

Proceedings of the Czech and Slovak Physiological Societies

(Prague 9–11, 1994)

I. Physiology of the Heart and Blood Circulation

COLD ACCLIMATIZATION-INDUCED CARDIOMEGALY AND PROTEIN REMODELLING OF THE FISH MYOCARDIUM. V. Pelouch, M. Vornanen¹, M. Milerová, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, and ¹Department of Biology, University of Joensuu, Finland.

Crucian carps (*Carassius carassius* L) were caught from local ponds in Eastern Finland and then randomly placed in tanks of recirculating water maintained at either 2 °C or 22 °C; the total acclimatization period was at least 14 weeks. The aim of the study was to find out whether adaptation to prolonged exposure to low temperature affects the weight of the ventricle and myocardial protein composition. Cold acclimatization increased the ventricle weight, while the body weight was not affected. The cardiomegaly was characterized by the following protein remodelling: concentration of total (sum of non-collagenous and collagenous) and collagenous (all proteins of extracellular matrix) proteins significantly increased and the concentrations of contractile proteins and total hydroxyproline significantly decreased. The proportion of a soluble collagenous fraction (newly synthesized collagen) was, however, elevated when compared with the control group. It follows from our results that the enlargement of the fish heart induced by cold acclimatization is accompanied by marked remodelling of interstitial proteins.

EXCITATION-CONTRACTION COUPLING IN THE MYOCARDIUM OF NEWBORN AND ADULT CATS. P. Pučelík, P. Barták, Department of Physiology, Faculty of Medicine, Charles University, Plzeň, Czech Republic.

The aim of this study was to find and compare the properties of excitation-contraction coupling (ECC) in the working ventricular myocardium (right-ventricular papillary muscles) of newborn (age under three days after birth) and adult cats. 1. Under interpolated extrasystole conditions the myocardium of the adult animals exhibited pronounced postextrasystolic potentiation. This frequency-dependent property was absent in the group of newborn cats. 2. The interruption of regular stimulation by a period of inactivity (Tp; duration of pause from 10 to 600 s) reduced post-rest contractions (MG) and prolonged the duration of post-rest action potentials proportionally to Tp in both age groups. The time when the maximum of the post-rest contraction was reached was unaffected in the adult myocardium. In the newborn cat myocardium this parameter was extended as a function of Tp. 3. The elevation of extracellular Ca²⁺ (from 2 to 6 mmol/l) caused a five-fold increase of MG in the newborn myocardium as compared with the adult heart. The myocardium of newborn cats is characterized by the sarcolemmal type ECC. During postnatal ontogeny the immature type of ECC converts to the reticular (adult) type.

ECG, VCG AND BODY SURFACE ISOPOTENTIAL MAPPING (BSPM) IN PATIENTS TREATED WITH SOME ANTIDEPRESSANT DRUGS AND IN HEALTHY CONTROLS. J. Slavíček, O. Kittnar, I. Paclt¹, A. Dohnalová, M.B. Horáček², Department of Physiology, ¹Department of Psychiatry, First Faculty of Medicine, Charles University, Prague, Czech Republic and ²Department of Physiology and Biophysics, Dalhousie University, Halifax, New Scotia, Canada.

Tricyclic antidepressant drugs (TCA) have some cardiovascular side effects (antimuscarinic, antihistaminic and quinidine-like effects). In the present work we compared the ECG, VCG and BSIM registered by the diagnostic system Cardiac in control subjects (n=24) of different ages (20–60 years) with patients (n=27) using both TCA, Amitriptyline, Nortriptyline, Prothiaden and the serotonin-reuptake blocker Citalopram (Seropram, n=10). In TCA patients both the antimuscarinic (tachycardia, shortening of QT and RR, p<0.01) and the quinidine-like effect (prolongation of QTc, p<0.05) was confirmed, while in Citalopram patients only a small quinidine-like effect (p<0.05) was present.

CARDIAC MICROPOTENTIALS EXTRACTED FROM A SINGLE ELECTRICAL SYSTOLE. Z. Drška, M. Polánková, P. Tíšer¹, O. Kittnar², Stress Research Centre, Academy of Sciences of the Czech Republic, Prague, ¹Department of Cardiovascular Diagnostics, University Hospital, Hradec Králové and ²Institute of Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

The non-dipolar residue concept led to a procedure of late potential extraction from single systoles. Eight simultaneously measured surface ECGs were processed by single value decomposition allowing the elimination of three first orthogonal (dipolar) components and white noise filtering. The RMS at individual time instants was then calculated. After this, signal processing was analogous to the standard late potential extraction. Data of 36 healthy subjects (mean age 42±21 years) and 43 patients (mean age 52±17 years) with CAD verified by coronarography were studied. Employing discriminant analysis the sensitivity, specificity and positive predictory values were found equal to 78 %, 76 % and 77 % respectively. In this way, the validity of the described single beat micropotential (non-dipolar residue) extraction has been verified.

DEVELOPMENTAL CHANGES IN SUBCELLULAR LOCALIZATION OF Ca²⁺ CHANNELS IN THE RAT HEART: ROLE OF THYROID HORMONES. F. Kolář, M. Wibo, L. Zheng, T. Godfraind, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and Laboratoire de Pharmacologie UCL, Brussels, Belgium.

Hyper- and hypothyroidism were induced in rats either by L-triiodothyronine (10 µg/100 g BW per day, s.c.) or by 6-n-propyl-2-thiouracil (0.05 % in the drinking water supplied to mothers), respectively for 3 weeks after birth. Cardiac sarcolemmal L-type Ca²⁺ channels and sarcoplasmic reticulum (SR) Ca²⁺ release channels were labelled with [³H](+)PN200-110 (PN) and [³H]ryanodine, respectively. The number of PN receptors (per unit tissue weight) in homogenates decreased both in hyper- (by 22 %) and hypothyroid (by 23 %) groups, as compared to the controls. The number of ryanodine receptors was unchanged in hyper- and reduced in hypothyroidism (by 31 %). Microsomal fractions, containing 30–50 % of the total number of receptors, were subfractionated by equilibration in linear density gradients. In control and hyperthyroid tissue, the majority of PN receptors were recovered in high-density subfractions, with ryanodine receptors, while in the hypothyroid group, they were found mainly in low-density subfractions, with β-receptors and Na⁺,K⁺-ATPase. Our results indicate that thyroid hormones are essential for the progressive concentration of L-type Ca²⁺ channels in junctional areas of T-tubules (linked to the SR) during postnatal development of the cardiac muscle.

THE ROLE OF TRANSMEMBRANE TRANSPORT OF Ca²⁺ IN THE CONTROL OF CARDIAC CONTRACTILITY. J. Šimurda, M. Šimurdová, G. Christé¹, P. Bravený, Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic and ¹INSERM U121, Lyon, France.

The dependence of the total amount of Ca²⁺ transported across the cell membrane during the cardiac cycle on the simultaneously released Ca²⁺ from the intracellular stores plays a key role in controlling the contraction of the cardiac cell. The aim of this study was to express this dependence quantitatively. The study was based on voltage clamp experiments in mammalian ventricular preparations and on the analysis of the quantitative model of the Na/Ca exchange system. It has been shown that the relationship is nonlinear and may be described by a quadratic polynomial with a dominant linear term. At the first approach, the quantitative model of beat-to-beat control of cardiac contractility complies with the linear approximation. The constant of the linear term including the feed-back effect of released Ca²⁺ on both main transmembrane currents (I_{Ca}, I_{NaCa}) may replace the conventional but less general function – recirculation fraction.

THE INFLUENCE OF CHRONIC HYPOXIA ON THE COMPOSITION OF PHOSPHOLIPIDS (PLP) IN RAT MYOCARDIUM DURING ONTOGENETIC DEVELOPMENT. *D. Smík, O. Nováková, V. Pelouch¹, F. Novák*, Department of Animal Physiology and Department of Biochemistry, Faculty of Natural Sciences, Charles University and ¹Institute of Physiology, Academy of Sciences of the Czech Republic, Prague.

The purpose of our study was to investigate the changes of PLP concentration in the myocardium exposed to conditions of intermittent high altitude (IHA) hypoxia. Four-day-old and adult male Wistar rats were exposed to IHA hypoxia. In both groups of animals IHA induced marked hypertrophy of the right ventricle (RV) and moderate hypertrophy of the left ventricle (LV). In the case of four-day-old animals, the hypertrophy did not disappear even 30 days after returning to normoxic conditions. The total amount of PLP in the myocardium of adult animals was lowered after IHA. Phosphatidylcholine and diphosphatidylglycerol (DPG) were responsible for this decrease. No change was observed in the concentration of PLP in the RV of four-day-old rats, whereas the concentration of DPG in the LV was decreased. The results indicate a higher resistance of the developing myocardium to IHA hypoxia.

DOES REPERFUSION FOLLOWING ACUTE CORONARY OCCLUSION IN THE LEFT VENTRICLE INJURE THE REPERFUSED REGION OF THE HEART ONLY? *A. Ziegelhöffer¹, O. Ondřejíková², J. Štyk¹, I. Gabauer¹, A. Breier³*, ¹Institute for Heart Research, ²Institute of Experimental Pharmacology and ³Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic.

After 60 min occlusion of LAD coronary artery (CO) and subsequent 20 min reperfusion of the occluded area, glutathione (GSH), its disulfide (GSSG), malodialdehyde (MDA) and the activity of γ -glutamyl transpeptidase (γ GTP) were studied in the ischaemic (I) and nonischaemic (NI) parts of the left (LV) and the right ventricle (RV) of the canine heart. An increase of GSSG and drop in GSH occurred similarly in all parts of the myocardium during CO. On the contrary, the MDA content remained unchanged in the I as well as in the NI parts of the LV. The activity of γ GTP increased in the well perfused RV only! Reperfusion of the I area of LV led to a similar rise in γ GTP activity and MDA accumulation, maintaining the previous GSH and GSSG profile in both, the reperused and continuously well perfused parts of the LV and RV. The results indicate that the CO- and reperfusion-induced effects in GSH, GSSG, MDA and γ GTP concern the whole myocardium and are not confined to a local area of the heart.

