontracted arterial rings. The arterial relaxation was significantly attenuated in rings from hypertensive rats. The maximum of reduced relaxations to acetylcholine and histamine were comparable and did not differ from each other. Residual relaxation in arterial rings from hypertensive rats was abolished by additional administration of L-NAME to the incubation mediam. Indomethacin, a cyclooxygenase inhibitor, did not affect the relaxant responses to acetylcholine and histamine in both control and L-NAME-treated rats. Relaxation induced by sodium nitroprusside was not affected by long-term treatment of rats with L-NAME. Compared to the control rings, contractile sensitivity to noradrenaline was increased in rings from hypertensive rats. The changes in reactivity of pulmonary arteries from hypertensive rats are due to a reduced availability of endothelium-derived nitric oxide.
This study was supported by the Pharmaceutical Factory Slovakofarma, Joint Stock Company, Hlohovec, Slovak Republic.

ROLE OF ENDOTHELIAL NITRIC OXIDE IN NEUROGENIC CONTRACTILE RESPONSES OF RABBIT CAROTID ARTERY DURING COOLING. *S. Čačányiová, J. Török,* Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Endothelium inhibits the responses of rabbit carotid artery to adrenergic nerve stimulation (1). The purpose of this study was to determine whether these neurogenic contractions are also modulated by the endothelium during cooling (26 °C). Isolated vascular rings were suspended in an organ bath and connected to a force transducer for the recording of isometric tension. Transmural electrical stimulation of rings caused frequency-dependent contractions. These were blocked by guanethidine or prezosine. Mechanical removal of the ednothelium or pretreatment of vessels with N^G-nitro-L-arginine methyl ester (L-NAME) augmented neurogenic contractions. Cooling the incubation bath from 37 °C to 26 °C moderately increased the magnitude of neurogenic contractions in intact rings. Contractions induced by exogenous noradrenaline in intact and denuded arteries were augmented during cooling. L-NAME increased the neurogenic contrations at 26 °C in intact arteries but not in denuded arterial rings. These results suggest that endothelial nitric oxide is involved in inhibition of adrenergic contractile responses at normal and reduced temperature.
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This study was supported by the Pharmaceutical Factory Slovakofarma, Joint Stock Company, Hlohovec, Slovak Republic.

HEART AND KIDNEY HYPERPLASIA IN NEWBORN RATS WITH SPONTANEOUS HYPERTENSION. *Z. Dobešová, J. Zicha, J. Kuneš,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Hypertrophy of the heart and kidneys is present in many forms of human and experimental hypertension. The aim of this study was to test whether early organ growth and/or cellular proliferation are related to subsequent blood pressure rise and whether they are controlled by the same genes as hypertension development. To achieve this goal we used a set of recombinant inbred (RI) strains obtained by systemic inbreeding of offsprings from the F_2 generation of two different progenitor strains – normotensive Brown-Norway (BN) rats and spontaneously hypertensive ones (SHR). In SHR newborns, the significantly lower body weight was accompanied by increased relative heart and kidney weights in comparison with BN. The relative DNA, protein and water contents in the heart of SHR newborns did not differ from those of BN ones. On the other hand, lower DNA and protein contents accompanied by a higher water content were found in the SHR kidneys. The average body weight of newborns in individual RI strains was continuously distributed between both progenitor strains but more strains resembled values of normotensive BN rats. The relative heart and kidney weights in RI strains derived by mating of hypertensive SHR males to normotensive BN females were close to values of SHR newborns. Correlation analysis did not reveal any significant relationship between blood pressure of adult males and the body weight or relative organ weights in newborns correlated positively with blood pressure of their mothers only in RI strains that were derived from SHR females. Finally, heart and kidney weights in adulthood can be predicted from newborns organ weights because a significant positive correlation was found between organ weights because a significant positive correlation was found between the weights of these organs in newborn and adult animals.
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III. Physiology of Behaviour

OLFACTORY IMPAIRMENT: A PATHOGNOMONIC SYMPTOM OF PARKINSON'S DISEASE? *J. Roth, T. Radil[1], E. Růžička, J. Tichý, C.J. Wysocki[2],* Clinic of Neurology, First Medical Faculty, Charles University, [1]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic and [2]Monell Chemical Senses Center, Philadelphia, USA.

In order to verify the hypothesis that olfactory dysfunction is an early marker of Parkinson's disease (PD), we studied olfaction in 16 patients with idiopathic PD and in 18 healthy controls. Amylacetate (banana smell) in 14 sequential dilutions (in 50 % steps) was used as odorant in four psychophysical conditions: A) binary ascendant force choice (odorant vs. pure solvent in random order); B) ascendant and C) descending limit thresholds; D) time course of desadaptation after olfactory adaptation. The testing showed anosmia in one patient; six more patients and one control subject were found to be hyposmic. Average olfactory thresholds were slightly higher in the patients compared with healthy controls. The decrease of olfaction was unrelated to the age of the patients, duration of disease, degree of motor impairment, dose and duration of L-DOPA treatment. A facilitatory effect of the ascendant stimulus ordering (lower B than C threshold), representing a modulatory or adaptive phenomenon related to stimulus expectancy, was observed in the patients as well as in healthy controls. No difference in the time course of desadaptation was found between the two groups. Our study revealed olfactory disturbances, when adopting rigorous criteria, in fewer than half of the PD patients. The dysfunction was mostly limited to slight hyposmia, and no impairment of modulatory-adaptive olfactory phenomena was disclosed. Olfactory impairment does not appear to be a pathognomonic symptom of PD.

SPATIO-TEMPORAL INTEGRATION IN "PURE OLFACTION". *T. Radil, C.J. Wysocki[1],* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic and [1]Monell Chemical Senses Center, Philadelphia, USA.

It was found impossible to detect the side of stimulation of "pure olfactory substances" (phenyl-ethyl alcohol resembling roses and vanilline) acting upon the olfactory epithelium only, when the odour was administered into one nostril and the "blank"-odourless solvent into the other nostril in a randomized way, or when stimulation had taken place on both sides simultaneously. We did not succeed in teaching the subjects to lateralize the stimuli by providing them feedback information, repetitively. It was neither possible to lateralize both the mentioned odours when administered to the right and left nostril simultaneously. All these tasks became simple, however, when the odorants and blanks entered both nostrils sequentially, during subsequent inspirations, We investigated, using a two-channel olfactometer, enabling the control of the duration of odours and blank air puffs into the nostrils, as well as their mutual timing, the shortest-threshold temporal intervals between stimuli, at which lateralization becomes possible (or, in a complementary way, the duration of the inability to lateralize more or less simultaneous stimuli). It was found that this interval lasted for about 400–250 ms depending on the duration of stimuli and on the circumstances whether two odours, or one odour with a blank, were applied. The temporal threshold in question was the same for the lateralization and for judging of the sequence of both stimuli.

SENSORY - TEMPORAL INTEGRATION IN A RHYTHMIC FINGER-TAPPING TASK IN SCHIZOPHRENICS. *R. Jirsa, P. Mohr[1], J. Libiger, M. Indra, T. Radil,* Institute of Physiology, Academy of Sciences of the Czech Republic and [1]Centre for Psychiatry, Prague, Czech Republic.

Thirteen patients with diagnosed schizophrenia and 12 healthy controls were asked to tap a button simultaneously with rhythmically presented auditory clicks. Interclick intervals were set on 400 ms. The results demonstrated that both patients and the controls tapped several tenths of milliceconds before the click-onset. This error representing integration of internal timing mechanisms with auditory stimuli denotes the known phenomenon called stimulus anticipation. The main value of anticipation did not differ significantly between schizophrenics (-71.4 ± 49 ms) and controls (-69.5 ± 29 ms); intraindividual variances were, however, significantly higher in schizophrenics (t-test, $p < 0.05$). Two modes could be detected upon the histograms of anticipations in both groups. The mean distance between modes was 24.0 ± 3.1 ms in healthy controls, and 19.1 ± 3.1 ms in schizophrenics, the difference being significant (t-test, $p < 0.01$). The intermodal value of 24 ms equals the duration of segments of synchonized neural activity revealed electrophysiologically by the authors for healthy subjects (the so called 40 Hz activity). This neural activity has been proposed to play an important role in neural integration. We suggest that our results prove a shortening of segments of the 40 Hz neural integration in schizophrenics. This might result in desynchronization among various neural integrators in the brain which in turn could be manifested by an increased variance of anticipations observed in our study, as well as by some psychotic symptoms characteristic for schizophrenics.

REPETITIVE FINGER TAPPING AND CORRESPONDING HEART RATE CHANGES. *Z. Bohdanecký, M. Indra,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

The rate heart changes (HR) related to specific sensorimotor events have been widely studied (1). Their conclusions reflect the vagal tonus modulation generally covering deceleration, acceleration and ultimate deceleration prior to the actual response initiation. The present experiment was designed to detect HR contingencies during a relatively slow finger tapping task accompanied with or without brief acoustic stimulation. Five men and 5 women were instructed to tap regularly (the inteval was 4800 ms) either in response to auditory stimuli (500 Hz tone, 50 ms duration) or spontaneously (without any acoustic stimulation), keeping the above interval between individual tappings. The following parameters were chosen to describe the expected changes: mean value of HR and the maximal value of HR between two subsequent key touchings. Both mean HR and maximal values of HR within these intervals were higher when no acoustic stimulation was given, HR values in men being always higher than in women. The HR acceleration in the first part of intervals was more rapid in women, while the deceleration before the key touching was faster in men.

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OLFACTORY THRESHOLD TUNING. *C.J. Wysocki, T. Radil[1],* Monell Chemical Senses Center, Philadelphia, USA, and [1]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Olfactory thresholds are usually higher when estimated by means of the descendent limit method (when the stimulus intensity is decreased gradually)-DT. In comparison with ascendant limit thresholds (based upon increasing the intensity of subthreshold stimuli gradually)-AT. It has been assumed that this effect might be caused by olfactory adaptation. The results of the present experiments in which different concentrations of amylacetate (AA), resembling banana smell, were presented by means of squeeze botles using different psychophysiological paradigms, might be summarized as follows: a) The difference between DT and AT (D/A) is not suppressed by decreasing the intervals between stimulus presentations. b) Stimulation by low concentrations of AA during the intervals between successive single testing trials is inneffective, high AA concentrations increase AT but do not influence DT under similar conditions. c) When randomizing the sequence of stimulus concentrations, the threshold is at the DT level. d) When alternating the sequence of descending concentrations with the ascending ones, the resulting threshold lies between DT and AT estimated separately. e) The binary ascendant forced choice threshold (odorant vs. pure solvent administered in random order in successive pairs, and the odorant detect) is close to DT. f) Stimulation with concentrations close to AP values (estimated before) only, reveals that the lack of the ascendant stimulus order increases the threshold (and stimulation with concentrations close to DP only, decrease it). It follows that the D/A phenomenon does not seem to be caused by olfactory adaptation.

CIRCANNUAL RHYTHM OF RECURRENCE IN AFFECTIVE DISORDERS. *A. Yamamotová,* Institute of Physiology and Clinical Physiology, Third Medical Faculty, Charles University, Prague, Czech Republic.

Until recently, individual predictions of the recurrence of affective illnesses have been considered impossible because of their irregularity. Some general rules have emeged for groups of patients but no satisfactory explanation has been put forward for the great individual variability. The course of this illness was analysed in a group of 52 patients with affective disorders (33 men, 19 women; mean duration of the afflication was 15.9 year (S.D. = 9.4); mean number of cycles was 7.4 (S.D. = 3.4). To discover the individual rhythm, a predictive computer program has been developed. Using this program, linear regression was applied to the cumulative values of the empirical cycles in order to find the best estimate of the period common to all cycles. The closeness of the fit was evaluated according to the mean quadratic error, calculated from the differences between the theoretical and empirical values. The period with the smaller error, taken relative to the corresponding cycle, was considered as the optimal basic cycle. The course of illness could then be expressed as the sequence of this rhythm which express the basic oscillatory pattern. The pattern is often repetitive, relatively rigid and characteristic for each patient. The basic cycles are polymodally distributed with a maximum frequency of about one year. The mean period is 346 ± 99 days (S.D.). The interindividual variability is relativelly large: the shortest observed period was 194 days and the longest 615 days. The mean absolute quadratic error was 25.7 ± 10.7 days, and the mean relative error was 7.9 ± 3.3 %. Eight cases out of 52 had a period close to one year, and in accordance with the study of Abe (1992) it could be considered circannual in its origin. The analysis of the natural course of the illness showed that in all cases studied, it was possible to find an individual rhythm responsible for the time structure of the illness. Because of the great interindividual variability and a great stability of these period in other patients, it can be hypothesized that this rhythm may be considered a free-running circannual rhythm.

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THE INFLUENCE OF L-ARGININE ON LEARNING AND MEMORY IN RATS DURING TWO PERIODS OF ONTOGENETIC. *J. Hassmannová, J. Mysliveček[1],* Institute of Physiology and Clinical Physiology, Third Faculty of Medicine, Charles University, Prague and [1]Institute of Pathophysiology, Medical Faculty, Charles University, Plzeň, Czech Republic.

The role of nitric oxide (NO) in learning and memory was studied mostly by a negative effect of NO elimination using an inhibitor of NO synthase (NOS). We have shown a positive effect of increased NO supply in early inhibitory learning (2). The aim of the present study was to investigate the NO impact on active avoidance at two distinct periods of rat ontogeny: 3 – 4 weeks of age, when learning and memory of this type of reaction reaches an optimum, and 7 – 8 weeks, when it displays a dip, as we have previously shown (1). NO supply was increased by i.p. injections of L-arginine, a substrate of NOS. In both the studied periods, L-arginine showed a positive effect, the most significant effect was established at the period of pessimum learning and memory in 24-hour memory retrieval. We can thus conclude that L-arginine, which plays a role as a NO donor, brings about a positive effect on learning and memory during the whole ontogeny.

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INTERACTIONS OF NITRIC OXIDE AND DOPAMINE IN NEONATAL INHIBITORY LEARNING AND MEMORY. *J. Mysliveček, J. Hassmannová, J. Šafanda,* Institute of Pathophysiology, Medical Faculty, Charles University, Plzeň and [1]Institute of Physiology and Clinical Physiology, Third Faculty of Medicine, Charles University, Prague, Czech Republic.