ISCHAEMIC-REPERFUSION INJURY OF THE MYOCARDIUM FROM THE FUNCTIONAL AND MORPHOLOGICAL POINT OF VIEW. *T. Stankovičová, M. Novotová¹, M. Ovečka, D. Magna², V. Knežl²*, Faculty of Pharmacy, Comenius University, ¹Institute of Molecular Physiology and Genetics and ²Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

The incidence of reperfusion dysrhythmias after ischaemic-reperfusion (I-R) injury of the myocardium was observed on the isolated perfused rat heart. A decrease of coronary flow and of contraction force was accompanied by an increase of enddiastolic pressure and reperfusion dysrhythmias. At the end of reperfusion a rise in the cAMP/cGMP ratio was observed. A decrease of cGMP concentration supported the dysrhythmogenic effect of cAMP. I-R injury led to increased vesiculation of sarcoplasmic reticulum membranes, dilatation of the T-tubular system and deformation of triads. Numerous vacuoles in the regions of Z-lines were found. Mitochondrial changes were manifested as vacuolisation and disruption of cristae. The morphological findings correlated with changes in myocardial function during I-R injury of the heart.

HISTOCHEMICAL PICTURE OF THE RAT HEART DURING POSTNATAL DEVELOPMENT AND ITS CHANGES INDUCED BY ISCHAEMIA. *N. Tribulová, B. Ziegelhöfferová, J. Slezák*, Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Republic.

We studied histochemically the distribution and enzyme activities of the markers of oxidative metabolism and glycolysis (SDH, β -HBDH, G-6PDH, LDH, phosphorylase), hydrolytic enzymes (ATPases, phosphatases, DAP IV, 5'-NC, ACHE) in the hearts of rats 0, 1, 2, 4, 8, 22 days old and of adults, and also in the hearts influenced by 1 h of global ischaemia at 37 °C. Enzyme activities increased during postnatal development, whereas the activities of alkaline phosphatase and DAP IV (markers of the capillary bed) were still very low in the second postnatal week. During this period, the differences in oxidative enzyme activities and ACHE between atria and ventricles, in comparison to the adults, were less pronounced. However, they were more expressed in the activity of 5'-NC. After 1 h of global ischaemia, the enzyme activities were decreased (particularly of phosphorylase and LDH) till the second postnatal week. Histochemical changes were more pronounced in the juvenile and adult rat hearts. The results indicate that neonatal hearts exhibit a higher resistance to ischaemia.

ISCHAEMIC PRECONDITIONING (PC) IN THE RAT HEART: THE ROLE OF CELL SIGNALLING. *T. Ravingerová, N.J. Pyne¹, J.R. Parratt¹*, Institute for Heart Research, Slovak Academy of Sciences and ¹Department of Physiology and Pharmacology, Strathclyde University, Glasgow, Scotland.

Our aim was to study the effect of PC with a brief episode of ischaemia on the incidence of arrhythmias during subsequent long-lasting ischaemia and to assess G-proteins in isolated rat hearts perfused (Langendorff) at a constant flow of 10 ml/min. In control hearts, 30 min occlusion of LAD induced a 100% and 67% incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF), respectively, as well as 541 \pm 84 extrasystoles (VPBs). PC (5 min occlusion, 10 min reperfusion) significantly reduced the number of VPBs (30 \pm 11, $p < 0.01$), the incidence of VT (17%, $p < 0.01$) and abolished VF. The measurement of G-proteins in myocardial tissue (Western blotting, ADP-ribosylation with bacterial toxins) revealed decreased amounts of Gs and increased amounts of Gi-proteins in the preconditioned hearts. Results indicate that PC renders the rat heart more resistant to ischaemic arrhythmias. The antiarrhythmic action of PC may be mediated by adrenergic receptors coupled to appropriate G-proteins with subsequent inhibition of adenylate cyclase activity and reduced production of cAMP.

CIRCASEPTAN RHYTHM IN CARDIOVASCULAR PARAMETERS IN NEWBORNS. *J. Dušek, J. Siegelová, B. Fišer¹, R. Nekvasil², J. Stejskal²*, Department of Pathophysiology, ¹Department of Physiology, ²Second Department of Pediatrics, Masaryk University, Brno, Czech Republic.

The aim of the study was to prove the hypothesis that the blood pressure (BP) in newborns has a 7 day (circaseptan rhythmicity) instead of 24-hour variability (Halberg). Long-lasting BP monitoring was performed using sphygmomanometric measurements once an hour. Twenty-three premature newborns (850 to 3250 g b.w.) in an intensive care unit were examined for 7 to 35 days after birth. The power spectra of systolic (SBP) and diastolic BP (DBP) and the heart rate (HR) were computed. Slow oscillations with variable periods between 5 to 10 days were found either in HR or in BP in all the newborns. Peaks of 24-hour periodicity were always smaller than those found in the circaseptan rhythm. Mean power spectra revealed a significant peak ($p < 0.05$, Student t-test) at 0.16 cycles per day in HR, SBP and DBP. Our results support the idea that the weekly period is not a mere feature of culture, but a part of nature.

BLOOD PRESSURE (BP) OF VERY PREMATURE NEONATES (VPN), PHARMACOLOGICAL ADJUSTMENT OF ITS ABNORMALITIES, AND ITS RELATION TO SUDDEN INTRAVENTRICULAR HAEMORRHAGE (IVH). *H. Janáková, J. Waterhouse¹, D. Minors¹, D.G. Sims², M.L. Chiswick², S.W. D'Souza², G. Appleton²*, Faculty of Sciences, Charles University, Prague, Czech Republic, ¹School of Biological Sciences, University of Manchester and ²St. Mary's Hospital, Manchester.

The survival of VPN threatened by IVH, closely related to an insufficient control of BP. Normative values of BP for VPN have not yet been determined. We observed a group of VPN (43) during the first ten days of their life. Intra-arterial BP was measured directly and monitored automatically every 15 min during 24 h. The time course of BP was compared with the medical record (IVH, death, drug applications). Thus, we assessed for VPN normative values of BP and their variations during 24 h. We also found that inotropes given to VPN (43 VPN during X days) did not increase BP sufficiently enough to reach the normative values of BP (Mann-Whitney, $p < 0.05$) that we established for "healthy" VPN (204 VPN during X days). We proved a close relationship between a marked change in the variability of the daily BP (as assessed by the 10%–90% range) on the day before IVH and the appearance of IVH afterwards. The programme has been approved by the Ethical Committee in U.K.

THE EFFECT OF DIFFERENT MATERNAL AND NEONATAL FACTORS ON THE BP AND HR IN THE FIRST POSTNATAL WEEK. *D. Andrášyová, E. Kellerová*, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

The effect of different maternal and neonatal factors on BP and HR was studied in 669 full-term neonates. Basal values of BP ($60 \pm 9/35 \pm 7$ mm Hg) on the first day of life increased during the 5 postnatal days by 17%, whereby HR (123 ± 13 beats/min) decreased by 2.5%. A difference in BP during the first postnatal hours was found between infants delivered vaginally or by Caesarean section ($P < 0.004$). No statistical correlation was observed between mother's and baby's BP. On the other hand, babies delivered from gestotic pregnancies had a significantly higher basal SBP ($P < 0.05$) and their postural BP increments were significantly higher in comparison with the controls. Newborns with disturbed fetoplacental circulation showed greater postural SBP reactivity as compared to the controls ($P < 0.01$). In the first hours of life, babies with a lower Apgar score had lower DBP in comparison to the controls ($P < 0.05$). There was a significant relation between birth weight and BP. Infants weighing < 3000 g had lower SBP $P < 0.01$ in comparison to those weighing > 4000 g. Family history, mother's age, order of pregnancy and sex had no effects on BP and HR. Different neonatal care modified the development of BP and HR in the first postnatal week.

VASOMOTOR RESPONSE TO BAROREFLEX STIMULATION DURING 24 HOURS IN MAN. *J. Siegelová, B. Fišer, J. Dušek, G. Cornelissen², F. Halberg²*, Department of Pathophysiology, ¹Department of Physiology, Masaryk University, Brno, Czech Republic and ²Chronobiological Laboratories, University of Minnesota, USA.

Occlusion of both lower extremities (5 min, 180 mm Hg) elicits reactive hyperaemia. An abrupt change in pressure in the occluding cuffs from 180 to 60 mm Hg decreases blood pressure, which is followed by an increase in heart rate. This manoeuvre was used to calculate baroreflex sensitivity (BRS). It was also possible to evaluate the rate of blood pressure recovery after the decrease and thus to obtain a vasomotor reflex response. The reaction was studied in 10 healthy subjects for 4 hours during a 24-hour period. An increase in BRS was not significant at night (20.9 ± 23.9 versus 11.8 ± 9.2 ms/mm Hg) and the rate of blood pressure recovery showed no difference. It is concluded that blood pressure regulation (after an abrupt decrease in peripheral resistance) depends on the vasomotor response, which is not related to BRS.

BAROREFLEX SENSITIVITY AND NON-SPECTRAL ANALYSIS OF HEART RATE VARIABILITY IN POST-INFARCTION PATIENTS. *N. Honzík¹, B. Semrád¹, B. Fišer, R. Lábrová¹*, Department of Physiology and ¹First Internal Clinic, Masaryk University, Brno, Czech Republic.

A decrease of heart rate variability (HRV) or of baroreflex sensitivity (BRS) are associated with sudden cardiac death after acute myocardial infarction. The aim of this study was to investigate the correlation of both parameters and their predictive value, if combined. In 76 patients, HRV was derived from 24-hour Holter recordings as SDANN (standard deviation of 5-minute mean R-R intervals) and as SD (mean of all 5-minute standard deviation of RRs). Noninvasive blood pressure records (the Peňáz method) of 3-minute duration were used for the determination of BRS by spectral analysis (1). The correlation coefficients between SDANN and BRS ($r = 0.10$, n.s.) and between SD and BRS ($r = 0.37$, $p < 0.01$) were low. In some patients, relatively low BRS and high HRV or vice versa were observed. However, in a subgroup of deceased patients ($n = 4$), both HRV (SDANN 29.9 ± 14.2 ms, SD = 15.8 ± 4.5 ms) and BRS (1.5 ± 0.8 ms/mm Hg) were low. It is concluded that the predictive value is increased if HRV and BRS are evaluated together.

1. Honzík¹, N., Fišer B., Honzík J.: Physiol. Res. 41: 31–37, 1992.

THE RELATION BETWEEN PERFUSION PRESSURE AND CAROTID BLOOD FLOW IN NORMOTONIC AND HYPERTONIC SUBJECTS. *B. Fišer, J. Siegelová¹, E. Savin², J.P. Martineaud²*, Department of Physiology, ¹Department of Pathophysiology, Masaryk University, Brno, Czech Republic and ²Department of Physiology, R. Descartes Université, Paris, France.

The relation between the mean arterial pressure (MAP) and cerebral flow was studied in 13 healthy (C) and 8 hypertonic (H) subjects during a rapid drop of MAP. The drop was elicited by occlusion of both lower extremities (180 mm Hg for 5 min) and successive abrupt lowering of the occluding pressure to 60 mm Hg (reactive hyperaemia). Minimum MAP was reached within 3 s. MAP (the Peňáz method) and the right carotid blood flow (pulsed Doppler velocimeter) were measured. Mean (\pm SD) MAP before (C: 93.8 ± 7.0 , H: 116.6 ± 17.7 mm Hg) and after the release of occluding pressure (C: 77.7 ± 9.2 , H: 102.8 ± 16.7) differed significantly in H (Wilcoxon, $p < 0.05$). Mean flow before (C: 316 ± 31 , H: 329 ± 48 ml/min) and after the release (C: 242 ± 33 , H: 258 ± 46) did not significantly differ in both groups. The slope of MAP - carotid flow curve was similar in H and C but in H the curve was shifted to higher pressure values (Wilcoxon, $p < 0.05$).

AN EXPERIMENTAL MODEL OF HYPERTENSION INDUCED BY ORAL ADMINISTRATION OF L-NAME IN RATS. *I. Bernáti¹, O. Pecháňová¹*, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Inhibition of NO synthesis by L-arginine analogues has been shown to induce hypertension (1). In the present study, cardiovascular and metabolic changes were investigated in the rats after long-term oral administration of the inhibitor of NO synthetase: NG-nitro-L-arginine methyl ester (L-NAME) in concentrations 20 mg/kg/day. The changes of cardiovascular parameters (blood pressure, heart rate) were measured by a specially prepared pressure transducer. The arrangement ensured measurement of heart rate as well as of the systolic pressure in the rat tail by a non-invasive method (tail-cuff plethysmography). The systolic pressure increased as compared with the controls by 37% after the first week, by 49% after the second week, by 41% after the third week and by 33% after the fourth week of L-NAME oral administration.

1. Moncada S.: Acta Physiol. Scand. 145: 201–227, 1992.

The work was supported by Slovakofarma a.s. Hlohovec, Slovakia.

ACTIVITY OF SOME ENZYMES OF ADENINE NUCLEOTIDE METABOLISM AFTER A SHORTLASTING PRESSURE OVERLOAD OF THE HEART. *O. Pecháňová, M. Gerová, I. Bernátová, V. Stoev, M. Kittová¹*, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences and ¹Institute of Physiology, Comenius University, Bratislava, Slovak Republic.

The activities of 5'-nucleotidase (5'-Nase), adenosine kinase (AKase), adenosine deaminase (ADase) and purine nucleoside phosphorylase (PNPase) were estimated after constriction of the abdominal aorta in dogs lasting 4 hours. The blood pressure increased from 147.3 ± 11.1 mm Hg to 204.6 ± 11.4 mm Hg during the constriction. The activity of total 5'-Nase increased by 31.8 % in the myocardium, by 29.4 % in the ramus interventricularis anterior (RIA) and by 20.0 % in the ramus circumflexus (RC) as compared with sham-operated dogs. The activity of AKase increased by 22.7 % in the myocardium, by 19.5 % in RIA, by 13.6 % in RC and the activity of ADase increased by 20.1 % in the myocardium, by 16.8 % in RIA and by 12.9 % in RC as compared with the corresponding values in sham-operated dogs. There was no change found in PNPase activity.

II. Physiology of behaviour

RELATIVE CONTRIBUTION OF EGOCENTRIC AND ALLOCENTRIC ORIENTATION TO PLACE NAVIGATION IN DARKNESS. *M. Moghaddam, J. Bureš*, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague.

Place navigation in the Morris water maze can be implemented either by the memory of the coordinates of the target relative to remote landmarks (allocentric) or by the memory of the start-target route (egocentric). The latter strategy prevails in the dark provided that the start and goal positions remain the same. In 10 Long-Evans rats trained under the above conditions (start S, goal NW) escape latencies gradually decreased during 20 sessions from the initial 45 s to 14 s. The possibility that the rats have solved the task by using non-visual beacons for allocentric target location was tested by rotating the start and goal position by 90° (start E, goal SW). Such rotation leaves the egocentric navigation unaffected but interferes with allocentric navigation. The change slightly prolonged escape latencies, but concentration of the search time in the SW rather than in the NW quadrant suggested that most rats used path integration. Place navigation under the above conditions is thus a suitable test of vestibular and kinaesthetic contribution to cognitive mapping.

RETRIEVAL OF OVERTRAINED PLACE NAVIGATION DURING OCCLUSION OF ONE EYE AND IPSI- OR CONTRALATERAL BLOCKADE OF RELEVANT BRAIN CENTERS. *L. Francis Turner¹, Z. Liu, J. Bureš*, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and ¹Centre of Neurological Restoration, Havana.

Place navigation acquired by the intact brain can be retrieved with either eye and is stored in both hemispheres. The retrieval circuitry was examined by testing an overtrained rat under lidocaine inactivation of the hippocampus (H), visual cortex (VC) and superior colliculus (SC). Thirty-three hooded rats with implanted cannulae aimed at the above structures were trained to find the target in the SW quadrant of the pool. Retrieval was tested during occlusion of one eye alone or combined with ipsi- or contralateral blockade (1 μ l 4 % lidocaine) of H, H+VC or H+VC+SC. The intact brain escape latencies (10.5 s) were only slightly prolonged by occlusion of one eye (to 14.2 s). Blockade of centers ipsi- or contralateral to the occluded eye increased escape latencies to 12.7 and 15.2 s for H, to 16.8 and 16.9 s for H+VC and to 23.6 and 17.4 s for H+VC+SC, respectively. Goal finding in the absence of direct visual input to the intact hemisphere is due to path integration supported by uncrossed visual projections.

SEPARATING THE START AND GOAL IN THE MORRIS WATER MAZE BY A ZERO VISIBILITY ZONE RETARDS BUT DOES NOT PREVENT PLACE NAVIGATION IN RATS. *Z. Liu, L. Francis Turner¹, J. Bureš*, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and ¹Centre of Neurological Restoration, Havana.

In an attempt to assess the contribution of visual (landmark sighting) and non-visual (path integration) mechanisms to place navigation in the Morris water maze ($r=1$ m) naive rats ($n=30$) were trained for 3 days to swim from starting points at the SW, S and SE rim of the pool to the escape platform in N. When the rat entered a 65 cm wide periequatorial belt extending from W to 45 cm from the E wall the tracking computer switched off the light but turned it on as soon as the rat had left the belt. Under the above conditions, rats learned place navigation significantly slower than in permanent light when the zero visibility belt was 65 cm but not when it was 32 cm wide. Although the rats could avoid the zero visibility zone by swimming close to the E wall they continued to cross the belt. The lack of landmark sighting deteriorated navigation on Days 2 and 3 for the wide belt but only on Day 2 for the narrow belt. The impairment was less expressed than when the goal was in the zero visibility zone.

EXPLORATORY ACTIVITY AND THE PROCESSING OF SPATIAL INFORMATION IN RATS. *E. Save*, CNRS, Laboratory of Cognitive Neuroscience, Marseille, France. Current address: Institute of Physiology, Czech Academy of Sciences, Prague.

Recent psychological studies have not provided evidence that exploration and spatial knowledge are closely related to each other by functional, reciprocal links. Furthermore, it has been demonstrated in rodents that exploratory activity is important for the building up of spatial maps. These experiments are based on a similar principle. Animals are allowed to explore an open field containing objects. After habituation, one or several objects are displaced. Animals usually react to the change by increased exploration, which indicates that during exploration, some information about the spatial relationships of the initial situation has been processed and stored and used as a reference to detect the change. Such a procedure is used to assess the nature of the spatial information processed by animals and to study the role of the brain structures (hippocampus, parietal cortex) involved in such processing.

TEMPORALLY GRADED RETROGRADE AMNESIA OF SPATIAL LEARNING AFTER MEDIAL SEPTAL INACTIVATION. *V. Bohbot, S.L. Thurm, L. Nadel, B.L. McNaughton, C.A. Barnes*, ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, USA.

After learning there is some time when memories can be disrupted by experimental manipulations such as electroconvulsive shock or therapy, drug infusions, lesions, or temporary inactivations. Humans suffering from lesions known to cause specific learning deficits also suffer from memory deficits for events that preceded the insult to the brain. The hypothesis posed here is that after acquisition of a spatial task, the hippocampus is needed to strengthen or "consolidate" the spatial memory traces, and that after some time, these memory traces get stored elsewhere in the brain, possibly the neocortex. This was tested by training 13 rats to criterion on a spatial task, after which the hippocampus was prevented from functioning normally by temporarily inactivating the medial septum with 2 % tetracaine at various delays after training (0 days, 14 days and 28 days). In the spatial task used, the rats had to locate a food reward from one of 16 cups located at the periphery of a circular platform. In order to prevent forgetting, rats were kept under a regular training schedule inbetween tetracaine injection days. We predicted that as the spatial memory traces are stored elsewhere than the hippocampus, the rats perform better during the inactivation, spatial information being now more accessible. During the inactivation, rats showed better performance by day 28 than they did at day 0 after reaching criterion. Our results favoured a consolidation hypothesis, but the rats' performance improved even without septal inactivation making the interpretation of these results unclear.

WORKING MEMORY IN MULTICHOICE SPATIAL TASKS; EFFECT OF TOPOGRAPHY AND THE ACCESS TO GOALS. L. Zinyuk¹, A. Zahálka, J. Bureš, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and ¹Institute of Physiology, Ukrainian Academy of Sciences, Kiev.