We have shown that newborn rats are able to learn and memorize an inhibitory reaction if it does not surpass their developmental abilities (1) and pointed to the important role of dopaminergic mechanisms in this kind of early learning and memory (2). Our experiments have also demonstrated the physiological role of nitric oxide (NO), known as a retrograde messenger in glutamergic synapses in early memory mechanisms (3). Here, we report on the interaction of NO with dopamine (DA) that is manifested as follows: the increased availability of DA and NO (after administration of L-arginine) significantly enhanced the efficacy of learning and memory, while the blockade of No synthase (NOS) by L-nitroarginine impairs these functions. The effects of NOS inhibition are partially eliminated either by NO or DA supply, and their positive effect is mutually potentiating. Thus, their interaction in learning and memory has been demonstrated.

Thus, their interaction in learning and memory has been demonstrated.
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DIFFERENT EFFECT OF PILOCARPINE-INDUCED EPILEPTIC SEIZURES ON NAVIGATION OF WISTAR AND LONG EVANS RATS IN THE MORRIS WATER MAZE. *J. Hort[1], E. Pavlišová, V. Komárek[2], P. Mareš[4], M. Langmeier[5], G. Brožek[3],* [1]Department of Neurology, [2]Department of Paediatric Neurology, [3]Department of Physiology, Second Medical Faculty, [4]Department of Pathophysiology, Third Medical Faculty and [5]Department of Physiology, First Medical Faculty, Charles University, Prague, Czech Republic.

The effect of the pilocarpine-induced (350 mg/kg i.p.) status epilepticus on memory functions during the following "silent period" (1) between induced and spontaneous seizures was tested in adult Wistar and Long Evans rats.. Methylscopolamine (1 mg/kg i.p.) was injected 30 min before pilocarpine to suppress peripheral cholinergic symptoms. The seizures were arrested after 2 hours by Clonazepam (1 mg/kg i.p.). During the "silent period", cognitive memory was tested in the Morris water maze. Navigation of pilocarpine-treated and control Wistar and Long Evans rats were mutually compared. Control Long Evans rats were more efficient in cognitive learning in the Morris water maze. Pilocarpine induced status epilepticus in all Long Evans rats which had to be discontinued with Clonazepam (in our experiments after 2 hours). On the contrary, in the Wistar strain, only a minority of rats developed status epilepticus, and most of them responded by temporary seizures. Status epilepticus deteriorated the memory of Wistar rats more than that of the Long Evans. Memory of Wistar rats with temporary seizures was influenced very little and they were ready for testing immediately after the seizures, whereas both strains with status epilepticus could not be tested 9 days after the seizures since they did not remain on the platform.
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Supported by grant UK 215/93/C.

KETAMINE EMELIORATES IMPAIRED NAVIGATION IN THE MORRIS WATER MAZE AFTER PILOCARPINE-INDUCED STATUS EPILEPTICUS IN RATS. *M. Šmejkalová[1], J. Hort[2], V. Komárek[2], P. Mareš[4], M. Langmeier[5], G. Brožek[1],* [1]Department of Physiology, [2]Department of Paediatric Neurology, [3]Department of Neurology, Second Medical Faculty, [4]Department of Pathophysiology, Third Medical Faculty and [5]Department of Physiology, First Medical Faculty, Charles University, Prague, Czech Republic.

The effect of single systemic administration of ketamine on Cavalheiro's (1) pilocarpine model of chronic spontaneous recurrent seizures was studied in adult Long Evans rats. Methylscopolamine (1 mg/kg i.p.) was injected 30 min before pilocarpine to suppress peripheral cholinergic symptoms. The status epilepticus was induced by an i.p. injection of pilocarpine (350 mg/kg) and stopped after 2 h by Clonazepam (1 mg/kg i.p.). In the experimental group, the injection of Clonazepam was immediately followed by administration of ketamine (100 mg/kg i.p.). The cognitive memory was tested in the Morris water maze. Navigation of ketamine treated rats was compared with navigation of the group of animals subjected only to the standard pilocarpine procedure and with a control group of animals without seizures. Immediately after status epilepticus (3 days in the ketamine-treated group and 6 days in the standard procedure group), testing was impossible because rats did not stay on the platform and were very aggressive. After the 4th day in the ketamine group and the 9th day in the standard procedure group till the beginning of spontaneous seizures, deficits of cognitive learning were observed in both groups but the differences were significantly less pronounced in the ketamine group. These results indicate than NMDA receptors play a certain role in Cavalheiro's model of epileptogenesis.
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Supported by grant UK 215/93/C.

COMBINATION OF FUNCTIONAL ABLATION AND ELECTRICAL BRAIN STIMULATION AS A TOOL FOR LOCALIZING THE CENTRAL RHYTHM GENERATOR IN FREELY LICKING RATS. *O. Vajnerová, L. Novotná, G. Brožek, J. Bureš[1],* Department of Physiology, Second Medical Faculty, Charles University and [1]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Rats were implanted with a cannula, aimed at the oral part of nucleus reticularis gigantocellularis. The cannulae were first used for introduction of the needle for tetrodotoxin (TTX) microinjection, and then for insertion of a concentric stimulating electrode. Location of the lick frequency generator was estimated by the effect of TTX microinjection into the nucleus reticularis gigantocellularis (AP 9, L 0, V 9 according to the atlas of Fifková and Maršala 1967) and its vicinity (AP 8, L 0, V 9, and AP 10, L 0, V 9) on spontaneous consummatory licking in freely moving rats. The rostrocaudal gradient of the TTX (1 ng) injection effect on spontaneous water consumption was demonstrated by significantly weaker disruption caused by TTX injection into the more caudal or more rostral locations. Electrical stimulation of the nucleus reticularis gigantocellularis was used to prove that the tested location is the proper licking frequency generator rather than some relay in its output. Spontaneously drinking thirsty rats were intracranially stimulated via the concentric electrode introduced through the same chronically implanted cannula that was used for the TTX experiment. Short-lasting (100 ms) stimulation (a train of rectangular pulses, 0.1 ms, 100 Hz) of adequate intensity applied during a period of continuous licking and synchronized by the onset or termination of every eight lick caused a phase shift of licks following stimulus delivery, but did not change the licking frequency. The induced phase shift of licking suggests that stimulation had reset this generator.
Supported by the grant GA ČR 309/93/0568).

TASTE FOLLOWED BY SUDDEN HYPERGLYCAEMIA ELICITS AVERSION RATHER THAN PREFERENCE TO THIS FLAVOUR. *M. Wesierska[1], G. Walasek[1], J. Bedrníček, J. Bureš,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic and [1]Nencki Institute of Experimental Biology, Warsaw, Poland.

Rapid progress of research into the neural mechanisms of conditioned taste aversion (CTA) contrasts with the poor knowledge of the circuits implementing conditioned taste preferences. The two processes interact in situations requiring relabelling of aversive into appetitive gustatory memories. In Experiment 1, rats trained a strong CTA to saccharin received repeated i.p. injections of 2 % saccharin in saline or in 9 % glucose. While saccharin alone elicited CTA extinction (saccharin preference SP 44 %), its combination with glucose tended to increase CTA intensity (SP 28 %). Experiment 2 attempted to enhance the attenuation of neophobia (AN) to diluted apple juice (AJ) by post-drinking intubation of 5 ml of water or of 25 % glucose. Whereas typical AN was observed in the first case (AJP 63 %), glucose elicited significant AJ aversion (AJP 33 %), which was even more marked when glucose was administered under thiopental (AJP 22 %) or ketamine (AJP 22 %) anaesthesia. In Experiment 3, an equivalent amount of polysaccharides intubated after AJ drinking in the form of 25 % potato starch had no eversive effects (AJP 56 %), whereas four i.p. injections of 5 ml of 6 % glucose applied at 30 min intervals elicited marked CTA (AJP 22 %). It is concluded that rats feel the rapid increase of glycaemia as an aversive visceral signal which can serve as US for CTA learning.

THE ROLE OF HIPPOCAMPUS IN CONSOLIDATION AND RETRIEVAL OF SPATIAL MEMORY. *Z. Liu, V. Bohbot[1], L. Nadel[1], J. Bureš,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic and [1]ARL-NSMA, University of Arizona, Tucson, USA.

The term consolidation is used to explain (1) the disruption of long-term engrams by chemical or electrical stimulation applied shortly after acquisition and (2) the differential effect of hippocampal lesions on recent and old memories. In order to examine the applicability of this concept (1) to spatial memory, 8 rats pretrained on the working memory version of the Morris water maze task immediately received a bilateral intrahippocampal injection of 4 % Lidocaine (1 μl) or an i.p. injection of Physostigmine (1 mg/kg) after the first swim to a new target location. Retrieval was impaired as long as the drugs were effective (8 min after the first swim) but returned to the control level after the drugs had become ineffective (33 min after trial 1). This indicates that the intervention disrupted retrieval but not consolidation of short term spatial memories. Concept (2) was examined in 8 rats trained for up to 10 weeks on the reference memory version of the Morris water maze task. Retrieval tests performed during bilateral inactivation of dorsal hippocampi by tetrodotoxin (5 ng/μl) showed full amnesia that did not start to subside until more than 10 weeks after acquisition. This seems to be the shortest time required for duplication of the spatial memory trace in an extrahippocampal storage site from which it is retrievable even after elimination of the hippocampus. The significance of the two consolidated concepts for current memory research is discussed.

ACTIVITY OF HIPPOCAMPAL PLACE CELLS: RECORDING TECHNIQUE, FIRING MAPS AND THEIR MODIFICATION BY TESTING CONDITIONS. *Yu. Kaminsky, L. Zinyuk, J. Bureš,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

The role of hippocampal local cells in spatial memory was tested by examining their activity under conditions interfering with place navigation. Rats were implanted with a driveable bundle of 8 nichrome wires (25 μ) aimed at the CA1 hippocampal region the activity of which was recorded while the animal searched for 20 mg pellets scattered in a large arena (2 m in diameter) and its movement was tracked with a computerized video system. Unit firing was amplified in the IC head stage amplifier (10x) and main amplifier (4000x), sampled at 32 kHz by a 12-bit ADC, detected by the DataWave Discovery software and stored on a re-writable optical disc (25 MB). Spike sorting was further refined by off-line template matching. Computer plotted maps of the time spent and of the number of spikes recorded in 20x20 cm squares of the floor were used to calculate firing rate maps allowing delimitation of place fields (PF). PF stability was tested in light and darkness, in partitioned segments of the arena and under the influence of discrete reward gradients. The results suggest that place cells respond not only to allocentric localization but also to other conditions of the test.

THE EFFECT OF SEXUAL EXHAUSTION ON SEROTONIN METABOLITES IN THE PREOPTIC AREA OF MALE RATS. *J. Petrinec,* Institute of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic.

Sexual behaviour and exhaustion after free exposure to sexual stimulation can cause significant changes in extracellular neurotransmitter levels in the brain areas related to sexual behaviour. These effects are accompanied by changes in metabolite levels, which in many cases are very marked. The medial preoptic area in regarded as being critical for the display of masculine behaviour together with its main neurotransmitter serotonin (5-HT). When sexually experienced male rats, implanted with an electrochemically pretreated carbon fibre voltametric microelectrode in the medial preoptic area, were freely exposed to receptive females, they copulated until a sexual exhaustion criterion was reached. During the subsequent "refractory period", which usually lasts 2 days, no further sexual behaviour was displayed. Animals were tested for both behavioural and neurochemical changes throughout the 3 following days during expose to sexually receptive females. Variously neurochemical substances were assessed by *in vivo* differential normal pulse voltametry (DNPV). DNPV signals were processed for numerical resolution of serotonin metabolites: 5-HIAA and uric acid (UA) components of peak 3. When animals were exposed to sexual behaviour both before entering and after recovery from the refractory period, we observed an increase in both 5-HIAA and UA values up to 140 % of basal extracellular levels. No significant changes were registered during the 2 days of sexual exhaustion. These data indicated that whereas brain serotonin and its metabolites play a role during sexual behaviour, they do not seem to be participating during the refractory period resulting from sexual and copulating satisfaction.

IV. Metabolism

AMBIVALENT EFFECT OF TERGURIDE ON GLUCOSE TOLERANCE. *V. Golda, L. Cvak*[1], Institute of Experimental Neurosurgery, Hradec Králové and 1GALENA a.s., Opava-Komárov, Czech Republic.

In a previous paper (1) we have shown that terguride (trans-DH-lisuride) alleviates a genetically based abnormality in glucose tolerance which was found in genetically hypertensive obese rats of the Koletsky type and in their non-obese siblings. On the other hand, terguride induced deviations in glucose tolerance in the normotensive rats of Wistar strain. In the present report we studied the effect of terguride on glucose tolerance in patients suffering from primary hyperlipidaemia. We found negative correlation between pre-existing values of glucose tolerance and the effect of terguride treatment in both animals and patients. In both cases we computed the inversion point of terguride effect; this is represented by the point of intersection of the regresion line with line Y = O. Analogous negative correlations and points of intersection were found in the parameters of lipid metabolism deviations (triglycerides, cholesterol, HDL, LDL, Apo A, Apo B). Within the scope of probability, when pre-existing values are lower than the point of intersection, terguride induced an elevation, and *vice versa*, where pre-existing values are higher than the point of intersection, terguride induced a decrease.

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THE INFLUENCE OF MERCURY ON LIPID AND LIPOPROTEIN LEVELS IN THE BLOOD SERUM OF SHEEP AFTER HYDRASINE MONOSULPHATE TREATMENT. *V. Eliáš, V. Rozdobuďková*[1], *J. Kollár*[1], *E. Michnová, D. Sopková, J. Várady, J. Legáth,* Department of Normal and Pathological Physiology, and [1]Institute of Experimental Medicine, Medical Faculty Šafárik University, Košice, Slovak Republic.

Development of the chemical and metallurgical industry, but especially the production of pesticides containg mercury have led to the incorporation of mercury into the environment. Furthermore, a higher incidence of intoxications of contamined individals with its compounds has to be taken into consideration. This problem has serious practical implications at present time. Therefore, the aim of our study was to investigate the response to peroral administration of sublethal doses of metal mercury on some biochemical parameters of lipid metabolism in the blood of mature sheep for 36 days and after hydrazine monosulphate tretament. The concentrations of TG (triacylglycerol), TCH (total cholesterol), PL (phospholipids), NEFA (nonesterified fatty acids) and LP (lipoproteins) in the blood were estimated. The results obtained suggest that the given doses of mercury did not significantly influence the TCH and PL levels and the percentage of some LP fractions. The TG level was increased and this can be explained by assuming that it is probably resynthesized from fatty tissue stores which would cover the energetic requirements of the total metabolism of the animals studied and, or for the blockade of catabolic lipolysis of LP. The increasing concentrations of NEFA in the blood may serve as indirect evidence that of fatty acids are being released from fatty reserves due to enzymes with lipolytic activity.