The striking working memory performance of rats in the 8-arm radial maze was compared with the capability of thirsty rats to successively visit 8 drinking spouts forming a 30x30 cm rectangle on the hind wall or ceiling of the choice compartment of a 80 cm long runway. The first visit to a spout was rewarded by 5 s drinking followed by opening of the floor and a fall into a lower runway providing return to the start. Revisiting the same spout elicited the fall immediately. During 15 days of training, the number of errors in the first 8 choices decreased from 1.5 ± 0.3 to 0.3 ± 0.3 in the radial maze, from 3.1 ± 0.5 to 2.5 ± 0.4 with the wall array and from 3.4 ± 0.5 to 2.3 ± 0.4 with the ceiling array. Whereas the rats performed significantly better in the radial maze than predicted by random choice, performance on the wall and ceiling arrays did not differ from the chance level of 2.75. The poor outcome was probably due to a preference for lower and nearer spouts, to a lack of distal cues and locomotor vectors specific for different goals in the radial maze.

ACQUISITION AND RETRIEVAL OF CONDITIONED TASTE AVERSION (CTA) IN RATS IS CONTROLLED BY IPSILATERAL CONNECTIONS BETWEEN CRITICAL BRAIN CENTRES. G. Roldan, E. Bielavksa¹, M. Gallo², J. Bureš¹, Medical Faculty UNAM, Mexico, ¹Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and ²Department of Psychology, University of Granada, Spain.

Acquisition and subsequent retrieval of CTA engram is implemented by cooperation of specific brain centers. The possible lateralization of the neural circuits involved has been tested by unilateral tetrodotoxin (TTX) blockade of the gustatory cortex (GC), amygdala (AM) and parabrachial nucleus (PBN). Inactivation of PBN and AM, PBN and GC or AM and GC in the opposite hemisphere during CTA acquisition impaired CTA formation, whereas inactivation of these centres in the same hemisphere did not prevent CTA learning. CTA acquired during ipsilateral blockade of PBN and AM has been examined during TTX blockade of GC alone, AM alone or GC and AM in the same of in the contralateral hemispheres. A clear amnesic effect was found only when contralateral structures were blocked. These results suggest that the CTA acquisition and retrieval are implemented by lateralized cortico-subcortical circuits.

GEOMETRY OF GOAL ACCESS AFFECTS PERFORMANCE IN A COMPUTERIZED DELAYED ALTERNATION TASK. Yu. Kaminski, L. Zinyuk¹, J. Bureš, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and ¹Institute of Physiology, Ukrainian Academy of Sciences, Kiev.

An apparatus for automation of delayed alternation consists of an upper runway separated by a trap door from the goal chamber with two drinking spouts on the hind wall and a collapsible floor the opening of which delivers the rat into a lower runway through which it returns to the start. Spout contact is detected by sensors connected to the I/O board of the computer which also unlocks the start door and the floor. Correct responses are rewarded by 3 s access to water, repeated visits to the same spout elicit the fall immediately. Computer runs the experiment in accordance with user defined parametric file setting the maximum session duration, number of correct trials, number of all trials, drinking time and response-start delay. Off-line analysis of records provides the ratio of correct reactions and length of correct or incorrect response sequences. After 15 sessions, rats scored 67 % correct when running straight from start to spouts and more than 80 % correct when they had to approach the spouts through a T-shaped insert.

MODIFICATION OF STERNBERG'S MEMORY TEST. M. Indra, Z. Bohdanecký, E. Růžička¹, V. Líněk¹, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and ¹Neurological Clinic, First Medical Faculty, Charles University, Prague, Czech Republic.

A package of standard IBM PC programs for presentation and evaluation of six different Sternberg memory test modifications has been prepared for clinical use. A series of 2-5 monochrome or coloured digits, or coloured rectangular targets only are displayed on the computer screen. At the end of each series, the target of the same type but accompanied by a short tone is presented. The task of the subject is to determine whether or not the target coincided with some of the events of last series by pressing the "Y" or "N" keys on the keyboard. Optionally, if the computer is equipped by an additional I/O card, two detached push buttons can be used. The reaction times (RT) of the subject's response are measured and evaluated together with the number of correct and false responses. The results are tabulated, the mutual dependence of RT and the length of the presented series are displayed in graphical form.

PERCEPTION OF THE TEMPORAL ORDER OF A BACKWARD-MASKED AUDITORY STIMULUS. R. Jirsa, T. Radil, M. Indra, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague.

Subjects were asked to assess the temporal order between the onset of a standard stimulus (STS) presented into the right ear and of a variable stimulus (VST) into the left ear. The VST consisted either of a weak stimulus followed 30 ms later by a strong one, or the weak and strong stimuli were presented separately. All subjects reported that they heard only one strong stimulus when the weak-strong stimulus pair was presented (backward masking). The results have shown that the distribution of correct judgments of order between VST and STS was controlled by the subconscious (weak), and conscious (strong) stimuli, respectively, depending on the objective temporal order between STS and VST in the case of backward masking. Our results suggest that neural processes determining the timing of stimuli differ from those determining the conscious representation of stimuli.

CHANGES OF NITRIC-OXIDE SYNTHESIS IN THE BRAIN INFLUENCE INHIBITORY LEARNING AND MEMORY IN NEWBORN RATS. J. Mysliveček, J. Hassmannová¹, J. Šafanda, Institute of Pathophysiology, Medical Faculty, Charles University, Pízeň and ¹Institute of Physiology and Clinical Physiology, Third Medical Faculty, Charles University, Prague, Czech Republic.

We reported previously (1) that blockade of nitric oxide (NO) synthesis by nitroarginine (NA) in newborn rats decreased the efficiency of inhibitory learning, memory processing and retrieval at the 24-hour interval after meeting the criterion; on the contrary, application of L-arginine (LA), a NO-synthase (NOS) substrate, enhanced these functions. In the present report, we studied the effect of 10 mmol NA and 20 mmol LA given into both cerebral lateral ventricles ($1 \mu\text{l} \cdot 10 \text{ g}^{-1}$ body weight) in a series of intervals from 0.5 h before learning up to 24 h after attaining the criterion, when memory retention was tested. The blockade of NO synthesis in the brain caused changes in all the studied functions: learning, memory processing and retrieval. Blockade of NOS impaired, whereas LA improved all of these functions. Several differences at various intervals were pointed out and their significance was discussed.

J. Mysliveček J., Šafanda J., Hassmannová J.: *Physiol. Res.* 43: 7P, 1993.

AN ONTOGENETIC STUDY ON THE INFLUENCE OF NITRIC OXIDE ON HIPPOCAMPAL POTENTIATION. *J. Barcal, J. Mysliveček, J. Záhlava*, Institute of Pathophysiology, Medical Faculty, Charles University, Plzeň, Czech Republic.

Nitric oxide (NO) takes part in many nervous functions, operating evidently as a retrograde messenger on glutamatergic NMDA synapses, e.g. in hippocampal potentiation, early learning and memory (2). In our previous work on newborn animals, an inverse effect of neurotransmitters was shown (3) and later confirmed (1). We therefore asked if the NO influence on hippocampal potentiation would exhibit similar ontogenetic changes. In Long Evans rats (equithesine anaesthesia, 0.3 ml/100 g), a NO-blocker L-nitroarginine (NA) (10 mmol) and sources of NO, such as L-arginine (LA) (20 mmol) or sodium nitroprusside (NP) (6 mmol), were applied to the exploratory electrode in microliter amounts. NA suppressed, whereas LA and NP enhanced the responses and potentiation in both adult and young (3-week-old) rats. However, in a part of the youngest pups the effect was reversed.

1. Ben Ari Y., Cherubini, Corradetti R., Caiarsa J.L.: *J. Physiol. (Lond.)* 416: 303, 1989.

2. Mysliveček J., Safanda J., Hassmannová J.: *Physiol. Res.* 43: 7P, 1994.

3. Záhlava J.: *J. Life Sci.* 2: 47, 1972.

FUNCTIONAL AND MORPHOLOGICAL EXPRESSIONS OF EARLY LEARNING. *F. Vožeh, J. Mysliveček*, Institute of Pathophysiology, Medical Faculty, Charles University, Plzeň, Czech Republic.

This work is focussed on functional brain morphology of newborn rats taught to avoid electric shocks in an inhibitory paradigm between day of birth and day 17; the response improved in a stepwise manner, as we had shown previously (1). Control pups were kept in the nest with their mother. The animals of both groups were killed at the same age of 11 or 17 days. Their brains were impregnated according to Golgi, in the modification of Ramón-Mölliner. Neurons in the hippocampal dentate nucleus and magnocellular nucleus of the basal forebrain (nc. Meynerti) were examined. The number of dendritic spines, total length of dendrites and dendritic branching were recorded. Dendritic spines were counted at defined sites of dendritic branching directly in the microscope and further parameters were evaluated by means of camera lucida (2). We found greater density of spines, total length and larger dendritic branching on dendrites of experimental animals as compared with the controls. Differences were statistically significant. These results show that learning in early ontogeny is accompanied by changes of functional morphology in the hippocampus and basal nucleus of Meynert.

1. Mysliveček J., Hassmannová J.: *Homeostasis* 33: 212–213, 1990.

2. Vožeh F.: *Plzeň. lék. Sborn. Suppl.* 64: 83–86, 1991.

FOUR HOURS OF PILOCARPINE-INDUCED STATUS EPILEPTICUS IN RATS ARE FOLLOWED BY IMPAIRED NAVIGATION IN THE MORRIS WATER MAZE. *R. Brábec¹, V. Komárek¹, P. Mareš², M. Langmeier³, G. Brožek⁴*, ¹Department of Pediatric Neurology and ⁴Department of Physiology, Second Medical Faculty, ²Department of Pathophysiology, Third Medical Faculty, ³Department of Physiology, First Medical Faculty, Charles University, Prague, Czech Republic.

The effect of the pilocarpine-induced (i.p. 360 mg/kg) status epilepticus on memory during the "silent period" (1) between induced and spontaneous seizures was terminated after 4 hours by clonazepam (i.p. 1 mg/kg). Methylscopolamine (i.p. 1 mg/kg) was injected 30 min before injection of pilocarpine. During the "silent period" the cognitive memory was tested in the Morris water maze. Navigation of pilocarpine-treated and control animals was compared. During the three days after status epilepticus, testing was impossible because rats did not stay on the platform and were very aggressive. From the 6th day until the beginning of spontaneous seizures (29th–32nd day) only deficits of cognitive learning were observed. Hippocampal lesions were confirmed histologically. (Supported by grant UK 215/93/C).

1. Cavalheiro E.A.: *Brass. J. Med. Biol. Res.* 23: 555–558, 1990.

CONDITIONED TASTE AVERSION AND PROTEIN KINASE-C IN THE PARABRACHIAL NUCLEUS OF RATS. *J. Krivánek*, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague.

Lesion studies (1) have shown that the parabrachial nucleus (PN) may be the site of the formation of conditioned taste aversion (CTA). To estimate a possible involvement of protein kinase-C (PKC) in CTA we have elaborated a method for dissection of the rat PN and for measuring its PKC activity (2). Forty-eight hours after pairing of saccharin consumption with LiCl poisoning, we found an enhanced PKC activity in the cytosol fraction of PN by 36% (1.02 ± 0.050 nmol P/min/mg prot. vs 0.73 ± 0.035). This increase could only be partially, if at all, accounted for by translocation of the membrane bound PKC (-0.06) to the cytosol. Thus the activity of total PKC (bound plus soluble) rose (1.35 as compared to 1.09 of the controls). The bound PKC portion of total PKC was taken then lower by 22%. Twenty-four hours after CTA formation the soluble PKC was higher by 10%, the portion of bound PKC was 19% lower than in the controls. Twelve hours and also 8 days after CTA induction no changes in PKC activity and distribution could be observed.

1. Ivanova S.F., Bures J.: *Physiol. Behav.* 48: 543–549, 1990.

2. Krivánek J.: *Brain. Res.* 629: 327–330, 1993.

THE INFLUENCE OF NGF, b-FGF AND CEREBROLYSIN ON THE SPATIAL MEMORY OF RATS AFTER DAMAGE OF THE SENSORIMOTOR CORTEX. *V. Valoušková, A. Gschaneš¹*, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and ¹Institute of Zoology, University of Graz, Austria.

Transplantation of embryonic CNS (ED 14) prevents memory impairment after bilateral lesions of the sensorimotor cortex of adult rats. The effect of some known trophic factors and commercially produced drugs on spatial memory was tested. NGF (38 ng/ml), b-FGF (100 ng/ml) and cerebrolysin (EBEWE Arzneimittel) were infused by a micro-osmotic pump (Alzet 2002, rate 0.5 μ l/hour, 14 days). At the end of drug application the rats were tested in a Morris water maze tank. All lesioned animals, irrespective of drug treatment, performed the task with a longer escape latency than non-lesioned controls ($p < 0.05$). Only NGF and Cer groups performed the task significantly better than lesioned control groups ($p < 0.01$). No short-term effect of b-FGF treatment was found. Seven to 8 months later the same rats were tested in the Morris tank (position of the platform was changed). The FGF group and lesioned control group (microinfusion of solution used for growth factor reconstitution – 1 mg BSA/1 ml PBS) performed the task on the same level as non-lesioned control animals. NGF and Cer groups significantly differ from non-lesioned controls ($p < 0.01$) and $p < 0.05$, respectively) and were comparable to rats with lesions only. The application of NGF and cerebrolysin acute improves the spatial memory deficit of lesioned rats but does not lead to permanent changes. The application of b-FGF did not protect the brain against the memory deficit but supported or initiated either biochemical or morphological changes leading to the compensation of spatial memory deficit.

A SENSORY-MOTOR PREFERENCE IN RESPONDING TO OUTWARD DIRECTION OF VISUAL MOTION. *W.H. Ehrenstein, B.E. Arnold, Z. Bohdanecký¹, L. Schlykova²*, Institut für Arbeitsphysiologie, Dortmund, Germany, ¹Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and ²CLI for Physiology, University of Leipzig, Germany.

We investigated the problem whether the shorter latency for motion towards the fovea obtained in a previous study for monocular observation and simple reaction times would occur under binocular conditions in a choice reaction task. The stimuli employed were two lights presented 5 degrees to the left or right of the central fixation position of which one moved leftward or rightward (2 deg. s^{-1}) in a given trial. Eight right-handed subjects had to respond by moving a joy-stick (leftward or rightward) with either their left or right hand in the direction or position of the visual motion. Responses to outward motion were shorter (23 ms on the average, $p < 0.01$) than to inward (centripetal) motion. This preference (resembling partially the motor preference for supination of the wrist) was more pronounced for the left visual field and for the non-dominant left hand.

III. Endocrinology

EXTRACELLULAR LEVELS OF CATECHOLS AND ASCORBATE IN THE RAT STRIATUM DURING ANAESTHESIA. *J. Petrinec, T. Guadalupe¹, J.L. Gonzales-Mora¹*, Institute of Physiology, Faculty of Medicine, Bratislava, Slovak Republic and ¹Department of physiology, University of Laguna, Spain.

Many pharmacological experiments on laboratory animals are performed during anaesthesia. The choice of anaesthetised drug for acute experiments can exert considerable influence on the levels of some neurotransmitters and related substances. We have compared four currently used anaesthetic drugs: pentobarbital (35 mg/kg i.p.), urethane (1200 mg/kg i.p.), chloral hydrate (450 mg/kg i.p.) and ketamine-xylazine (85–15 mg/kg i.m.) and their effects on extracellular levels of dopamine (DA), its main metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) and ascorbic acid (AA) in the rat striatum. The levels of substances were monitored by Differential Normal Pulse Voltametry (DNPV) with carbon fibre electrodes. We observed that urethane injections increased AA up to 200 % and DA up to 250 % of the basal levels. Pentobarbital only caused a shorter increase of DA levels up to 150 % and also a later reduction of AA levels to 70 % of basal values. Chloral hydrate significantly enhanced catechol levels (DA up to 280 %), but it had no influence on AA levels. Ketamine-xylazine increased DA and AA levels (DA up to 200 %) after 30 min with a subsequent considerable decrease, and even disappearance, of the dopamine signal.

EFFECT OF EXTREMELY LOW TEMPERATURES ON CORTISOL LEVELS, MILK YIELD AND FEED CONSUMPTION OF DAIRY COWS. *J. Brouček, C.W. Arawe¹, Y. Nakanishi², S. Mihina*, Research Institute of Animal Production, Nitra, Slovakia, ¹Utah State University, Logan, USA and ²Kyushu University, Oita, Japan.

Two groups of dairy cows were observed. The trial group was housed in an open free stall barn with minimum temperatures ranging from -9.4 °C to -14.8 °C, the control group was kept in a warm barn with minimum temperatures from 0.2 °C to 2.2 °C. Blood samples were withdrawn four times. No significant differences were found in the levels of cortisol. During the fourth week, the concentration of cortisol levels in cows kept in the environment with extremely low temperatures was decreased to 0.47 µg/dl but during the eleventh week it returned to the initial level. There were no significant differences in milk production, which was gradually decreased in the course of lactation from 34.8 kg to 30.8 kg in the trial group and from 35.5 kg to 30.8 kg in the control group. The feed intake in the two groups significantly differed only during the second and eleventh week. Low temperatures of -15 °C were found not to stress dairy cows that are optimally fed and did not cause any significant changes in cortisol levels or decrease the milk yield.

EFFECTS OF CALF SUCKLING AND MILKING ON THE SECRETION OF OXOTOCIN AND CORTISOL OF DAIRY COWS. *V. Tančin, J. Brouček, S. Mihina, L. Harček, M. Uhrinčar*, Research Institute of Animal Production, Nitra, Slovak Republic.

The responses of oxytocin and cortisol were studied during machine milking in the presence of calves in three first-calver cows, during suckling of calves and following machine milking after weaning. Each cow suckled four calves during the four weeks after delivery. The experiment lasted five days. Blood samples were taken with a permanent catheter prior to, during and after sucking or milking. The mean levels of oxytocin concentrations were slightly lower during the first milking than in a situation when calves were present and significantly lower during suckling and 3rd milking after weaning. During the first milking, a significantly higher amount of cortisol was released compared with suckling and the third milking. Milk production and flow of milk were significantly lower during the first milking when compared with the third milking after weaning. The results have shown that the first milking after weaning of calves is a strong stress for dairy cows.

CHANGES OF INSULIN AND GLUCAGONE RECEPTORS IN HEPATOCYTES DURING REGENERATION AFTER PARTIAL HEPATECTOMY IN RATS. *L. Macho, M. Ficková, J. Knopp, S. Zorad*, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

The binding of glucagone (GL) and insulin (INS) on isolated hepatocytes was determined in rats during the process of regeneration after partial hepatectomy. Adult male rats were subjected to two thirds partial hepatectomy, control animals were sham operated. The isolated hepatocytes were prepared 1, 2, 3 and 5 days after the surgery and the binding of radioiodine labelled INS and GL was determined. The plasma levels of INS and glucose and also the microviscosity of liver plasma membrane were measured. It was found that the binding capacity for INS was decreased 1, 2 and 3 days after the operation. On day 5 no changes in INS binding were noted. The increase of GL binding was found on day 3 after hepatectomy, however, on day 5 no changes in GL binding were observed. The plasma insulin and glucose levels were similar in hepatectomized and sham-operated animals. An increase of microviscosity of plasma membrane of hepatocytes was noted during the process of liver regeneration and a negative correlation was found between INS binding and microviscosity of the membrane. These results demonstrated that there are significant changes in INS and GL receptors in hepatocytes during the liver regeneration after partial hepatectomy.

THE INFLUENCE OF EWE BREEDS ON SUPEROVULATION STIMULATION. *M. Molnárová, J. Arendarčík, J. Várady*, Department of Physiology, University of Veterinary Medicine, Košice, Slovak Republic.

The Merino ewe breed is sensitive to superovulation but varies considerably in individual sheep. Ewes react to treatment with increased doses of PMSG by a statistically significant increase in the average ovulation number, particularly after joint treatment with chloresuperlutine (Ageline vaginal sponges). When we compare the Merino breed (M) with the Wallachian breed (W), after treatment with Ageline (9 days), and then with 2000 IU PMSG, we can see a higher average ovulation number and a smaller ratio of nonovulating protruding 5–10 mm follicles to follicles > 10 mm in M ewes (M: 3.25±2.1, 1.53, W: 1.16±1.4, 4.43 – ovulation/ovary ratio). The average changes in antiproteolytic activity (AA) of blood plasma (BP) and follicular fluid (FF) after stimulation were also different. There were high average values AA BP W (P<0.02) among M ewes prior to the experiment. After stimulation, changes of AA BP in MW ewes were statistically higher (P<0.001), while changes of AA BP in W ewes were nonsignificant. Similar results were obtained in E₂ ositive (E₂/P₄>1) follicular fluid follicles 2–10 mm where antiproteolytic activity had increased after stimulation (P<0.02).