UPTAKE OF ^{14}C-LYSINE IN THE RUMEN EPITHELIUM IN SHEEP. *Z. Faixová, J. Várady,* University of Veterinary Medicine, Košice, Slovak Republic.

The uptake of ^{14}C-lysine was measured in two parts of the ovine rumen epithelium *in vitro*. A comparison of the distribution ratios at various concentrations of lysine (0.95, 4.95 and 9.95 mmol.l^{-1}) in the dorsal sack after 15 min incubation revealed a significant difference in the concentrations of 4.95 and 9.95 mmol.l^{-1} (1.619 ± 0.078 vs 0.952 ± 0.089, $P < 0.05$). At the 0.95 mmol.l^{-1} concentration of lysine the distribution ratio was 1.255 ± 0.127. After 30 and 60 min incubation no significant differences were found. A comparison of the distribution ratios at various lysine concentrations (0.95, 4.95 and 9.95 mmol.l^{-1}) in the ventral sack, after 15 min incubation, revealed a significant difference between the concentrations of 0.95 and 9.95 mmol.l^{-1} (1.536 ± 0.085 vs 1.182 ± 0.089, $p < 0.05$, respectively). At the lysine concentration of 4.95 mol.l^{-1}, the distribution ratio was 1.182 ± 0.131. After 30 and 60 min incubation, no statistically significant differences were found. A comparison of the lysine distribution ratios in both the dorsal and ventral sacks after 15 min incubation revealed a significant difference at the 4.95 mmol.l^{-1} concentration (1.619 ± 0.087 vs 1.182 ± 0.131, $p < 0.05$, respecively). At the concentrations of 0.95 and 9.95 mol.l^{-1}, the following distribution ratios were obtained: 1.255 ± 0.127 vs 1.536 ± 0.085 and 0.952 ± 0.089 vs 1.182 ± 0.089 (NS). After 30 and 60 min incubation no significant differences were found. Our results have shown that the absorption of amino acids in the rumen of sheep is dependent on the concentration and the duration of incubation.

CYCLOSPORIN A AMELIORATES CHEMICAL HEPATIC INJURY IN ISOLATED HEPATOCYTES. *H. Farghali, J. Martínek*[1], *V. Bencko*[2], *L. Kameníková, S. Hynie,* Institute of Pharmacology, [1]Histology and Embryology and [2]Hygiene and Epidemiology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

The immunosupressive compound cyclosporin A (CS) has been shown to have a hepatoprotective effect on the liver injured by various insults. In the present study, aimed to investigate the beneficial effect associated with CS pretreatment (CONSUPREN Galena, the i.v. prepration given twice i.p. 5 mg/kg on two successive days), we used isolated immobilized perfused hepatocytes from control rats and animals injured by carbon tetrachloride (CCl_4, 1 ml/kg i.p., 18 hours before hepatocyte isolation and 6 hours after the second CS dose). CS pretreatment increase trypan blue exclusion from 73 ± 4 % in CCl_4 injured cells to 87 ± 2 % which is comparable to 88 ± 4 % viability in normal rats hepatocytes. Liver cells obtained from CCl_4 treated rats showed a 60 % reduction in urea production as compared to the controls. CS pretreatment, however, increased the urea production in CCl_4 injured cells by 40 % over the relevant controls (CCl_4). CS pretreatment before CCl_4 intoxication reduced the amount of lipid droplets in the cytoplasm and partially preserved the cytoplasmic organelles. The preservation of the granular endoplasmic reticulum is indicative of higher protein synthesis activity in most observed CS pretreated cells. The hepatoprotective effect of CS against the damaging action of CCl_4 on some cellular structures and functions has been demonstrated.

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RESPONSE OF IMMOBILIZED HEPATOCYTES IN A PERFUSION SYSTEM TO ANOXIA/REOXYGENATION: EFFECT OF CYCLOSPORIN A. *L. Kameníková, H. Farghali, V. Bencko[1], J. Martínek[2], S. Hynie,* Institute of Pharmacology, [1]Hygiene and Epidemiology and [2]Histology and Embryology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

The present study was directed to follow up urea biosynthesis in perfused hepatocytes system under physiological conditions and during their exposure to anoxia/reoxygenation, and to study the effects of cyclosporin A (CS) pretreatment of rats on urea formation and cell integrity. CS (CONSUPREN Galena, the i.v. preparation) was given i.p. in a dose of 5 mg/kg per day for 2 successive days. Urea production by immobilized hepatocytes, perfused with RPMI 1640, was evident 30 min after perfusion and increased steadily as time progressed. Anoxia of two hours' duration (exposure of cells to N_2 instead of O_2) reduced the rate of urea production to one quarter of the original values and after reoxygenation it was restored to approximately 80 % of preanoxia values. Plasma mebrane integrity of cells, as measured by lactate dehydrogenase (LD) leakage, was stable during oxygenation and increased to 250 % by anoxia and to 400 % by reoxygenation. CS pretreatment did not affect urea synthesis, however, LD leakage was significantly reduced (the increase was only 80 to 100 % above the controls). The data suggested that the used system is convenient for studying urea production and that CS partially protected the liver cells from anoxic damage, but did not affect urea synthesis under the present conditions.

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POLYUNSATURATED FATTY ACIDS OF THE N-3 SERIES IMPROVE INSULIN ACTION IN THE HEREDITARY HYPERTRIGLYCERIDAEMIC (HHTG) RAT. *D. Raučinová-Gašperíková, S. Šeböková, I. Klimeš, P. Langer,* Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovek Republic.

The hHTG rat is a unique animal model of the human metabolic syndrome X (1,3). Several components of the syndrome are associated with hypertriglyceridaemia (HTG) and/or with an increased TG content in skeletal muscles and the heart. n-3 polyunsaturated fatty acids (PUFA) inhibit the production and accumulation of TG. The aim of this study was to test the effect of raised dietary intake of n-3 PUFA on insulin action under conditions of hereditary fixed (basal diet = BD) and diet-induced (sucrose diet = HS) HTG. hHTG rats were fed for 2 weeks 4 types of diets: a) BD, b) BD with marine fish oil (BD+FO), c) HS (70 cal %) and d) HS+FO. Then, euglycaemic hyperinsulinaemic (6.4 mU/kg/min) clamps were carried out in conscious rats equipped with permanent cannulas in the carotid artery and jugular vein (2). Skeletal muscles and the heart collected after the clamp were used for analyses of $GLUT_4$ gene expression.

Results	BD	BD+FO	HS	HS+FO
Triglycerides*	3.95 ± 0.56^a	0.78 ± 0.17^b	10.6 ± 0.26^c	1.64 ± 0.37^b
Glycaemia *§	6.04 ± 0.19^a	5.74 ± 0.16^a	5.51 ± 0.85^a	5.28 ± 0.13^a
Insulinaemia **§	319.2 ± 34.5^a	317.8 ± 27.3^a	339.4 ± 23.1^a	339.9 ± 32.2^a
GIR 1.§	25.3 ± 1.1^a	28.5 ± 0.1^b	21.6 ± 07^c	30.5 ± 0.4^d
GLUT4 mRNA***				
soleus	13.0 ± 1.0^a	9.8 ± 0.5^b	7.0 ± 0.2^c	15.6 ± 1.5^a
heart	3.2 ± 0.3^a	4.5 ± 0.3^b	1.8 ± 0.3^c	3.4 ± 0.1^a

Correlation GIR vs TG: r = −0.840 n = 20 p < 0.001
Correlation mRNA in soleus m. vs TG: r = −846 n = 14 p < 0.001
* mmol/l; ** lU/ml; *** arbitrary units; § = clamp data; 1 = mg/kg/min

It may be concluded that a) the *in vivo* insulin action and glucose transport in muscles change in an indirect dependence on the concentration of circulating TG, as b) the decrease of endogenous HTG and c) exogenously potentiated HTG (which are seen after raised dietary intake of n-3 PUFA) were accompanied by d) increased insulin sensitivity (see changes of GIR) and by e) changes of GLUT4 gene expression.

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THE COMPOSITION OF DIETARY POLYUNSATURATED FATTY ACIDS (PUFA) CHANGES THE METABOLIC EFFECT OF INSULIN IN RAT ADIPOCYTES. *M. Ficková, C. Leray[1], P. Hubert[2], G. Cremel[2],* Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic, [1]CNRS-CEPE and [2]INSERM U 338, Strasbourg, France.

The insulin effect is modulated at the cellular level by the properties of plasma membranes (PM), the lipid composition of which corresponds to the nature of dietary lipids (1). We studied the effect of short time (7 days) *ad libitum* intake of two isocaloric diets (16.3 % proteines, 53.8 % saccharides and 21.4 % lipids), with a modified content of PUFA: n-3 (diet M) – 32.4 mol % and n-6 (diet S) – 37.8 mol % respectively. Dietary PUFA induced the incorporation of appropriate fatty acids (FA) into PM phospholipids with the consequence of decreased membrane fluidity of PM in fat cells of rats fed the M diet (vs S). Values of the double-bond index (DBI) and the content of total and esterified cholesterol in PM were similar in both dietary groups. The increased content of n-3 PUFA in lipid compartments of adipocytes (vs n-6 PUFA) significantly inhibited a) the antilipolytic effect of insulin, b) stimulatory effect of insulin on glucose transport and lipogenesis, c) the number of insulin binding sites (IR). The decline of insulin-stimulated phosphorylation of IR β-subunit (tyrosine kinase activity) in adipocytes of the M dietary group (vs S) paralleled the decline of IR and could explain the diminution of binding signal transduction to the metabolic response. The number of IR does not correlate with insulinaemia, it depends on the n-6 PUFA content and the ratio of unsaturated/saturated FA in fat cells phospholipids.

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EFFECT OF CHOLECYSTOKININ ON FOOD INTAKE IN BROILER AND LAYER CHICKS IN THE EARLY POSTINCUBATION PERIOD. *E. Baranyiová, R.L. Hullinger[1],* Department of Biochemistry and Biophysics, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic and [1]Department of Anatomy, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana, USA.

Exogenous cholecystokinin (CCK) causes a short-term decrease of food intake in adult mammals and birds. To elucidate its satiating potency in the early post-incubation period of chicks, we administered the synthetic sulfated octapeptide CCK-8 to two strains of chicks: broilers which are selected for rapid growth accompanied by high food intake and layers with a growth rate about 1/3 that of broilers during the first three weeks after hatching. A total of 192 chicks were divided by strain and sex in 8 groups of 6 males and 6 females each. On days 3, 6 – 7, 10 – 11, 14 – 15 and 18 – 20, four groups were treated with single i.p. injections of CCK ($5\ \mu g.kg^{-1}$) and 4 control groups were treated with saline. Their food intake was measured 30, 60 and 180 min, and 24 h after the treatment. Multiple ANOVA (model including factors day, sex, food intake and treatment) revealed that CCK decreased food intake of treated broilers, both males and females ($P < 0.01$), compared to that of the controls during the entire experimental period. On the other hand, the food intake of layers remained unaffected by this dose of CCK at all ages in both sexes. These findings indicate that the selection for different production traits also selects for factors involved in food intake control.

METABOLIC CHANGES IN CONTINUOUSLY IRRADIATED FEMALE RATS: MODIFICATION BY DIMETHYLBENZ/A/ANTHRACENE AND MELATONIN ADMINISTRATION. *I. Ahlers, E. Ahlersová, P. Solár, M. Sabol, M. Kassayová, B. Šmajda,* Institute of Animal Physiology, Faculty of Sciences, Šafárik University, Košice, Slovak Republic.

Within the frame of the project, dealing with mammary carcinogenesis induced by continuous irradiation, some parameters of lipid and carbohydrate metabolism in young virgin female rats were followed. 50-day-old female Wistar rats were continuously irradiated by a daily dose of 100 mGy of gamma rays for 15 days. Low (5 mg per animal) dose of 7, 12-dimethylbenz/a/anthracene (DMBA) was administered between 55 – 60th postnatal day to a part of the animals by the intragastric route, another part of animals drank melatonin (20 μg per ml of tap water) during the exposure. The analyses were done 48 h, 30 and 100 days after irradiation. DMBA administration increased serum glucose and lipid levels and increased the lipid content in the liver and thymus and the glycogen content in the liver, especially in non-irradiated rats. Myocardial glycogen and the concentration of triacylglycerols (TG) in the bone marrow were decreased by DMBA. The additional drinking of melatonin modified some DMBA-induced changes: the increase of liver TG and the cholesterol content and the decrease of myocardial glycogen and bone marrow TG were inhibited in both irradiated and non-irradiated rats. Melatonin did not alter DMBA-induced changes in other parameters, or (rarely) augmented them.

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KETAMINE: EFFECTS ON GLYCIDE METABOLISMUS AND ADRENERGIC LIPOLYSIS IN RATS. *D. Mišeková, D. Lincová, S. Hynie,* Institute of Pharmacology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

In our previous paper we have shown that ketamine, applied i.p. to adult rats of both sexes, decreased plasma cholesterol. This effect was

significant in female rats. We also found that *in vivo* injected ketamine enhanced the *in vitro* lipolytic response of isoprenaline. The aim of the present study was to determine whether ketamine also influences glycid metabolism and whether the potentiating effect of ketamine on adrenergic lipolysis is produced by its action on the adipose tissue. An i.p. injection of ketamine (100 $mg.kg^{-1}$ b.w.) to adult rats of both sexes significantly increased the glycogen content of skeletal and cardiac muscles not only during anaesthesia (10 min after injection of ketamine), but also in the post-anaesthetic period (30 min after injection of ketamine). The hepatic glycogen content was not changed 10 min after the administration of ketamine and no significnat changes were found in plasma glucose levels. Ketamine, in concentrations 30 and 100 $\mu g.ml^{-1}$, added directly to the incubation medium with rat epididymal adipose tissue, decreased the *in vitro* lipolytic response of isoprenaline. The results of our study suggest that i.p. injected ketamine does not influence only lipid but also glycid metabolismus in rats. In order to explain the opposite effect of *in vivo* and *in vitro* administered ketamine on adrenergic lipolysis in rat adipose tissue *in vitro*, further experiments will have to be carried out.