IMMUNONEUTRALIZATION AND HYPOTHALAMIC NEUROPEPTIDE SECRETION. *V. Štrbák, M. Nikodémová, J. Benický, M. Grino¹, C. Oliver¹*, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic and ¹INSERM U 297, Faculty of Medicine, Nord, Marseille, France.

Passive immunization is a commonly used approach for eliminating endogenous biologically active substances. Binding to a specific antibody usually prevents the binding of a neuropeptide to its receptors, this making it possible to study the effect of its neutralization on physiological functions. An injection of labelled monoclonal antibodies against AVP to rats revealed that their distribution volume is 73.2 ml/kg BW and their circulation half-time is 24 h. High saturation of the AVP antibody suggested excessive secretion of the AVP and its binding to the antibody. Injection of polyclonal antibody against Corticotiberin resulted in high plasma CRH levels, related to the amount of the antibody injected. CRH mRNA levels in ncl. paraventricularis were significantly increased thus suggesting that CRH biosynthesis was enhanced. Examination of hypothalamic *in vitro* Thyroliberin secretion in rabbits actively immunized against TRH revealed its high secretion in relation to the titre of produced antibody. Immunoneutralization may thus also represent a potent stimulus for secretion of the studied substance.

TRH SECRETION IN DIFFERENT REGIONS OF NEURON. M. Nikodémová, V. Štrbák, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

At the previous conference we presented a new methodological approach for the investigation of TRH secretion from the hypothalamic structure median eminence (ME) and paraventricular nucleus (PVN). These structures represent one functional system regulating the pituitary – thyroid axis. The TRH is synthesized as a precursor in the PVN (perikarya), transported to the ME (nerve terminals) and secreted into the portal circulation. Repeated membrane depolarization by 56 mM KCl was used to stimulate TRH secretion. This release is Ca^{2+} dependent. After incubation, the TRH amount in the PVN decreased, but did not change in the ME. It seems that ME, unlike the PVN, maintains a constant level of TRH by immediate restoring of TRH content most probably by maturation of the precursor. The time course of the secretion during depolarization revealed the most rapid secretion in the first 5 minutes. Secretion was more rapid in the ME than in the PVN. Moreover, the exchange of the medium during depolarization (removing of released TRH) increased TRH secretion from the ME, but not from the PVN. This suggests that either TRH or some other released substance act at the level of ME by a negative feedback mechanism. Our results suggest that there are differences in the regulation of TRH in the perikarya and nerve terminals.

EXPRESSION OF PROLACTIN IN RAT LYMPHOCYTES. J. Jurčovičová, R.N. Day¹, R.M. McLeod¹, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic and ¹Department of Internal Medicine, University of Virginia, Charlottesville, Virginia, USA.

We investigated whether prolactin (PRL) mRNA is produced by cells of the rat immune system. Analysis of total RNA from PRL-production MMQ cells and spleen by the solution hybridization method resulted in protection of rat PRL cRNA probe from nuclease digestion. In the spleen, the protected fragment presented as a weak band, indicated a low incidence of the message. The transcript was further characterized by a polymerase chain reaction (PCR). Oligonucleotide primer sets complementary to the rat pituitary PRL gene sequence were used to amplify cDNAs from the pituitary, MMQ cells, peripheral lymphocytes, spleen and Rat-1 fibroblast cells. No product could be detected in peritoneal macrophage or rat mesangial cell RNA. Restriction endonuclease digestion of the PCR products revealed identical fragment suggesting the same pituitary PRL gene sequence in all the studied preparations. These results show that rat lymphocytes and fibroblasts produce pituitary PRL that may function as a local growth factor.

POSSIBLE MECHANISMS OF ACTION OF SALMON MELANIN-CONCENTRATING HORMONE (sMCH) ON THE FROG SKIN SODIUM TRANSPORT. M. Smruga, P. Bakoš, D. Ježová, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Salmon melanin-concentrating hormone (sMCH) is a cyclic heptadecapeptide reported to control skin pigmentation both in fish and amphibia. The effects of sMCH alone, in combination with dDAVP, a vasopressin agonist, or with forskolin were studied on active sodium transport across the frog skin. Standard measurements of transepithelial short-circuit current (I_{sc}) were performed. Salmon MCH (0.5–10 μM) applied to the basolateral side of the epithelium failed to modify active sodium transport as reflected by unchanged levels of I_{sc} across the skin. However, sMCH significantly reduced the stimulatory effect of dDAVP (10 μM), did not change its enhancing effect on sodium transport. The inhibitory action of sMCH is probably the result of its modulating influence at the membrane level. We assume that cAMP, being involved in the effects of dDAVP and forskolin, does not participate in the mediation of sMCH action in the frog skin.

EARLY CHANGES OF PINEAL MELATONIN AND CATECHOLAMINES IN LETHALLY GAMMA-IRRADIATED RATS. E. Ahlersová, M. Kassayová, B. Pástorová¹, I. Ahlers, M. Sabol, Institute of Animal Physiology, Faculty of Science, Šafárik University and ¹Department of Comparative Physiology, University of Veterinary Medicine, Košice, Slovak Republic.

Male Wistar SPF rats were subjected to whole body irradiation after adaptation to an artificial light-dark regimen 12:12 h with 14.35 Gy gamma rays. The rats irradiated in the night were analysed 30, 60 and 120 minutes after exposure, from 2040 h to 0130 h. Control rats were sham-irradiated. Radiation did not change the N-acetyltransferase activity, the key enzyme of melatonin synthesis, but decreased the concentration of melatonin during the observed period. The level of norepinephrine was decreased after 30 and 120 min; epinephrine was elevated 30 min after the exposure; subsequent values markedly decreased. The irradiation did not influence the melatonin levels but changed the corticosterone and thyroxine concentrations in the serum.

Project No. 2-990719-92 was supported by the Slovak Grant Agency.

THE EFFECT OF EXOGENOUS MELATONIN ON METABOLIC AND HORMONAL CHANGES IN RATS IRRADIATED CONTINUOUSLY WITH GAMMA RAYS. I. Ahlers, M. Sabol, E. Ahlersová, M. Kassayová, Institute of Animal Physiology, Faculty of Science, Šafárik University, Košice, Slovak Republic.

Male Wistar SPF bred rats were continuously irradiated with a daily dose 100 mGy of gamma rays for 15 days. A part of irradiated and control animals drank melatonin in the concentration 20 μg/ml of tap water during exposure. The analyses were performed 1, 30 and 100 days after the irradiation had been discontinued. Continuous irradiation (by analyses 1 and 30 days after the end of the exposure) increased the concentration of glucose, total cholesterol (CH), phospholipids (PL), thyroxine (T₄) and triiodothyronine (T₃) in the serum, the content of glycogen, triacylglycerols (TG) and Ch in the liver. This was accompanied by a decrease in the content of PL and TC in the thymus and an increase of TG in the bone marrow. Exogenous melatonin prevented the liver glycogen accumulation and decreased T₄ and T₃ levels. In this manner, melatonin ameliorated some consequences of the radiation injury.

Project No. 2-990719-92 was supported by the Slovak Grant Agency.

AMILORIDE-SENSITIVE SODIUM CONDUCTANCE IN ANTRAL EPITHELIUM OF *NECTURUS MACULOSUS* AND MECHANISMS OF ITS REGULATION. P. Bakoš, E. Frömter¹, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic and ¹Zentrum der Physiologie, Klinikum der J.W. Göthe Universität, Frankfurt/M., Germany.

In the isolated antrum of *Necturus maculosus*, a time-dependent increase in sensitivity to the sodium channel blocker amiloride (10^{-5} M) has been found. Maximum inhibition of the transepithelial potential (V_{ms}) and short-circuit current (I_{Na}) by amiloride was determined after 1–2 h, while practically no sensitivity to this blocker was detected at the beginning of the experiments. Amiloride-sensitive I_{Na} was higher in fed animals. An increase of intracellular calcium concentration by ionomycin (10^{-6} M) markedly inhibited sodium conductance of the apical membrane. A decrease of amiloride-sensitive sodium transport was detected both after application of a protein kinase C (PKC) stimulator-phorbol esters and trifluoperazine. The results indicate that at least two regulatory mechanisms of epithelial sodium transport may be involved: 1) activation of calmodulin and secondary stimulation of Na^{+} -conductance, 2) activation of mobile PKC in the cell, its insertion into the apical cell membrane, followed by a degradation of sodium channels and inhibition of Na^{+} -conductance.

THE EFFECT OF LIGHT IN THE EARLY AND LATE NIGHT PERIOD ON THE PHASE OF THE CIRCADIAN CLOCK AND C-FOS EXPRESSION. *H. Illnerová, A. Sumová, J.W. Schwartz¹, Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic and ¹University of Massachusetts Medical Center, Worcester, USA.*

Light pulses in the early night delay and in the late night advance the rat circadian pacemaker in the hypothalamic suprachiasmatic nuclei (SCN). These phase shifts might be mediated by c-fos expression in SCN after a light stimulus at night. To test this hypothesis, we studied the relationship between shifts and c-fos expression. As a phase-marker we used the pineal rhythm in N-acetyltransferase (NAT) controlled by SCN. C-fos mRNA was determined by *in situ* hybridization. Male rats maintained in a regime with 16 h of light and 8 h of darkness were exposed to 30-min light pulses either in the early night or in the late night. Pulses around the usual dark onset induced various c-fos expression; the intensity of expression correlated with the magnitude of the delay in the NAT rise. Pulses around the usual light onset also induced various c-fos expression; the intensity of expression correlated with the magnitude of the advance in the NAT decline. The data indicate that the NAT rhythm may be reset *via* c-fos expression in the SCN.

PHASE SHIFTS OF THE HUMAN CIRCADIAN CLOCK FOLLOWING SINGLE LIGHT PULSES APPLIED AT NIGHT. *L. Samková, M. Burešová, H. Illnerová, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague.*

When humans are exposed to a sufficiently bright light during their subjective night for several days, their circadian clock driving daily rhythms delays or advances. Our study was undertaken to find out, whether even a single light pulse may phase shift the pacemaker and if so, how the phase shift is affected by the illumination time. Subjects were exposed to 3–6 h of bright light (2000–3000 lux) at various night times. The next day, the phase shifts of the evening rise and of the morning decline in the serum or salivary melatonin values were followed as markers of shifts of the underlying pacemaker. Altogether, we obtained 17 values for the rise and 18 values for the decline shifts. The light pulse in the early night delayed the evening rise and the morning decline by 1–2 h. Following a light pulse in the late night, the melatonin rise and the decline were phase advanced; the advance of the morning decline was, however, much larger than that of the evening rise. It appears that, in humans, the evening melatonin rise and the morning decline do not necessarily phase shift in parallel and thus the underlying pacemaker might be rather complex.

THE ROLE OF ADRENOCORTICAL AND SEROTONERGIC ACTIVATION IN NON-PHOTIC CIRCADIAN ENTRAINMENT IN THE SYRIAN HAMSTER. *A. Sumová, E.S. Maywood¹, F.J.P. Ebling¹, J. Herber¹, M.H. Hastings¹, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and ¹Department of Anatomy, University of Cambridge, UK.*

Handling and injection of animals held under constant darkness at circadian time (CT) 10 has been found to be a potent non-photic cue able to phase advance the free-running activity rhythm. Injection at CT6 is ineffective (1). Repeated injections at CT10 caused an accumulated phase advance, confirming true entrainment. The non-photic phase shifts were correlated with adrenocortical activation. When delivered at CT10, the non-photic cue significantly enhanced secretion of cortisol in the plasma above baseline levels. The same stimulus failed to elicit cortisol levels at CT6. A serotonergic antagonist, ketanserin, blocked the phase advances induced by the non-photic cue. These results demonstrate the involvement of adrenocortical activation and serotonergic neurotransmission in non-photic entrainment of the circadian clock. 1. *Hastings M.H., Mead S.M., Vidlacheruvu R.V., Ebling F.J.P., Maywood E.S., Grosse J.*: Brain Res. 591: 20–26, 1992.

MELATONIN RHYTHM IN HUMAN MILK. *M. Burešová, H. Illnerová, J. Presl¹, Institute of Physiology, Academy of Sciences and ¹Institute for the Care of Mother and Child, Prague, Czech Republic.*

The pineal hormone melatonin inhibits a circadian rhythm in body fluids (blood, urine, cerebrospinal fluid, saliva) with nighttime values considerably higher than daytime levels (1). No data on melatonin in human milk have hitherto been available. The present study was undertaken to determine whether melatonin is detectable in human milk and, if so, whether it exhibits a daily rhythm. Blood and milk were sampled between 1400–1700 h and again between 0200–0400 h from 10 mothers 3–4 days after delivery. Melatonin in both fluids was beyond the limit of detection during the day, whereas during the night its concentration was 280 ± 34 pmol/L in the serum and 99 ± 26 pmol/L in the milk. Six mothers collected milk after each feeding throughout a 24-h period within 3 months after delivery. Melatonin in the milk of all subjects exhibited a pronounced daily rhythm, with high levels during the night and undetectable levels during the day. The presence of the rhythm in milk suggests that melatonin fluctuations in the milk might communicate information about the time of day to breast-fed infants.

1. *Illnerová H.*: Pineal Res. Rev. 6: 173–217, 1988.

CAN MATERNAL MELATONIN ENTRAIN THE CIRCADIAN PACEMAKER OF NEWBORN RATS? *Z. Trávníčková, H. Illnerová, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague.*

The rat circadian pacemaker is entrained to the external environment by light. Administration of melatonin immediately prior to the dark onset may, however, cause its phase advance. The pacemaker of newborn rats is entrained by their mothers; light does not affect the pacemaker until 6 days after birth. Melatonin found in maternal milk might serve as an entraining factor. To test this hypothesis, we studied the entraining effect of melatonin administered just prior to the dark period on phase of the pacemaker in 5-, 15- and 27-day old rats. We used the rhythm of N-acetyltransferase (NAT) activity which drives melatonin production as a marker of pacemaker's phase. Melatonin administration phase advanced the evening NAT rise in all age groups. As the NAT rise in intact 5-day-old rats was advanced by 2 hours as compared with that in their mothers, it appears that maternal melatonin does not entrain the newborn rat pacemaker in the sense of advance. The NAT rise in 15- and 27-day-old rats was already in phase with that of their mothers.

IV. Physiology of Blood Vessels, Blood and Respiration

ORNITHINE DECARBOXYLASE ACTIVATION IN THE CORONARY ARTERY DURING INCREASED AFTERLOAD. *M. Gerová, M. Juráni¹, M. Kittová², Institute of Normal and Pathological Physiology, ¹Institute of Biochemistry and Genetics, Slovak Academy of Sciences and ²Institute of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic.*

Ornithine decarboxylase (ODC) is a rate-limiting enzyme involved in the production of polyamines which control the DNA and RNA levels and proteosynthesis. A 4 hours lasting cardiac pressure overload increases RNA and ¹⁴C leucine incorporation in the coronary wall. The question arose whether ODC activation preceded the increase in proteosynthesis. In a group of 4-hour hypertension due to aortic constriction (group 1), and noradrenaline infusion (group 2), the ODC activity increased in the later group only. Thus, if hypertension is induced *via* autonomic mediator and respective receptors, the ODC activation is involved in triggering the process of proteosynthesis. The ODC activation may be considered as one link of the trophic effect of sympathetic nervous system.

TORBAFYLLINE DOES NOT IMPROVE THE RESPONSIVENESS OF CHRONICALLY LIGATED ILIAC ARTERY. A. Holéciová, O. Hudlická¹, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic and ¹Department of Physiology, University of Birmingham, Birmingham, UK.

Dawson et al. (1) showed that the xanthine derivative, torbafylline (TBF), improves the reactive changes in skeletal muscle microvessels caused by chronic ligation of the iliac artery. The present study was concentrated on the question of whether TBF could also influence the impaired responsiveness of the conduit artery. Sprague-Dawley rats with ligated right common iliac artery were given torbafylline (25 mg/kg/day) for 2 weeks. After this period, the isometric tension to vasoactive drugs was monitored in the ligated vessels. The results indicate that neither the depressed contractility to noradrenaline and potassium chloride, nor the reduced relaxation to acetylcholine in response to ligation were influenced by TBF.

1. Dawson J.M., Okuyuz-Baklouti I., Hudlická O.: *Int. J. Microcirc. Clin. Exp.* 9: 385–400, 1990.

MYO-ENDOTHELIAL JUNCTIONS IN DOG CORONARY ARTERY DURING ONTOGENESIS. F. Kristek, M. Gerová, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Myo-endothelial (ME) junction in the coronary artery have been suggested to have an important role in the control of smooth muscle (SM) tone (1). Since it has been reported that adrenergic terminals in foetuses are absent, the role of the endothelium (E) and contacts to SM in early ontogenesis became more urgent. Using standard electron microscopy techniques the ME area was studied in foetuses (1 week before birth), newborns (1–4 weeks old) and adult dogs. Significant differences ($P < 0.001$) in the number of ME junctions were found among these groups: in foetuses one junction/ 7.2 ± 1.0 E sections (cca 60 junctions/E), newborns one junction/ 31.5 ± 6.7 (cca 16 junctions/E) and in adults one junction/ 154.3 ± 25.9 (cca 5 junctions/E). The findings support the hypothesis about the decisive role of E in SM tone in early ontogenesis.

1. Kristek F., Gerová M.: *J. Vasc. Res.* 29: 29–32, 1992.

The work was supported by the Slovakofarma Joint Stock Company, Hlohovec, Slovak Republic.

ENDOTHELIAL AND NEUROGENIC CONTROL OF CANINE THORACIC AORTA IN ONTOGENESIS. J. Török, M. Gerová, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

The nerve terminals in vessels appear in the late gestation period and develop further after birth. In the prenatal period, the endothelium covers the luminal surface of the vessels completely. The purpose of this study was to compare the range of endothelium relaxation and the neurogenic contraction of the aorta in dog foetuses (1 week before birth), in newborns (1, 2, 4 and 6 weeks old), and in adult dogs. Acetylcholine caused a dose-dependent relaxation of aortic rings precontracted by phenylephrine. The magnitude of relaxation was comparable in foetuses and all newborns and was greater than in adults. Transmural nerve stimulation (1–64 Hz) induced only slight contractions in foetuses at 32–64 Hz. The neurogenic contractions increased at all frequencies with age, and the maximum was found in adults. The results suggest that the endothelium-dependent control of vascular tone precedes neurogenic control in the canine aorta.

BREED DIFFERENCES IN THE GROWTH KINETICS OF RAT AORTIC SMOOTH MUSCLE CELLS (SMC) IN CULTURE. L. Bačáková, V. Mareš, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague.

Migration and proliferation of SMC of the vessel wall play a key role in the pathogenesis of several vascular diseases. In this study, the growth capacity of SMC from rats raised under conventional or specific pathogen-free conditions was compared in cultures obtained by explantation from the thoracic aorta of male Wistar rats (180–200 g, 8 weeks old). The SMC from conventionally raised rats migrated from the explants earlier by 1 to 3 days and the number of cells which moved out was higher. In the 3rd passage, these cells had a shorter doubling time (by 35%), a higher mitotic index (by 37%), a higher ³H thymidine labelling index (by 53%), a shorter mean cell cycle duration (by 46%) and they attained a higher maximum population density (by 45%). These results suggest that breeding conditions have an important impact on the growth capacity of vascular SMC, which may be due to a different spectrum of commensal microorganisms.

EFFECT OF PERINATAL AND ADULT EXPOSURE TO HYPOXIA ON DISTRIBUTION OF PULMONARY VASCULAR RESISTANCES IN RATS. J. Herget, V. Kuklík, Department of Physiology, Second Faculty of Medicine, Charles University, Prague, Czech Republic.