TOCOLYSIS AND FATTY ACIDS IN THE BRAIN AND BLOOD OF PREGNANT RATS AND THEIR FOETUSES. *J. Mourek, L. Šmídová, J. Baše,* Institute of Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

The beta-mimetic tocolytic preparation (Partusisten-Boehringer) was administered to six pregnant female rats subcutaneously on day 19, 20 and 21 of pregnancy (0.1 mg per 250 g of body weight). This dose corresponds to that administered in clinical practice. Pregnant controls (n=6) received saline only. On the 22nd day of pregnancy, the experimental and control pregnant females were decapitated. From the brain cortex and mixed blood as well as from the pooled brain tissue and blood of foetuses (4–5 foetuses), using gas chromatography (in Baše's modification), a wide spectrum of fatty acids (FA) was found. It was ascertained that the administration of the beta-mimetic tocolytic to pregnant rats significantly affected their spectrum of unsaturated FA of the n-3 series. The decreased of these FA in the blood of females as well as the significant deerease of n-3 unsaturated FA in the brain tissue of foetuses were found. We also confirmed our previous data concerning the effect of beta-mimetic tocolyticum (Partusisten) on the presence of unsaturated FA in the human newborn and their mothers (2,3). The deficiency of the n-3 unsaturated FA series endangers the future development in such tissues as the brain, retina, germinative tissue etc. The beta-mimetic (Tocolytic) preparation could stressed the general shortage of n-3 FA in immature babies endangered by preterm delivery. Subsequent recovery could therefore be complicated and the longlasting effects of such defects could be not eliminated.

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BOVINE SOMATOTROPIN (bST) TREATMENT *IN VIVO* AND CONTROL OF LIPOGENESIS BY INSULIN, bST AND CORTISOL *IN VITRO* IN ADIPOSE TISSUE OF GOATS. *J. Škarda, P. Krejčí,* Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Trials have been conducted on 4 placebo and 4 bST-treated young castrated male goats as described recently (1). The rate of lipogenesis was determined before and after culture by measuring the amount of (^{14}C)acetate incorporation into total lipids per mg protein of omental adipose tissue explants over 2 h incubation in a modified Krebs-Henseleit buffer containing sodium acetate alone (KHBA) or acetate with glucose (KHBAG) as the substrate. The lipogenesis in explants incubated before culture in KHBA was low (1.69 sothewr1.72 $nmol.mg^{-1}.h^{-1}$). Addition of glucose increased acetate incorporation into lipids in tissue from the controls to 56.2 and bST treated animals only to 40.2 $nmol.mg^{-1}.h^{-1}$ ($P<0.01$). Using chronic cultures (24 h) of adipose explants in modified Waymouth's medium, the *in vitro* effects of insulin (I), bST, cortisol (F), IF, IbST or IFbST were evaluated. The lipogenesis was always lower in explants from bST treated animals than from the controls. The addition of I resulted in increased lipogenic activity of explants incubated in KHBAG only in tissues from the controls. F alone decreased lipogenesis, but acted synergistically with I to enhance the rate of lipid synthesis more than when I alone had been used in both groups of animals. The addition of either bST alone or in combination with I or IF decreased lipogenesis in KHBAG in both groups of animals, but this decrease was more profound in tissues from bST-treated animals.

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EFFECT OF BOVINE SOMATOTROPIN TREATMENT *IN VIVO* ON THE EFFECTIVENESS OF CATECHOLAMINES IN LIPOGENESIS INHIBITION *IN VITRO* IN GOAT ADIPOSE TISSUE. *J. Slaba, J. Škarda, P. Krejčí,* Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Bovine somatotropin (bST) increases lipolytic responsiveness of adipose tissue to an *in vivo* adrenergic challenge. We studied the *in vitro* effect of noradrenaline and isoprenaline on lipogenesis in omental adipose tissue of castrated male goats treated *in vivo* with recombinant bST (1). The effectiveness of noradrenaline in lipogenesis inhibition in explants from control and bST-treated animals incubated before culture in the Krebs-Henseleit buffer containing only sodium acetate (KHBA) as substrate was higher (50.4 % and 68.9 % respectively) than that of isoprenaline (35.6 % and 41.5 %). However, in explants incubated in KHBA containing also glucose (KHBAG) as substrate the effectiveness of isoprenaline was higher (84.8 % and 83.1 %) than that of noradrenaline (66.2 % and 53.3 %) in control and bST-treated animals respectively. The responsiveness to noradrenaline was the same or increased in explants from control animals incubated in both KHBA and KHBAG after culture (24 h) in Waymouth's medium alone or with insulin (I), bST, IbST, I plus cortisol (F), IbST and decreased with F alone. In bST-treated animals, the responsiveness to noradrenaline decreased in KHBA and KHBAG in all cultures with the exception of culture with IbST incubated in KHBAG. The responsiveness of all cultures to isoprenaline was increased to the same level in explants of control and bST-treated animals incubated in KHBAG.

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EFFECT OF RECOMBINANT BOVINE SOMATOTROPIN (bST) ON THE PLASMA SOMATOTROPIN (ST) PROFILE, GROWTH AND CARCASS CHARACTERISTICS IN YOUNG MALE GOATS. *P. Krejčí, J. Slaba, J. Škarda, J. Picka,* Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

One of the new techniques for improving growth and carcass characteristics utilizes the administration of ST in growing pigs, sheep and cattle. Our trials have been conducted on 22 male Czech-white goats castrated during the first three weeks of postnatal life. Starting between 2 and 3 months of age, 11 animals received weekly s.c. injections of olive oil (control) and 11 animals received weekly recombinant methionyl bST in a sustained release vehicle (100 mg per animal) for 5 months. Blood plasma ST concetrations in the controls were 5.3 ± 1 $ng.ml^{-1}$ throughout the 7-day injection interval. In bST-treated animals (following the 20th application of bST), the concentration of ST in the plasma increased to 68.8 ± 25.0 $ng.ml^{-1}$ 24 h after the injection of bST and declined by 72 h postinjection to 10 ± 2 $ng.ml^{-1}$ and at this level remained till 144 h postinjection. Application of bST improved average body weight gain by 22 % and the relative weights of the liver, kidney and adrenal glands were increased 16, 19 and 20 % respectively. The fat content in m. longissimus dorsi and omentum was smaller in bST treated animals than in controls by 29.5 and 44.4 % respectively; the fat content of the liver, heart and kidney was not changed. The significant increases in protein content of the omentum can be attributed to the reduction of the fat content.

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DEVELOPMENT OF LIPOGENESIS IN PERIRENAL ADIPOSE TISSUE FROM KIDS. *J. Škarda, P. Krejčí, D. Müllerová, J. Hovorková,* Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

The rates of lipogenesis in perirenal adipose tissue explants of kids (Czech-white breed) were compared at the age of 2–3 days and 25–35 days. The rate of lipid synthesis was determined by measuring the amount of (^{14}C)acetate incorporated into total lipids expressed per mg protein of adipose explants over a 2 h period of incubation in a modified Krebs-Henseleit buffer containing as a substrate sodium acetate (KHBA; basal lipogenesis) or acetate with glucose (KHBAG; glucose-stimulated lipogenesis). In uncultured explants, both basal and glucose-stimulated lipogenesis were lower in perinatal than in older kids by 54.3 % and 92.5 % respectively. The addition of glucose to KHBA increased lipogenesis 3.2 times in perinatal kids and 19.9 times in older kids. Basal lipogenesis was not affected by catecholamines in adipose tissue (at that time brown one) of perinatal kids, while noradrenaline in older kids decreased lipogenesis by 51.9 % but isoprenaline had no effect. However, glucose stimulated lipogenesis was decreased by the addition of isoporenaline but not by noradrenaline in both perinatal and older kids. Lipogenesis was not affected in explants of perinatal kids incubated in KHBAG after culture (24 h) in Waymouth's medium with insulin (I), cortisol (F) or bovine somatotropin (bST) alone, but in older kids was stimulated by I

and inhibited by F. The effectiveness of catecholamines in lipogenesis inhibition was higher in explants cultured with bST or F, than in those cultured in a medium without hormones or with I.
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FREE OXYGEN RADICAL GENERATION DURING POST-ISCHAEMIC PERFUSION OF THE ISOLATED RAT HEART AS MONITORED USING VARIOUS TECHNIQUES. *H. Vavřínková, M. Tutterová, P. Stopka[1], M. Vidláková*, Institute of Clinical and Experimental Medicine and [1]Institute of Inorganic Chemistry, Czech Academy of Sciences, Prague, Czech Republic.

Oxygen-derived free radicals (FR) are regarded as important mediators of cell injury in the post-ischaemic heart. Their content in tissues is very difficult to determine because of their short life, low concentrations and the danger of artifacts when obtaining tissue samples and their preparation for measurement. Our experiments were designed to compare three methods used in FR generation monitoring: 1. Electron spin resonance (ESR); 2. Spin trapping using DMPO; 3. Malondialdehyde (MDA) measurement in cardiac extracts. FR were determined in isolated hearts of genetically hypertriglyceridaemic rats perfused according to Langendorff. The rats, weighing 250 g, were fed before decapitation for three weeks with a standard diet (SD), a high-saccharose diet (65 %) with 10 % lard (HS-L), and high-saccharose diet with 10 % fish oil (HS-FO). ESR and MDA determinations were performed in the heart at minute 7 of reperfusion after 20-minute ischaemia, after freezing in liquid N_2. DMPO-.OH adducts were assessed in perfusate of the same heart between minutes 1 and 5 of reperfusion. Recordings obtained using ESR revealed 3 signals of FR associated with substrate mitochondrial oxidation: signals $g=1.94$ and $g=2.005$ are assigned to mitochondrial dehydrogenases, and signal $g=2.0045$ to ubisemiquinone. Signals $g=2.025$ and $g=2.0045$ were 100 % higher in HS-FO rats than in SD and HS-L. Signals of radicals $.O_2$ and .OH cannnot be demonstrated without mathematical analysis of the recordings. MDA values were also significantly higher in HS-FO compared to HS-L and SD (9.7 ± 0.3, 7.9 ± 0.7 and 5.7 ± 1.5 respectively). By contrast, signals of DMPO-.OH adducts were the highest in HS-L. Under conditions of minimizing the possibility of artificial FR development, using ESR methods, we were able to detect the generation of FR compounds of the mitochondrial respiratory chain, probably giving rise to free oxygen redicals and, using MDA, to demonstrate that lipid peroxidation had occurred. When measuring DMPO-.OH adducts, we measure rather the FR generated on membranes since DMPO does not penetrate into the cells.

EFFECT OF ENTERAL ADMINISTRATION OF FAT EMULSIONS CONTAINING DIFFERENT AMOUNTS OF MCT ON THE COURSE OF LIVER REGENERATION IN PARTIALLY HEPATECTOMIZED RATS. *Z. Červinková, R. Svátková, L. Hadaš*, Department of Physiology, Faculty of Medicine, Charles University, Hradec Králové, Czech Republic.

The nutrtitional preference of lipids in the early phase of liver regeneration is a fact well known from the literature and also from our previous results. This study was aimed to assess the effect of lipid substrates rich in MCT (triacylglycerols containing medium chain fatty acids). These fatty acids were used because of their prompt accessibility for tissue oxidation, so they could provide energy necessary for liver regeneration. The experiments were carried out on male albino Wistar rats with initial body mass of 250–300 g. During the experiment, all rats had free access to a standard laboratory diet. Liver regeneration was induced by partial hepatectomy (PH) – resection of 2/3 of liver tissue. Fat emulsions of different content of MCT (Miglyol 812 – Dynamit Nobel, Germany; Lipofundin MCT/LCT 10 % – B. Braun Melsungen, Germany; mixture Lipofundin/Miglyol 812 in ratio 1/1) were administered by stomach tube twice a day started always immediately after PH, lasting till the end of the experiment. It was impossible in this experimental design to administrate isoenergetic amounts of lipids due to the fact that the energy content in the used fat emulsions is very different. Therefore, body weight and average food intake were also measured daily. The extent of liver regeneration 24, 48 and 72 hours after PH was checked by liver DNA synthesis, total content of liver DNA, liver weight and the mitotic activity of hepatocytes. Respiratory activity of isolated liver mitochondria was also measured. Fat emulsions in all experimental groups caused an increase of liver regeration markers in comparison to control rats which received saline. The most effective was Miglyol 812 – pure MCT-oil. The acceptability of using different fat emulsion in order to support liver regeneration is discussed in our contribution.

5-BENZYLOXY-2-THIOCYANATOMETHYL-4-PYRANONE: A NEW COMPOUND WITH AN INHIBITORY EFFECT ON PROLIFERATION OF MURINE LEUKEMIA CELLS L1210. *J. Bransová, J. Brtko, M. Uher[1]*, Institute of Experimental Endocrinology, Slovak Academy of Sciences and [1]Department of Organic Chemistry, Faculty of Chemical Technology, Slovak Technical University, Bratislava, Slovak Republic.

We report a new chemical 4-pyranone compound 5-benzyloxy-2-thiocyanatomethyl-4-pyranone which possessses properties of biologically active prepartions with antileukemic activity. 5-benzyloxy-2-thiocyanatomethyl-4-pyranone was prepared from 5-hydroxy-2-hydroxymethyl-4-pyranone according to Uher *et al.* (1). While 5-benzyloxy-2-thiocyanatomethyl-4-pyranone had no effect on cell growth of L1210 murine leukaemia, a significant inhibitory effect of 5-benzyloxy-2-thiocyanatomethyl-4-pyranone was found on cell proliferation. The dynamics of cell proliferation were evaluated by cell counting, and the trypan blue exclusion test was used for cell viability determination. The IC_{50} value of 5-benzyloxy-2-thiocyanatomethyl-4-pyranone (2.6 μM) was extrapolated from the growth inhibition curve at compound concentrations ranging from 0.1 μM to 100 μM. Effects of the compound studied on 1. the activity of cAMP independent protein kinases and 2. DNA, RNA and protein synthesis in L1210 murine leukemia cells were evaluated *in vitro*. Since the effect of 4-pyranone derivatives on growth of neoplastic cells have not yet been described, our preliminary experiments suggest that 5-benzyloxy-2-thiocyanatomethyl-4-pyranone may represent a new compound among 4-pyranone derivatives with antileukemic activity that might advantageously be used as a constituent of biologically active preparations.
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METABOLIC RESPONSE TO NONCONVENTIONAL FOOD IN THE DIET OF SHEEP. *Z. Zeleňáková, J. Várady, I. Zeleňák*, Department of Physiology, University of Veterinary Medicine and Institute of Animal Physiology, Slovak Academy of Sciences, Košice, Slovak Republic.

Ruminant animals are able to ferment and utilize such kind of food that other animals and people are not. This is important especially from the point of view of such new potential food sources as wooden materials. The aim of this research was to investigate the effect of wood sawdust included into the diet fed to sheep on some representative rumen microorganisms, volatile fatty acid (VFA) production and the molar percentage of individual acids. The results showed that the numbers of Lactobacillus sp., total bacteria and protozoa were lower in animals fed the diet with 20 % treated beech sawdust. The numbers of sp. Enterobacterium and Streptococcus in control and experimental animals were not different. In the experiment with 5, 10, 15, 20 % untreated beech sawdust in the ration fed to sheep the concentration of total VFA in the rumen of control animal was the highest. In mol% of acetate, there were no significant differences between control and experimental animals with the exception of those receiving 10 % sawdust when it was higher 7 h after feeding ($P<0.02$). Mol% of propionate was higher with diets containing 5 and 15 % sawdust while in diets containing 10 and 20 % sawdust it was slower 7 h after feeding. Mol% of butyrate was significantly lower with diets containing 5 % ($P<0.05$) and 15 % ($P<0.01$) proportions of sawdust. It can be concluded that beech sawdust included in the diets of sheep partly influenced the microbial populations and VFA production in the rumen.

DIFFERENTIAL REACTIONS TO DEPRIVATION IN THE EARLY ONTOGENY IN TWO RAT STRAINS. *J. Záhlava, J. Hassmannová[1], J. Mysliveček*, Institute of Pathophysiology, Faculty of Medicine, Plzeň and [1]Third Faculty of Medicine, Charles University, Prague, Czech Republic.

Development of the conditioned appetitive reaction, namely the pup's approach to the anaesthetized dam differs in the Long Evans (LE) and Wistar (W) strains (1). In the present communication, the speed of the pup's approach to the dam and its oxygen consumption of 8-hour and 24-hour mother deprivation (sensory and nutritional) was compared in 11-day-old pups. After 8-hour deprivation, the pigmented LE pups learned to approach the anaesthetized dam most rapidly, whereas, after the 24-hour deprivation, their approach was much slower. In the albinotic W pups, there were practically no differences in the approach speed after the two deprivation durations. After the 8-hour deprivation, the oxygen consumption was significantly increased as compared with the initial value in the LE pups, which might underlie the increased speed, whereas in the W pups it was increased. After 24-hour deprivation, the oxygen consumption in both strains was reduced. Although a causal relation between the two phenomena cannot be definitely proved, we conjecture that in LE pups after the 24-hour

deprivation exhaustion of metabolic resources has taken place which brings about a slowing down of the approach to the dam.
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DEVELOPMENT OF SOLUTE TRANSPORT IN EMBRYONIC NEPHRONS. *Z. Zemanová, E. Ujec,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

The distal nephron in 7-day chick embryos is equipped with a sodium pump and exhibits a higher transtubular potential (TP) than the proximal tubule, thus indicating a possibility of its use in reabsorption of Na^+ (2). Other data on transport properties of embryonic nephrons in the chick mesonephrons are lacking. For this reason, we have studied the development of tubular functions in relation to the differentiation of mesonephrons. Our aim was to access the way in which the TP develops in individual segments of the oldest population of nephrons situated on the surface of the kidney using the method published previously (3) and to correlate the results with K^+-NPPase activity detected histochemically (1). We showed that the proximal tubule mostly exhibited a lower TP than the distal and collecting tubules and that this difference increased during development. An analogous increase of TP was recorded in the allantoic sac (structure homologous to the urinary bladder) and in the Wolffian duct, through which urine is conveyed there. The K^+-NPPase activity was demonstrated in distal and collecting tubules as well as in the Wolffian duct wall at all the stages studied. The results indicate that, in the embryonic mesonephric nephrons, not only filtration of solutes and water might take place but also their resorption. The proximo-distal gradient of TP supports the assumption that the course of mesonephric nephron maturation is analogous to the mammalian metanephrons.
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V. Muscle and Sports Physiology

ANAEROBIC POWER AND CAPACITY IN 30 s AND 60 s ALL-OUT TESTS (WINGATE) IN TRIATHLETES. *J. Heller, J. Novotný, V. Bunc[1], J. Horčic[1],* Biomedical Research Centre and [1]Sports Research Centre, Faculty of Physical Education and Sport, Charles University, Prague, Czech Republic.

The classical Wingate test (30 s) seems to be brief to exhaust the anaerobic energy stores, however, the longer the duration of the test, the more aerobic it is. The aim of the present study was to compare the maximal anaerobic power (AP) and anaerobic capacity (AC) from 30 s and 60 s Wingate tests in endurance athletes, non-trained specifically for anaerobic performances. Altogether, 19 triathletes, both males (n = 11, age 18 ± 2 years, VO_2max 72 ± 4 ml.min^{-1}.kg^{-1}, lactate (LA) max 12 ± 2 mmol.l^{-1}) and females (n = 8, age 17 ± 2 years, VO_2max 61 ± 4 ml.min^{-1}.kg^{-1}, LAmax 10 ± 2 mmol.l^{-1}) were tested for VO_2max on a treadmill and for both 30 s and 60 s all-out cycle ergometer tests (Wingate). In males, AP in the 30 s test was higher than in 60 s test ($p < 0.01$, 14 ± 1 and 12 ± 1 W.kg^{-1}, respectively), similarly to females (10 ± 1 and 9 ± 1 W.kg^{-1}, respectively)]. AC in 30 s supramaximal exercise was lower ($p < 0.01$) than in 60 s exercise (males: 341 ± 30 and 531 ± 55 J.kg^{-1}; females: 259 ± 23 and 442 ± 43 J.kg^{-1}, respectively) as well as the peak blood LA (males: 10 ± 1 and 12 ± 1 mmol.l^{-1}, females: 10 ± 1 and 11 ± 1 mmol.l^{-1}). The corresponding values of AP and AC correlated poorly with each other and the rate of LA production in the 30 s test was higher (0.44 mmol.l^{-1}.kJ^{-1} in males and 0.63 mmol.l^{-1}.kJ^{-1} in females) than in the 60 s test (0.34 and 0.44 mmol.l^{-1}.kJ^{-1}, respectively), corresponding thus to the sex differences in VO_2max. Prolongation of the exercise from 30 to 60 s only exhibited low LA production of 0.14 and 0.15 mmol.l^{-1}.kJ^{-1} (in males and females, respectively), indicating an important increase of aerobic energy metabolism. It could be concluded that the 60 s Wingate test understimulates maximal AP and is not sufficiently advantageous for the determination of AC, especially in endurance athletes.

ENERGY EXPENDITURE OF RUNNING IN SUBJECTS DIFFERING IN BODY FAT. *V. Bunc*, Faculty of Physical Education and Sport, Charles University, Prague, Czech Republic.

The utilizable sources of energy in man are relatively restricted, whereas interindividual differences are not substantial. The differences in physical performance are extreme. The energy requirements of running, as one of the basic human locomotor activities, may be characterized by the energy cost coefficient c, which indicates how much energy is needed to carry the body mass of 1 kg over a distance of 1 m. It is generally accepted that the lower is c, the better is the capacity to transform chemical energy of human into mechanical work. By human's physical activities, the mechanical energy is mainly used for transfer of the body over a certain distance, an inactive body mass may be one of the limiting factors of physical performance. The aim of this study was to assess the influence of body fat on the energy expenditure of running. The coefficient *c* was determined by running on a treadmill in a group of men (n = 54, mean age 42.1 ± 6.3 years, body mass 44.7 ± 8.3 kg, height = 175.7 ± 9.2 cm) with varying amounts of physical activity, differing significantly in percentage of body fat (% BF) (15.9 – 27.2 %). The % BF was assessed by means of 10 skinfold measurements. The lowest values of *c* were found in subjects with the lowest % BF (*c* ranged from 3.92 to 4.22 J.kg^{-1}.m^{-1}). We found a significant positive correlation of *c* and % BF ($r = 0.806$, $c = 0.024$*% BF + 3.579) and close negative correlation of VO_2max.kg^{-1} and % BF ($r = -0.883$, VO_2max.kg^{-1} = −1.598*% BF + 74.721). It can be concluded that with increasing % BF, the energy cost of running increases, the prerequisities for effective use of human energy potential are reduced and the work capacity of the organism thus declines.

LATERAL PREFERENCES OF EFFECTIVE FORCE ON THE ISOKINETIC CYCLE ERGOMETER. *J. Lipková, L. Hamarová, D. Noga,* Faculty of Physical Education and Sports, Research Institute of Sport Sciences, Comenius University, Bratislava, Slovak Republic.

The aim of the present study was to detect and quantify lateral differences between the force of the right and left leg during cycling and to evaluate their relationship to the parameters of laterality. Twenty-seven children – pupils of a primary school (mean age 13.07 ± 0.28 years) were tested on a fully computerized isokinetic cycle ergometer. The isokinetic principle makes it possible to perform the time and position analyses of the effective pedal force and power produced at various revolution rates. Laterality was tested by the test of Matějček and Zlab and expressed in a percentage score. The results indicated that the differences between the average as well as the maximal force of the stronger and weaker limb were significant. Correlation analysis suggested that there was no relations between the score of laterality and the functional deficit. The result indicates that the differences between the force production by a certain leg does not depend on laterality as that detected by physiological methods.

MODAL GATING OF THE CARDIAC SARCOPLASMIC RETICULUM CALCIUM RELEASE CHANNEL. *I. Zahradník, A. Zahradníková,* Laboratory of Electrophysiology, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Three modes of activity above the resting state were revealed in gating of the cardiac sarcoplasmic reticulum calcium release channel in planar lipid bilayers. The two open modes, M1 and M2, differ by one order of magnitude in their opening probability. The difference is due to the dominance of short ($\tau_o = 0.38$ ms), isolated openings in mode M1 opposed by long ($\tau_o = 2.77$ ms), aggregated-burst forming openings in mode M2. In mode 0, the channel has a bound calcium ion but is not available for opening. Mode lifetimes are on the order of 1 s (0.61, 1.60 and 0.86 s for modes 0, 1 and 2, respectively). Intermodal transitions are almost exclusively between modes M1 <---> M0 and M1 <---> M2. Direct intermodal M2 <---> M0 transitions could not be proved in either direction. These findings can be explained by a gating scheme in which the channel upon binding Ca^{2+} first enters fast-access states of the high-open-probability mode 2 and only then it can equilibrate into the slow-access states of the modes 1 and 0 (in series). This interpretation of the observed patterns of channel gating can explain several features of calcium release channel behaviour, such as channel inactivation and modulation by drugs and cell metabolism.

ANALYSIS OF MODELS DESCRIBING CALCIUM CURRENTS IN ISOLATED CARDIOMYOCYTES. *J. Pavelková, I. Zahradník, A. Zahradníková,* Laboratory of Electrophysiology, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic.

The time course of L-type Ca channel currents was analyzed in cardiomyocytes contracting to stimulation. The currents were recorded from isolated rat ventricular myocytes using the whole-cell patch clamp technique under conditions yielding pure Ca currents. Calcium currents were approximated by different models using software written for an IBM RISC/6000 computer with use of the IMSL library. The currents could not be approximated by equations based on a Hodgkin-Huxley type model, since they were inactivated by two independent processes. Incorporation of another inactivation

exponential term enabled an adequate fit, however, it provided non-realistic values of the paramaters. The fast component of inactivation could not be described by current- or voltage-dependent mechanisms (either singly or in combination), as has already been described for non-contracting myocytes. The only suitable solution could be reached using a model implementing Ca current inactivation by calcium ions released from the sarcoplasmic reticulum. The model assumes, in agreement with the morphological features of the tubulo-reticular junction, that only a portion of the channels is exposed to action of Ca ions released from the terminal cisternae. The entire time course of the calcium current could be best approximated by models with the exponent of activation variable m = 4.

STEREOLOGICAL MEASUREMENTS OF THE AREA AND NUMBER OF MUSCLE FIBRES IN CROSS-SECTIONS OF RAT SOLEUS MUSCLES. *G. Zacharová, L. Kubínová,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

At present methods based on image analysis belong to those most widely used in 2-D muscle morphometry. However, the main difficulty is fully automatic identification (segmentation) of individual muscle fibres. Therefore, we have applied stereological methods, which have already proved to be effective in many types of biological materials, for the estimation of muscle parameters in cross-sections of rat soleus muscles. Our results show that two-dimensional stereological methods using the principles of point counting and unbiased counting frames can be reliably employed and with high efficiency for the estimation of cross-sectional muscle area, number of fibre profiles, and total and mean area of fibre profiles of each fibre type. Their precision is sufficient and is comparable with that achieved by conventional manual and image analysis methods. Two-dimensional stereological measurements can be performed directly on specimens under the microscope and do not require expensive technical equipment in the simplest implementation. Furthermore, unbiased results are obtained, no segmentation and edge effect problems arise and the quantity of work invested in stereological estimation is reasonable.
Supported by grants from the Academy of Sciences of the Czech Republic (No. 511103 and No. 71158).

IV. Endocrinology

EFFECT OF COLD STRESS ON CATECHOLAMINES, BEHAVIOUR AND MILK COMPOSITION OF DAIRY COWS. *J. Brouček[1], C.W. Arave[2], Y. Nakanishi[3], P.H. Stewart[2], Š. Mihina[1],* [1]*Research Institute of* Animal Production, Nitra, Slovakia, [2]Utah State University, Logan, USA and [3]Kagoshima University, Kagoshima-shi, Japan.

Thirty Holstein cows were randomly assigned to two housing treatment groups. The first group (outside) was kept in an open barn and the second group (inside) in a warm enclosed barn. The experiment lasted twelve weeks. An average of the minimum temperatures in the open barn was lowest during the eighth week (−19.4 °C) and during the seven out of twelve weeks of the experiment averages below −10 °C were recorded. The averages of noradrenaline (1290 pg/ml vs. 981 pg/ml) and dopamine levels (708 pg/ml vs. 510 pg/ml) were slightly higher in outside cows than those inside. On the contrary, the levels of adrenaline were higher in the second group from warm housing (562 pg/ml vs. 883 pg/ml). During three 24 h observation periods, cows from both groups spent longer periods of time lying on their left than on their right side. During the first observation (coldest temperature), the outside cows spent significantly more time eating than the inside cows (15.5 % vs. 8.5 % of total time). Outside cows were observed ruminating slightly longer than inside cows (32.6 % vs. 30.6 %). The frequency of meals was greater for both groups during the third observation. The duration of standing as well as of lying down did not differ between the groups. Feeding behaviour appeared to be most affected by the type of housing. In both groups, the fat content had a similar trend. A significant increase was recorded during the sixth week. The protein content the milk in the first group was significantly higher during the whole experiment. The contents of lactose and non-fat solids expressed in percentages had a similar trend, with a rapid decrease during the sixth week. The concentrations of these parameters were slightly lower in the outside group compared with the inside group. The somatic cell count was higher in the first group during the whole period of observation.

EFFECT OF ATRAZINE ON GLUCOCORTICOID RECEPTOR BINDING IN THE RAT LIVER. *M. Alexandrová, M. Vargová[1],* Institute of Experimental Endocrinology, Slovak Academy of Sciences and [1]Institute of Preventive and Clinical Medicine, Bratislava, Slovak Republic.

The herbicide atrazine, used in great quantities for treating corn fields might negatively affect any body tissue through contaminated drinking water. The effect of short term administration of atrazine on glucocorticoid receptor binding and tyrosine aminotransferase (TAT) activity was studied in the rat liver. Adrenalectomized rats were treated trazine orally in two different doses (8 mg or 12 mg/100 g) for 14 days. The animals were killed on day 15 and the glucocorticoid receptor concentration, TAT activity and TAT inducibility with dexamethasone were measured in rat liver cytosol. The higher dose of atrazine significantly inhibited ^{3}H-dexamethasone binding to the glucocorticoid receptor in liver cytosol, while the lower dose was without effect. The apparent dissociation constants as calculated by Scatchard analysis were in the nM range and remained unchanged. Atrazine did not affect basal TAT activity. In spite of the fact that a higher dose of atrazine inhibited ^{3}H-dexamethasone receptor binding almost by 30 %, TAT inducibility by dexamethasone was not affected. These results suggest that the concentration of glucocorticoid receptor in the liver did not fall below treshold levels and that functioning receptors in the cells were present in amounts sufficient for triggering the cascade of biochemical events followed by a biological response.

CIRCANNUAL FLUCTUATIONS OF FREE TESTOSTERONE CONCENTRATION IN MEN AND WOMEN. *D. Ostatníková, Z. Putz[1], P. Matějka[1],* Institute of Physiology, Faculty of Medicine, Bratislava, and [1]Endocrinological Institute, Ľubochňa, Slovak Republic.

Only a free fraction of testosterone, not bound to sex-hormone binding globulin, is present in the saliva, since saliva is an ultrafiltrate of the plasma. It is assumed that only free testosterone is biologically active and can be taken up and metabolized by target tissues including the brain. We investigated the circannual fluctuations of salivary testosterone in both sexes. Forty-seven healthy students (age 20 – 22 years) participated in our study. The saliva was collected in April and in November of the same year, during the same time of the day, under constant conditions. Radioimmunoassays were used to determine the concentrations of free testosterone. We found significant differences in testosterone levels between men and women both in the spring and autumn. November levels were higher than April levels in both sexes. There was a significant difference between testosterone levels in women obtained in the spring and autumn, but the difference in hormonal levels in men were not significant. As it is known that the free testosterone fraction is in close correlation to plasmatic testosterone concentrations, these results confirm other studies concerning circannual fluctuations of testosterone in the plasma.

POSTNATAL DEVELOPMENT OF 11β-HYDROXYSTEROID DEHYDROGENASE (OHSD) IN THE RAT INTESTINE. *J. Pácha, I. Mikšík,* Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic.

The developmental pattern of OHSD was studied in various intestinal segments. Slices of the duodenum, jejunum, ileum, caecum, proximal and distal colon were incubated *in vitro* in the presence of corticosterone (B) or 11-dehydrocorticosterone (dehydro-B). 11β-dehydrogenase activity (B to dehydro-B) was very low in proximal parts of the intestine, medium in the ileum and caecum and high in the proximal and distal colon. Significant dehydrogenase activity was already measured 2 days after birth and increased moderately during the suckling period. In contrast, 11-oxo-reductase activity (dehydro-B to B) was negligible both in the immature and adult intestine. Hypothyroidism, induced by drinking a methimazole solution instead of water, significantly decreased OHSD in 24-day-old rats. Replacement therapy of these animals with T_3 stimulated OHSD in hypothyroid animals. We conclude that 1) intestinal OHSD is active not only in adulthood but also in the immature intestine the maturation of which is regulated by glucocorticoids, 2) OHSD may decrease but not increase corticosterone availability in the intestinal epithelium and 3) thyroid hormones may be important in the regulation of OHSD.
This study was supported by grant No. 305/94/1719 from the Grant Agency of the Czech Republic.

CHANGES IN THE HYPOTHALAMUS AND EPENDYME IN THE THIRD VENTRICLE OF THE BRAIN IN SHEEP AFTER SUPEROVULATION. *A. Staníková, J. Halagan V. Rajtová, B. Pástorová, M. Molnárová, V. Eliáš, J. Várady,* Department of Physiology, University of Veterinary Medicine, Košice, Slovak Republic.

Our attention was directed to the changes in neurosecretion and the study of changes in the cellular nucleus volume of neurones from the nucl. supraopticus, nucl. infundibularis and nucl. tuberomamillaris in sheep after hormonal stimulation. Twenty eight sheep in the anoestrous period were used. They were synchronize with Agelin and hormonally stimulated with serum gonadotrophin, Oestrophan and Folistiman.The ependymal lining was studied under the scanning electron microscope. In the rostral hypothalamus the neurosecretory structures were slightly stimulated to increased neurosecretion by the hormonal influence. More pronounced changes occured in the central hypothalamus. No qualitative changes were found in the caudal hypothamus in the analysed nucleus. The evaluation of karyometric analysis, i.e. the changes in the volumes of hypothalamic nuclei observed, correlated with neurosecretion. The ependyma in anoestrous sheep is monolayered, cubic up to cylindrical. After hormonal stimulation, its focal repletion occurred in the suprachiasmatic and infundibular area. Local proliferation of the tanycyte ependyma occurs in the recessus infundibuli. Ependymocytes desquamated and were released into the cerebrospinal fluid. Because the ependyma in the central part of the hypothalamus reacted very sensitively to all processes connected with sexual activity under physiological conditions, the effect of the administered hormones was most probably only indirect, acting *via* the hypothalamus or ovaries.

CATECHOLAMINE LEVELS AND ACTIVITY OF MONOAMINE OXIDASE IN OESTRUS EWES AFTER TREATMENT WITH PMSG AND ANTISERGONE. *B. Pástorová, J. Halagan, A. Staníková, M. Molnárová, J. Várady, V. Eliáš,* Department of Physiology, University of Veterinary Medicine, Košice, Slovak Republic.

The effects of 1000 IU serum gonadotrophine (PMSG) on the catecholamine levels (norepinephrine and dopamine) and monoamine oxidase activity (MAO) in the sheep hypothalamus (area preoptica, eminentia mediana, corpus mamillare) were studied by radiochemical assays. We attempted to eliminate the negative effects of PMSG by administration of anti-PMSG serum (Antisergon) 24 hours after PMSG. In the area preoptica of the sheep hypothalamus, the hormonal stimulation significantly reduced dopamine concentrations ($p<0.05$) in both experimental groups. The administration of Antisergon (As) did not exhibit any appreciable effect in the given area. A significant increase in dopamine levels ($p<0.001$) was recorded in the corpus mamillare and median eminentia of sheep treated with 1000 IU PMSG. An insignificant increase in the concentration of norepinephrine (NE) was recorded in the area preoptica. The most marked changes in the concentration of NE ($p<0.001$) were observed after PMSG application in the corpus mamillare. After combined administration of PMSG with As changes in catecholamine levels in the hypothalamus were not pronounced. In the corpus mamillare, a significant decrease ($p<0.01$) in MAO activity occurred after PMSG administration. These changes are considered to be connected with an increase in oestrogens after treatment with PMSG. The results obtained after PMSG and As confirm this assumption.

THE INFLUENCE OF GAMMA IRRADIATION ON PINEALOCYTE NUCLEI VOLUME AND OVARY FOLLICULAR ATRESIA IN SHEEP. *J. Halagan, A. Staníková, B. Pástorová,* Department of Physiology, University of Veterinary Medicine, Košice, Slovak Republic.

The changes of pinealocyte nuclei volume and atresia of antral follicles in the ovary of merino ewes were studied after exposure to 4. 8 Gy of gamma irradiation for 5 days in the anoestrous period. The trial was performed in May on 24 ewes, divided into four groups as follows; 1) intact – nonirradiated ewes (5); 2) irradiated ewes, fed an ordinary diet (8); 3) irradiated ewes on a diet with a polyvitamine supplement (8); and 4) ovariectomized ewes (3). Irradiated ewes were killed 5 or 10 days after irradiation. The pineal gland and ovary were perfused and immersed in paraformaldehyde, and stained with haematoxylin – eosine. Histological changes were evaluated under the light microscope. The pinealocyte nuclei volume was estimated by direct karyometric evaluation of 100 pinealocyte cells after 3000 x magnification on a Plotter Tablet using the Complete Computer System evaluation Program (JADRO). The atretic and non-atretic antral follicles were subjected to quantitative histological differentiation (1). The number of non-atretic follicles was found to have decreased significantly in the irradiated ewes of group 2 (37 % : 13 % : 19 %). A significant decrease of the pinealocyte nuclei volume in the ewes after irradiation was also confirmed (226. 91 nm^3:133. 31 nm^3). The administration of vitamins after irradiation seems to reduce the occurrence of atresia but it was not effective to prevent this inhibitory effect on pineal gland.

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BINDING CHARACTERISTICS OF INSULIN RECEPTORS IN RAT ADRENAL GLAND. *Š. Zórad, M. Ficková, M. Rusnák, P. Blažíček[1], R. Kvetňanský, L. Macho,* Institute of Experimental Endocrinology, Slovak Academy of Sciences and [1]Military Hospital, Bratislava, Slovak Republic.

Until recently only limited data have been available with regard to insulin (I) binding in the adrenal (A) gland. Specially in the rat, the presence of I receptors (IR) in A tissue was doubtful (1). We developed a sensitive assay for determination of I binding in A plasma mebranes. The I binding reached an equilibrium after 5 h of incubation at 10 °C. The binding was displaced by unlabelled I in a similar manner as in liver membranes and adipocytes (Kd in the nM range) and was present in both the A medulla and cortex. In addition, the pattern of displacement of I binding by desoctapeptide I, IGF 1 and IGF 2 was similar in the A gland and liver suggesting the presence of specific IR in A tissue. However, in the A gland, the pH optimum for I binding differed from that in the liver; 6 vs. 7.5. Moreover, immobilization stress (single IMMO for 2 h) upregulated the IR in the A gland despite increased plasma I level. Under the same conditions, the IR in adipocytes were downregulated. Single I treatment (5 I.U. per 1 kg of body weight) also downregulated the IR in adipocytes, but no change was noticed in A plasma membranes. In conclusion, we characterized the I binding sites in the rat A gland which seem to be very similar to those in typical I sensitive tissues. The physiological significance of different pH profile of I binding and the different pattern of IR regulation in the A gland is unknown and awaits for further investigation.

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THE EFFECT OF PREMATURE WEANING ON INSULIN AND GLUCAGON RECEPTORS IN THE RAT LIVER. *L. Macho, M. Ficková, Š. Zórad,* Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

During the weaning period, young mammals consume food with a decreasing proportion of fat from milk and an increasing content of carbohydrates from solid diet. In was demonstrated, by using glucose clamp studies, that hepatic insulin resistance is present in suckling rats and this disappears after weaning (1). Because this could be related to changes in the number of insulin receptors, the aim of our experiments was to study the effect of early weaning on insulin and glucagon binding in rat hepatocytes. The number of newborn rats per litter was adjusted to 8 rats on the second postnatal day. Early weaned rats were separated on the 18th postnatal day, control animals remained with a lactating dams up to the 30th day. Plasma insulin and glucose levels, and insulin binding capacity of isolated hepatocytes were determined at the age of 18, 22, 30 and 120 days. In some age groups, the degradation of insulin and the binding capacity for glucagon were also determined. A decrease of plasma insulin and glucose levels was found in 22-day-old prematurely weaned rats as compared to 18-day-old and to controls of the same age. The age-dependent increase of insulin binding was observed in hepatocytes from 18 to 30-day-old control rats. However, a highly significant elevation of insulin binding capacity was found in prematurely weaned animals 22-days-old. At the age of 30 and 120 days, no significant differences in insulin binding capacity of hepatocytes from prematurely weaned and control rats were noted. A decrease of insulin degradation was observed in 22-day-old prematurely weaned rats. The values of glucagon binding to hepatocytes were similar at the age of 30 days in control and premature weaned rats. These results showed that a sudden change in the diet in prematurely rats affected the insulin binding capacity of hepatocytes.

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EFFECT OF 3,5,3'-L-TRIIODOTHYRONINE ON THE HEPATIC ORNITHINE DECARBOXYLASE AND THYMIDINE KINASE ACTIVITY IN THE RAT AFTER PARTIAL HEPATECTOMY. *J. Knopp, J. Brtko, M. Juráni, I. Jaroščáková, J. Jurčovičová,* Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Partial hepatectomy (60 %) caused a biphasic increase (one after 4 h and the second after 48 h) of ornithine decarboxylase activity in the rat liver remnant. Daily administration of 3,5,3'-L-triiodothyronine (T3) (10 μg/100 g) to rats for seven days before partial hepatectomy had no effect on enzyme activity. After five days, ornithine decarboxylase

activity declined to control levels (sham controls) and its activity was significantly enhanced by T_3. Ornithine decarboxylase gene expression in the rat liver examined by Northern blot analysis using poly A+ mRNA began to increase 4 h after partial hepatectomy, remained elevated for 48 h, depressed after five days and was not altered by T_3 treatment. The thymidine kinase activity increased progressively after partial hepatectomy, however, its peak value was delayd by T_3 administration. Plasma prolactin levels increased within 5–15 min of liver resection, then declined to control values and again increased 24 h after the surgery. The data demonstrate that the changes in ornithine decarboxylase activity in the rat liver after partial hepatectomy might be a result of the direct effect of prolactin on the activity of the enzyme rather than its induction by the hormone. Triiodothyronine administration altered both ornithine decarboxylase and thymidine kinase activities suggesting that T_3 appears to regulate ornithine decarboxylase activity at the posttranslational level.

THE EFFECT OF 3,5,3'-L-TRIIODOTHYRONINE ON SURVIVAL, CELL MORPHOLOGY AND INTRACELLULAR CALCIUM CONCENTRATION OF EMBRYONIC RAT SEPTAL AND HIPPOCAMPAL NEURONES. *H. Saito[1], P. Filipčík, H. Katsuki,* Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic and [1]Department of Chemical Pharmacology, The University of Tokyo, Tokyo, Japan.

The effect of 3,5,3'-L-triiodothyronine (T_3) on survival, cell morphology and intracellular calcium concentration ($ICCa^{2+}$) was studied in primary cultured neurones of the foetal rat brain. Under defined conditions of serum-free culture media we found that high concentrations of T_3 in hippocampal and septal neurones cultivated at high initial densities of plating (10^5 cells/cm^2) prologend the survival in pure neuronal populations. Moreover, the septal neurones also responded positively at normal physiological concentrations of T_3 (1 nM) in low density cultures ($5x10^3$ cells/cm^2). The supporting effect of glial cells under such conditions was eliminated. The treatment of septal neurones with 10 nM of T_3 caused statistically significant changes in their morphology especially in axon elongation (194.5 ± 15.7 % in comparison with the control neurones). In the artificial neuronal network of hippocampal neurones growing 15 days *in vitro*, a modulating effect of T_3 on intracellular calcium levels after depolarization by a high concentration of KCl (50 mM) was observed. T_3 and 1.0 μM inhibits the KCl-induced increase of $ICCa^{2+}$ by about 29.1 ± 11.8 %. These findings suggest that the direct effect of T_3 on a pure population of septal and hippocampal neurones derived from embryonic rat brain and support the evidence about the role of this peripheral hormone in neuritogenesis and neurotransmission.

DISTINCT DOSE-RESPONSE RANGES IN REGULATION OF DIFFERENT BIOLOGICAL RESPONSES IN RAT PITUITARY GH_4C_1 CELLS BY 3,5,3'-L-TRIIODOTHYRONINE (T_3). *P. Filipčík, J. Brtko, V. Štrbák,* Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

The GH_4C_1 pituitary cell line is generally considered as an excellent model for the study of thyroid hormone action. The cells respond to T_3 in several different ways. We have studied the relationship between thyroid hormone receptor occupancy (THRO) and intensity of several biological responses: a) cell proliferation; b) prolactin (PRL) production; c) thyrotropin (TSH) inhibition, in a wide range of T_3 concentrations. The intensity of none of the observed responses was directly proportional to thyroid hormone receptor occupancy. The most sensitive response to T_3 seems to be cell growth, followed by stimulation of PRL production and TSH inhibition. The concentration of T_3 and percentage of the THRO corresponding to maximal intensity of the above biological responses were as follows: a) the maximal growth rate was achieved at 0.1 nM T_3 corresponding to T_3 receptor occupancy of less than 20.0 %; b) the maximal stimulation of PRL production was observed at 1.0 nM T_3 which corresponds to 66.0 % of THRO; c) inhibition of TSH production occurs at 20.0 nM of T_3 when more than 95.7 % receptors are occupied by T_3. We conclude that each of the observed biological events operates over distinct dose-response ranges in cultured GH_4C_1 cells. These data also suggest that this cell line is a suitable model for clarifying the mechanism leading to different sensitivity of pituitary cells to T_3.

SEASONAL INFLUENCES ON THE SUPEROVULATION RESPONSE AND ANTIPROTEOLYTIC BLOOD PLASMA ACTIVITY OF EWES. *M. Molnárová, J. Arendarčik, P. Molnár, A. Staníková, B. Pástorová, J. Várady, V. Eliáš,* Department of Physiology, University of Veterinary Medicine, Košice, Slovak Republic.

Merino ewes do not produce such a strong anoestrous period as those of other breeds. However, application of vaginal sponges impregnated with Ageline (Ag 20 mg chlorsuperlutine/animal) during the anoestrous period increased the ratio of atretic to no-atretic terciary follicles (A/N) from 1.85 to 2.21, but as a result the mean number of ovulations was higher after 1000 IU PMSG (2.6 ± 1.7 – 5.7 ± 4.4). During oestrous, after Ag, 1000 IU PMSG changed A/N from 3.6 to 3.5, while the mean number of ovulations increased from 0.8 ± 0.8 to 2.5 ± 1.6. After synchronization with prostaglandine F_2a,(2x125 lg) A/N = 1.29, after 1000 IU PMSG A/N = 1.83, and after synchronous application of 50000 IU of vitamin A with 1000 IU PMSG decreased A/N = 0.95 and the mean number of ovulations increased (1.6 ± 0.5; 2.6 ± 1.9; 3.6 ± 3.1). Antiproteolytic activities (Aa) of blood plasma and the cervical mucus were influenced differently by synchronization and stimulation in both the anoestrous and oestrous period. The mean Aa of blood plasma decreased during anoestrus after Ag treatment (90 % of initial values); stimulation with 1000 IU PMSG decreased these values to 67 % of mean initial values. During the oestrous period stimulation increased these values to 108 %. Aa of the cervical mucus were higher after Ag treatment during the anoestrous period, subsequent stimulation decreased these values. During the oestrous period, Ag treatment and stimulation of ovary decreased antiproteolytic activities of the cervical mucus.

THE INFLUENCE OF STRESS ON THE GROWTH OF EXPERIMENTAL TUMOURS IN SPRAGUE-DAWLEY (SD) RATS. *M. Mráz, B. Otová[1], K. Blažek[2], M. Starec[3], S. Hynie,* Institute of Pharmacology, [1]Institute of Biology, First Faculty of Medicine, Charles University, [2]National Institute of Health and [3]Institute of Pharmacology, Third Faculty of Medicine, Charles University, Prague, Czech Republic.

The influence of stress on tumour growth was studied on the inbred strain of SD (Prague) rats, in which spontaneous acute lymphoblastic leukaemia occurs. By inoculation of cells from the spleen of leukaemic animals, solid tumours develop at the site of their administration. The subcutaneous administration of tumour cell suspension (10^6 cells) to young animals of the inbred strain leads to standard tumour growth, which can be used as a model for studies on the influence of various interventions of drugs on tumour growth. Using this model, we studied the effect of 4-hour water immersion stress on tumour growth in 4 groups of rats: A. Inoculated controls, B. after 2 stress sessions starting on the 4th day after inoculation, C. after 3 stress sessions starting on the 6th day after inoculation and D. after 9 stress sessions starting the 6th day. The rats were sacrificed on the 20th day or the 34th day, or left-alive. Tumour weight, organ histology and survival time were evaluated. As compared to the controls, rats of group B had twofold larger tumours on day 20, but the difference was smaller on day 34. However, in group C, no differences were found and in group D the tumours were larger on day 34 and surviving animals lived shorted than the controls. The test for tumour specific antibodies was negative in all groups. Our data suggest that stress might increase tumour growth and shorten the time of survival of animals.

HIGH PROGESTERONE LEVELS IN THE BLOOD DURING EARLY LIFE OF CALVES. *V. Tancin[1], G.J. Garssen[2], J.H.J. van der Werf[2], T. van der Lende[2], S. Mihina[1],* [1]Research Institute of Animal Production Nitra, Slovak Republic and [2]Institute for Animal Sciences, Research Branch Zeist, Zeist, The Netherlands.

Surprisingly high progesterone (P4) levels, corresponding to those of the luteal stage of the oestrous cycle or of pregnancy, were found in the first (prechallenge) sample of a serial blood collection during challenge test of 17 week-old female calves. The aim of the trial was, therefore, to describe in more detail the P4 origin on the basis of cortisol (CS) levels. Blood samples were taken through a jugular catheter during challenge tests - 30 samples (one test a day, with GRF, adrenaline and glucose), and P4 and CS were detected in female Holstein calves. Calves fasted for four days preceding the test. On the base of the first samples of P4 levels, 37 out of 152 calves exhibited P4 levels >2ng/ml and 8 animals of them showed levels >8ng/ml. Six to seven animals with the highest and lowest P4 levels in the first sample of a test day were selected and of those animals P4 and CS were determined in all test day samples. We found that 5 out of 7 calves with highest P4 levels in the first sample showed a decrease to prepubertal levels in the last samples on the same test-day. Calves with lowest P4 levels in first sample exhibited low values above the whole test day. CS values highly correlated with the P4 values, above the test day samples over animals as well as above test-day samples compared with the whole group of animals. We assume that high P4 levels seen during early life of calves most probably are a result of adrenal activity.

SODIUM SELENITE: A MODULATOR OF LIGAND BINDING PROPERTIES OF THE NUCLEAR THYROID HORMONE AND THE ALL-TRANS RETINOIC ACID RECEPTORS. *J. Brtko, P. Filipčík, J. Bransová, S. Hudecová,* Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Selenius is a trace element that plays an important role in the metabolism of higher organisms; it is a constituent part of glutathione peroxidase. Moreover, 5'-deiodinase, type I has recently been identified as a selenoenzyme. Selenium in the form of selenite (Se^{IV}) is also known as a catalyst of oxidation of the sulphydryl groups of proteins. The thyroid hormone and retinoic acid nuclear receptors are members of the steroid/thyroid hormone receptor family which acts as "ligand inducible transcription factors" capable of forming homodimers or heterodimers and thus yield selective transcriptional responses to the respective ligands. In the present study, we demonstrated the significant inhibitory effects of sodium selenite on nuclear thyroid hormone receptors (TR) as well as on nuclear all-trans retinoic acid receptors (RAR). According to our findings, both the TR > the RAR are sensitive to low concentrations of SeIV, *in vitro*. Sodium selenite at 0.1 μmol/l inhibits the specific binding of 3,5,3'-triiodothyronine (T_3) to nuclear receptors in the rat liver, moreover, it also incerases the rate of dissociation of T_3 from the hormone-receptor complex. We found a similar inhibitory effect of 1 μmol/l Se^{IV} on RAR binding properties. Furthermore, the relationship was found between Se^{IV} concentration and the TR gene expression. It is concluded, that the above data may yield further insight into the action of T_3 via its cognate receptors.

HIBERNATION TRIGGER AND OTHER CRYOGENS - MODE OF ACTION. *S. Vybíral, M. Šimková,* Department of Physiology and Developmental Biology, Faculty of Science, Charles University, Prague, Czech Republic.

A hibernation trigger (HT) is a hypothetical substance supposedly occurring in the blood of hibernating animals. Literary data indicate that HT can evoke a cascade of physiological and behavioural responses not only in hibernating but also in nonhibernating animals (1). The thermoregulatory effects of centrally (POAH) and peripherally (intravenous) administered plasma from control and hibernating European hamsters and of two naturally occurring brain opioid peptides (D-ala^2-D-leu^5-encephalin (DADLE) and met-encephalin) have been studied in rabbits using the method of intestinal cooling. While intrahypothalamic and intravenous injections of control and hibernating plasma were without a thermoregulatory effect, central injections of DADLE led to a dose-dependent, shortlasting (60 min) hypothermia due to the shift of the thermoregulatory threshold for induction of shivering to lower body temperatures. The thresholds for panting and vasodilation remained elevated, the consequence being a dissociation of thresholds for the cold and warm defence. Peripheral administration of DADLE was without any thermoregulatory effect. The question of identity of DADLE and of HT still remains open. In contrast to DADLE, the central application of met-encephalin evoked hyperthermia in rabbits due to the shift of all thermoregulatory thresholds to higher central temperatures.
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VII. Methods

SOFTWARE AND HARDWARE SYSTEMS FOR QUANTITATIVE ANALYSIS OF MOTOR LEARNING IN RATS ON MS-DOS PC AT COMPUTERS. *Yu. L. Kaminskij[1], G. Brožek, J. Bureš[1],* Department of Physiology, Second Faculty of Medicine, Charles University and [1]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

The acquisition of new skills can be modelled in rats trained to lick at a retractable spout which is automatically withdrawn after every lick but can be returned by presing a lever placed below the spout. Rats learn to perform presses synchronized with the lick cycle in a way allowing continuous drinking without any change of licking frequency. Licking was monitored with a photoelectric drinkometer detecting the protrusin of the tongue across the gap between the cage wall and the spout. The expriment was controlled and evaluated with a PC AT computer which measured the duration of the lick signals (LD) and of the bar presses (PD), the intervals between successive lick (LLI) and bar press (PPI) terminations, and the time between termination of one lick to the next termination of the bar press (LPI). The results were displayed in blocks of 500 licks and the appropriate number of bar presses in the form of histograms covering in 8-ms bins the distributions of LD, PD, LLI, PPI and LPI over a 1024 ms post-event interval. The LD and PD histograms express event duration. The LLI and PPI histograms reflect the distributions of intervals from the termination of one to the termination of thge next event of the same class. The abscissa of LPI histogram starts from termination of a lick to termination of the subsequent press. The overall performance of the lick-press synchronization task was expressed by the press-lick ratio. For early stages of training, the raw data, i.e. actual position of the tongue, of the spout, and of the lever can be continuously displayed on the scope.
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THE USE OF COMPUTER TECHNOLOGY IN PHYSIOTHERAPY OF NON-STRUCTURAL DEFORMATIONS OF THE TRUNK. *M. Chalupová, D. Valová, J. Otáhalová[1],* Faculty of Physical Education and Sports, Charles University and [1]Economical University, Prague, Czech Republic.

Systematic exercise if performed correctly, regularly and for a sufficiently long period of time has proved to give good results in the treatment of early postural and non-structural reversible deformations of the trunk. For suitable exercises selection it is necessary to know both the shape of the trunk and spine and muscle contractures and painful areas on the patient's body surface. Muscle tonus and painful areas have a relation to individual vertebral blocks due to the state of muscle tone occurring in typical sequences and they are accompanied by the hypersensitivity of muscles and other structures in the whole trunk and extremities. Special software and information database concern both the described phenomena. They show and evaluate the space form of the spine and some other structures, distribution of muscle tonus and painful areas on a schematic human body figure. On the base of this information, the software system offers a list of exercises with the number of necessary daily repetitions, and after this the list is checked by an expert (physiotherapeutist or physician) and corrected. This printed list of instructions and comments turns attention to the most frequent errors which are likely to occur the patient's practice at home.
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SERIAL CHANNEL AS AN INTERFACE BETWEEN CLINICAL LABORATORY INSTRUMENTS AND PC. *M. Jirků, J. Hemer,* Institute of Nuclear Medicine, First Faculty of Medicine, Charles University, Prague, Czech Republic.

The efficiency of many clinical laboratory instruments can be extended by computer processing of their output data. Among suitable interfaces between these instruments and computers, i.e. parallel or serial and their control, i.e. flag, interrupt or DMA, is the serial, flag controlled channel most readily available, since the RS 232 or RS 422 ports belong, at present, to standard options of clinical instruments. We are presenting a developed interface between IBM PC/AT and systems for measurement radioactivity in samples of biologoical fluids - JKA 1102 TEMA (Cz) and automaton LB 2104 - RIA Plus (Berthold - G). In both cases, a simple flag controlled (DATA READY) data transfer is used. It allows for transfer rate up to 10 kb/s and a distance of 10 m. The transfer is terminated either automatically after the whole output data set had been transmitted or manually from the keyboard. The described trivival transfer routine, written in C, is included in both data processing programmes (CLEARANCE, DATA) which provide data output on a screen, formatted printer and a data base. Both systems have proved useful for clinical application since they can operate unattended and provide data applicable to medical information systems. It should be underlined that the described approach can be easily applied to a number of other instruments in laboratories and clinics and a single PC can be equipped with multiple serial ports.

IBM-PC BASED SYSTEM WITH A SINGLE TV CAMERA FOR ON-LINE TRACKING IN 3D. *J. Chrasková, Yu. Kaminskij, I. Krekule,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Demands for an automatic computer supported tracking system operating in a defined 3D space are encountered in ecology and biological sciences are increasing, e.g. for the study of movement activities of flies or fishes, etc. Current systems of this kind are based on the application of a pair of TV cameras, each tracking in the 2D plane, both being in a mutually perpendicular position. We are presenting a system with a single TV camera which captures the image in one plane directly while the picture in the perpendicular plane is inspected in a mirror. Because these pictures never coincide, a simple, on-line videosignal amplitude threshold based algorithm can be used for the detection of the object in both pictures. The original TV frame is divided into halves, along the midline (row). The x and y coordinates of the object are evaluated directly from the directly viewed picture, while the z coordinate is evaluated from the picture observed in the mirror. Thus the pictures of the same object have the same x coordinate, which helps detection. Moreover, the object in different

pictures is detected at different time instants. Pilot implementation of the described system was accomplished with a CCD TV camera Tesla (Piestany) with 256^2 pixels providing a resolution of 256x128x128 (x,y,z), the IBM-PC was enhanced by the Universal I/O card which has been described elsewhere (1). The modified SW system RT-, developed for automation of the Morris water maze experiments was used. The mirror was slanted by 45 degrees. A LED marker was tracked in the pilot experiments.

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A STUDY OF THE GEOMETRY OF THE CAPILLARY BED OF A TERMINAL VILLUS IN THE HUMAN PLACENTA. *I. Krekule, L. Kubínová, M. Jirkovská[1], P. Hach[1], D. Palouš[1], P. Karen,* Institute of Physiology, Academy of Sciences of the Czech Republic and [1]Institute of Histology and Embryology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

The geometry of the capillary bed of a terminal villus in the human placenta was accomplished within the framework of a study of villi in healthy women and in diabetes mellitus patients. The capillary bed was studied in thin optical sections of a villus obtained by a confocal laser scanning microscope (BioRad MRC 600). A villus was cut into 60 – 100 thin slices, 1 μm apart. The image of the capillary lumen was enhanced by eosin staining. The capillary profiles were interactively segmented by using a high resolution graphic table connected with an IBM PC. The capillary bed was visualised by its wireframe reconstruction and by its transformation into a sample of a branching fibre process by connecting the centers of gravity of consecutive profiles. Unbiased, design-based stereological methods, (see e.g. (1)) were applied to estimate the main geometrical parameters of the capillary bed. Its volume was estimated by applying the Cavalieri principle to a series of profiles. The surface area of capillary lumen was estimated by the spatial grid method. Capillary length was estimated by counting capillary profiles in sections. The number of loops of the capillary bed was given by the Euler number, estimated by using the method of the sweeping plane.

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ESTIMATION OF SURFACE AREA OF APICAL AND BASOLATERAL MEMBRANES OF EPITHELIAL CELLS IN THE RAT COLON USING STEREOLOGY AND ELECTRON MICROSCOPY. *L. Kubínová, R. Vágnerová[1], J. Pácha,* Institute of Physiology, Academy of Sciences of the Czech Republic and [1]Institute of Histology and Embryology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

The surface area of intestinal epithelial cells can be influenced by various hormones and dietary factors. It was demonstrated that aldosterone (ALDO) or a low salt diet modulate Na^+ transport not only by increasing the number of transport molecules but also by the increase of apical and basolateral surface areas. However, it still remains unclear whether ALDO can change the surface area of colonocyte membranes during early postnatal life. Therefore, methods of measuring the apical or basolateral surface area per unit volume of epithelial cells as well as the apical surface area per unit of colon luminal area were developed. These parameters are estimated by the stereological method of vertical sections (1) from electron micrographs of sections of systematic samples of the colon tissue. Other parameters, e.g. the volume density of colon epithelial cells in the colon wall, are estimated by the pointcounting method from semi-thin sections observed under the light microscope.

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SOFTWARE FOR COMPUTER ANALYSIS OF UNIT CELLULAR ACTIVITY. *R. Hetka, J. Mareš,* Institute of Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

The software product IDR is an open powerful system for electrophysiological studies, especially for intracellular and extracellular single unit activity recording and analysis. Viewing and recording of the signal can be performed simultaneously in two different and independent sampling rates. The stimulator can be triggered directly by the program. All on line is the basic feature of the IDR software. A large part of the program is written in assembly language and high technologies are used for graphic operations (including 16-bits access to video-RAM). This introduces extremely high speed in viewing of the signal on the screen. During on line viewing in the full screen mode and in the line quality the maximal sampling frequency is 10 kHz (on PC 486 even higher). The signal could be continuously observed on the monitor and no other oscilloscope is needed. During on-line viewing, an adjustable period of the passed signal is retained in the memory. This provides an opportunity to store any interesting activity on the disk if required and no shape will be lost. Minimal hardware requirements: PC AT 286 (recommended is 386 DX and higher), 2MB RAM, SVGA 512 kB with VESA bios, simple AD board, MS DOS 3.3 and higher.

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VIII. Blood and Respiratory Physiology

CROSS RESISTANCE AGAINST CYTOSTATICS WITH DIFFERENT STRUCTURE IN THE "MULTIDRUG RESISTANT" LINE L1210 OF MOUSE LEUKAEMIC CELLS. *A. Breier, Z. Štefanková[1], M. Barančík[1], P. Dočolomanský, A. Ziegelhöffer[1],* Institute of Molecular Physiology and Genetics and [1]Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Republic.

P-glycoprotein (P-GP) is responsible for the ATP-dependent efflux of lypophilic substances (pharmaca) through the plasma membrane of animal cells. Recent results indicate that P-GP may be considered as a magnesium-dependent and vanadium-sensitive ATPase. The "multidrug" character of P-GP-mediated resistance is ensured by the capability of P-GP to transport cytostatics with different structure. In the present study the cytotoxicity of different cytostatics was tested on the L1210/VCR line of mouse leukaemic cells in which an overexpression of P-GP has been previously induced by long-term adaptation to vincristine. It was found that the cells are resistant to less polar cytostatics which are without any charge of the physiological pH range. L1210/VCR cells are less sensitive to cytostatics with high total molecular energy, i.e. to cytostatics with enhanced flexibility in the spatial arrangement of their molecules. The latter type of resistance concerns cytostatics with diverse structure. This, however, makes it difficult to assess those structural determinants on the molecules of cytostatics, which may be responsible for their interaction with and transport by the P-GP.

IN VITRO He-Ne LASER EFFECT ON PHAGOCYTIC ACTIVITY OF THE POLYMORPHONUCLEARS (PMN), AND MONOCYTES (MO) IN RABBITS. *J. Luža, J. Hubáček[1],* Institute of Physiology and [1]Clinic of Otorhinolaryngology, Faculty of Medicine, Palacký University, Olomouc, Czech Republic.

Recently, the laser influence on various physiological functions of the animal or human body has been studied (3). The available information in the literature about the relationship between the laser beam and the reaction of the immune system is controversial. The aim of this study was to evaluate *in vitro* the effect of helium-neon (He-Ne) laser irradiation on the viability and phagocytic activity of the PMN and MO of peripheral blood in rabbits. Phagocytic activity was examined by the classical method using the so-called Hema-particles (1) and the viability was examined by a simple method according to Hanks and Wallace (2). He-Ne laser in low dosage (<0.8 J) increases the phagocytic activity of PMN and MO by about 25 % and, after higher laser irradiation dosage (>0.8 J), the phagocytic activity of both PMN and MO is decreased. The viability of the PMN and MO after higher laser irradiation dosage (>1.2 J) is gradually decreased.

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THE INHIBITION ADHERENCE OF LEUKOCYTES IN RELATION TO SPECIFIC RABBIT ANTIBODY AND TO RNA POLYMERASE. *A. Jandová, J. Bendl, M. Nedbalová[1], S. Trojan[1],* Second Clinic of Gynaecology and Obstetrics and [1]Institute of Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

The defence mechanism of the organism is mainly based on normal physiological reactions, i.e. inflammation, fever, phagocytosis, etc. These physiological reactions are responsible for the removal of undesirable and noxious agents, i.e tumor cells, infectious agents, products of cell destruction, etc. In the course of evolution of higher organisms, systems have been specializing which, due to their memory, were able to stimulate in a purposeful and functional way the reaction of these basic physiological mechanisms. These specific immunological reactions ensure, for example, a timely and directed reaction of non-specific physiological defence mechanisms, e.g. the activation of phagocytic cells in the sense of the stimulation of their killer and

digestive functions. We distinguish substantially two kinds of these specific memory reactions: the antibody type of immunity and cell-mediated immunity. Landsteiner and Chase (1942) were able to show that some immunological manifestations other than those of the antibody type can be transferred passively by lymphoid cells. Thus the basis for the study of cell-mediated immunity was set. We observed CD_4 lymphocytes purified from the sera of 11 patients with ca endometrii (index of positivity IP = 2.6). We inactivated the positive reaction of the leukocyte adherence inhibition test using specific anti-LDH viral antibodies (IP = 0.7) and RNA polymerase (IP = 0.8). We examined the negative reaction in CD_4 lymphocytes in a control group (10 blood donors) after application of a specific tumour antigen (IP = 0.7), specific antibody (IP = 0.6) and RNA polymerase (IP = 0.6). Using these facts we will define the receptor-activity of CD_4 lymphocytes taken from cancer patients.

EXAMINATION OF LDH VIRUS BY MEANS OF CELL MEDIATED IMMUNITY IN CLINICAL PRACTICE (IN PATIENTS WITH CA MAMMAE). *M. Nedbalová, A. Jandová[1], M. Smejkal[2], J. Šorfová[2], S. Trojan, J. Vachoušek[2],* Institute of Physiology, First Faculty of Medicine, Charles University, [1]Second Clinic of Gynaecology and Obstetrics and [2]First Clinic of Gynaecology and Obsterisces, First Faculty of Medicine, Charles University, Prague, Czech Republic.

Senology is a relatively new interdisciplinary branch dealing with questions of the complex care of diseases of the breast. Hence, a medical superstructure has been formed requiring close collaboration of a gynaecologist, surgeon, radiodiagnostician, oncologist, pathologist, psychologist and/or other specialists involved in these problems. When a surgical intervention is indicated, on the day of admission, blood is taken from the woman patient for hormonal screening, tumour markers, cholesterol fractions and LDV virus. In dependence on the histopathological diagnosis, concentration of hormonal receptors, levels of ca mammae antigen and LDV virus, women are subjected to a target conservative treatment by means of hormonotherapy or chemoprophylaxis and/or combination of the two methods. The cell mediated immunity was examined in CD4 lymphocytes by leukocyte adherence inhibition (LAI) test in 129 patients with precarcinoma and carcinoma mammae. Our own LDV antigen and organ-specific antigens were used. The test was highly specific in comparison with a control group of healthy blood donors. The ever increasing curve of the incidence of cancer of the mammary gland fully justifies our attention paid to the immunological problem in carcinoma mammae from the point of view of the LDH virus.

HYPOVENTILATION AND VENTRICULAR FIBRILLATION THRESHOLD. A CHRONOPHYSIOLOGICAL STUDY. *P. Švorc, P. Wilk, I. Podlubný[1], Š. Kujaník, I. Bračoková, F. Vlasatá,* Department of Physiology, Medical Faculty of Šafarik University, [1]Technical University, Košice, Slovak Republic.

The circadian rhythm of the ventricular fibrillation threshold (VFT) was investigated in female WISTAR rats under conditions of normal ventilation (NV) (17 animals) and hypoventilation (HV) (10 animals). The animals were adapted to a daily light – dark cycle 12:12 hours with dark period from 1800 h to 0600 h under constant conditions. The experiments were performed under pentobarbital anaesthesia (40 mg/kg i.p.) on open chest animals. During NV ($pH = 7.51 \pm 0.05$, $PaCO_2 = 2.9 \pm 0.5$ kPa, $PaO_2 = 12.7 \pm 3.1$ kPa) VFT in female rats showed a circadian rhythm with the maximum values in the active phase. The values of VFT were independent on the heart rate (HR). A relatively serious respiratory acidosis ($pH = 7.04 \pm 0.03$, $PaCO_2 = 6.6 \pm 1.3$ kPa, $PaO_2 = 9.3 \pm 3.7$ kPa) was induced in group HV. The circadian course of VFT showed the double-peak character with the first smaller one between 1500 – 1800 h while the second higher peak occurred between 2400 – 0300 h of the daily regime. HV significantly decreased ($p < 0.001$) VFT at all the measured intervals. The values of VFT were also independent on HR. It is concluded that the electrical stability of the heart measured by VFT in female WISTAR rats exhibits a significant circadian rhythm. HV decreases VFT during the whole 24-hour period, where the circadian course is changed and becomes characterized by a double-peak. VFT is probably independent of changes of HR during both types of ventilation.

EFFECT OF HYPERTHERMIA ON DEFENSIVE AIRWAY REFLEXES IN RABBITS. *K. Javorka, A. Čalkovská, V. Gecelovská, M. Petrášková,* Department of Physiology, Jessenius Medical Faculty, Comenius University, Martin, Slovak Republic.

The effects of different body temperatures (range 39 – 42 °C) on Kratschmer's apnoeic reflex, laryngeal chemoreflex, sneezing and laryngopharyngeal coughing and/or expiratory reflex were studied in 22 anaesthetized rabbits. All stimulations were repeated at different levels of body temperature up to the panting level (41.88 ± 0.04 °C). Control rabbits were stimulated in the same periods after a sham-operation without overheating. Defensive airway reflexes were changed in hyperthermia. With elevated body temperature and increased respiratory rate, the duration and amplitude (assessed as the ratio of the apnoeic pause to the mean duration of the previous 5 breaths) of the nasal apnoea and laryngeal chemoreflex were decreased mainly in panting. The excitability of sneezing and coughing was diminished and the intensities of respiratory efforts in these reflexes were inhibited. The expulsive reactions evoked by mechanical stimulation of the glottis were replaced by very short lasting inhibitions of breathing during panting. The results indicate that the ability to eliminate irritants from the airways – mainly from the larynx – is diminished during hyperthermia and panting in rabbits.