Pulmonary vascular changes induced by perinatal hypoxia are permanent (1). Three groups of adult male rats were studied: controls (C, n=7), rats born in hypoxia ($F_{iO_2} = 0.12$) but raised in air (PH, n=6) and rats born in normoxia and exposed to hypoxia when adult (AH, n=8). Distribution of vascular resistances was measured by the double occlusion technique (2) in isolated lungs ventilated with air or 3% O₂. Total, arterial, middle segment and venous resistances were significantly higher in AH than in PH and C. In acute hypoxia, vascular resistances did not change in AH, the resistance in the middle segment increased in C and both, arterial and middle segment resistances increased in PH. Perinatal hypoxia produces permanent lung vascular changes which may explain increased hypoxic reactivity found earlier (1). (*Grant of Charles University No. 214*).

1. Hampl V., Herget J.: *Am. Rev. Resp. Dis.* 142: 619–624, 1990.

2. Hakím et al.: *J. Appl. Physiol.* 54: 1298–1302, 1983.

EFFECT OF 1,4-DHP ON LIPID PEROXIDATION IN POSTISCHAEMIC RAT BRAIN. INFUSION AND PER OS APPLICATION. P. Noskovič, V. Faberová, Department of Biochemistry, Drug Research Institute, Modra, Slovak Republic.

Free radicals have been implicated in a wide variety of diseases and degenerative processes such as aging and Parkinson's disease. In our study we investigated the possible effect of an original substance VULM 993 (calcium channel blocker) on lipid peroxidation using a four-vessel occlusion model of transient ischaemia. We have compared the effect of infusion and *per os* application. It was found that the original substance VULM 993 has a protective effect on lipid peroxidation (decline concentration of malondialdehyde – MDA) after 30 min recirculation. The infusion reduced the concentration of MDA in the cortex, striatum and hippocampus. The *per os* application reduced MDA in the cortex and striatum.

MULTIDRUG RESISTANCE (MDR) OF NEOPLASTIC CELLS MEDIATED BY P-GLYCOPROTEIN (P-GP). 1. L1210 MOUSE LEUKAEMIC CELLS ADAPTED TO VCR IN A *IN VITRO* MODEL. A. Breier, M. Barančík¹, Z. Štefanková¹, Institute of Molecular Physiology and Genetics and ¹Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Overexpression of P-GP, that is believed to ensure ATP-dependent drug efflux across the plasma membrane, was developed by adaptation of L1210 mouse leukaemic cells to vincristine (VCR). The subline obtained in this way not only induces resistance to VCR, but also cross-resistance to vinblastine and actinomycin-D. Resistant cells were found to accumulate lower amounts of [³H]-vincristine ([³H]-VCR) than sensitive L1210 cells. At first accumulation of [³H]-VCR started at the same velocity in both sensitive and resistant cells. [³H]-VCR accumulation was run to equilibrium in the sensitive cells. On the contrary, the amount of accumulated [³H]-VCR by resistant cells was found to be stabilized after the initial phase at values (reflecting probably the affinity of P-GP) smaller than those observed for sensitive cells. Several drugs such as calcium channel blockers, calmodulin inhibitors and local anaesthetics, depressed the MDR. Efficiencies of these drugs in MDR reversal were found to correlate with their ability to interact with calmodulin or with their local anaesthetic efficacy, but not with their capability to block the L-type of calcium channel.

MULTIDRUG RESISTANCE (MDR) OF NEOPLASTIC CELLS MEDIATED BY P-GLYCOPROTEIN (P-GP). 2. CULTIVATION OF RESISTANT CELLS IN A MEDIUM CONTAINING VINCRISTINE ENHANCES CELL RESPIRATION. Z. Sulová, A. Breier¹, B. Uhrík¹, A. Ziegelhoffer², Institute of Chemistry, ¹Institute of Molecular Physiology and Genetics and ²Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Overexpression of P-GP is an often observed biochemical characteristic of cells with MDR. P-GP is an integral glycoprotein of the plasma membrane of animal cells that secure the ATP-dependent efflux of lipophilic substances (drugs) across the plasma membrane. More condensed mitochondria were observed when a MDR subline of L1210 cells (1) was cultivated in the presence of vincristine. This phenomenon was coupled with a significant increase in the cell respiration rate. Stimulation of oxygen consumption induced by the presence of vincristine observed in resistant cells, was accompanied by a decrease in [¹⁴C]-glucose accumulation. Contrary to these findings, a decrease of both oxygen consumption and glucose accumulation were observed in parental sensitive cells. Thus, vincristine probably induced more effective glucose catabolism in resistant cells via a prevalence of the mitochondrial aerobic pathway accompanied by a depression of anaerobic glycolysis by the Pasteur effect.

1. Breier A., Barančík M., Štefanková Z.: See preceding abstract at this conference.

MULTIDRUG RESISTANCE (MDR) OF NEOPLASTIC CELLS MEDIATED BY P-GLYCOPROTEIN (P-GP). 3. EFFECT OF PENTOXIFYLLINE (PTX) ON P-GP MEDIATED MDR. Z. Štefanková, M. Barančík, A. Breier¹, B. Uhrík¹, Institute for Heart Research and ¹Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Several drugs termed as chemosensitizers were found to inhibit the drug efflux capacity of P-GP. The drugs influencing calcium homeostasis such as calcium entry blockers and calmodulin antagonists have often been described as potent chemosensitizers. PTX, similarly as caffeine, influences calcium homeostasis by stimulating calcium release in the endo(sarco)plasmic reticulum and by mobilization of intracellular cAMP levels by inhibiting phosphodiesterase activity. The reversal effect of PTX on the vincristine (VCR) resistance of the MDR subline of mouse leukaemic cell line L1210/VCR (1) was observed. Higher amounts of [³H]-VCR accumulated by resistant cells were observed in the presence of PTX. PTX did not exert any effect on VCR accumulation by parental sensitive cells. The ability of PTX to restore VCR sensitivity of the resistant cell line was confirmed by observable ultrastructural damage of resistant cells, cultivated in the presence of both VCR and PTX, in comparison to undamaged cells cultivated in the presence of either PTX or VCR. These facts indicate that PTX may be considered a MDR reversal agent.

1. Breier A., Barančík M., Štefanková Z.: See preceding abstract at this conference.

REGULATORY ROLE OF THE ARACHIDONIC ACID CASCADE IN STIMULATED AND INHIBITED BLOOD PLATELETS. R. Nosál, V. Jančinová, M. Petříková, Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Blood platelets (BP) participate substantially in the regulation of blood homeostasis and blood clotting. BP respond to stimulation by shape changes and aggregation. We demonstrated that stimulation of BP *in vitro* liberated arachidonic acid (AA) from membrane phospholipids and also increased the production of malondialdehyde (MDA) and thromboxane (B₂ TXB₂). Cationic amphiphilic drugs (CAD) from the group of beta-adrenoreceptor blocking (BAB) drugs, depending on their physico-chemical properties, inhibited stimulated aggregation, AA liberation, MDA formation and TXB₂ production in BP. Inhibition of stimulated aggregation by BAB drugs correlated with their inhibition of the investigated AA pathway. We suggested from our results that CAD, at least in part, inhibited stimulated BP aggregation at the level of membrane phospholipase A₂, which is the crucial enzyme participating in triggering the AA cascade in BP.

POSTPERFUSION SYNDROME: ACTIVATION OF GRANULOCYTES DURING EXTRACORPOREAL CIRCULATION (ECC). F. Kovářů^{1,2}, R. Wagner³, H. Kovářů⁴, R. Halouzka⁴, Z. Knotková⁴, J. Černý³, A. Španová², ¹First Medical Faculty, Charles University, Prague, ²Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Brno, ³Cardiosurgery Centre, Medical Faculty, Masaryk University and ⁴University of Veterinary Sciences and Pharmacology, Brno, Czech Republic.

Pathophysiological and immunological consequences of ECC were studied using a pig model with the aim to demonstrate possible activation of circulating granulocytes part A of our experiments. Separation of cells and the plasma using a blood separation apparatus was included in part B of the experiments. In both A and B parts we estimated superoxide radical (O₂⁻) production, phagocytosis and surface neutral protease (NP) activity. The values before ECC were taken as reference for three intervals during ECC: 10 min after starting ECC (I), 10 min after aortic X-clamping (II), and after discontinuing ECC (III). Experiments lasted 3.5 h (A) and 5 h (B), respectively. Interval I and II in part A without separation was characterized by a marked increase of O₂⁻ production with further elevation in phase III. On the other hand, no changes were observed in phagocytosis (particle engulfment). High NP activity was found during phase I and II with a subsequent decrease of enzyme activity during III. Alteration of capillary endothel with cell infiltration was also demonstrated. However, granulocyte and plasma separation in part B of ECC prevented the activation of granulocytes at all intervals I, II and III and estimated values of tested parameters were comparable with reference data.

MODIFIED C-6 GLIOMA LINE - TARGET CELLS FOR NK PIG LYMPHOCYTES. H. Kovářů^{1,2}, F. Kovářů^{3,2}, H. Kozáková², ¹First Medical Faculty, Charles University, Prague, ²Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague and ³University of Veterinary Sciences and Pharmacology, Brno, Czech Republic.

Cell-to-cell interactions are also represented by reactions between natural killer (NK) activity of lymphocytes as effector cells and target cells. In the initial phase of NK activity cell surface interaction of effector and target cells may be assumed with strong adhesion of appropriate surface structures. Lymphoma K 562 cells are mostly used as targets for tests of NK lymphocyte activity in postnatal pigs in contrast to no NK cytotoxicity of fetal cells with the same targets (1). Our aim was to assay fetal NK activity with other target cell types. We prepared modified C-6 glioma cells with inserted T lymphocyte surface markers and the cell line was stabilized until the 85th passage. Then modified C-6 cells were employed in tests of fetal NK cytotoxicity. Results of ⁵¹Cr tests of fetal NK lymphocytes with modif. C-6 cells indicated a high cytotoxicity in contrast to no NK activity using original C-6 or K 562 employed as targets in tests of NK activity. The results of ⁵¹Cr tests of NK cytotoxicity with modified C-6 cells indicated high NK activity of fetal lymphocytes, in contrast to no NK activity with original C-6 cells or K 562 cells.

1. Kim Y.B., Ichimura O: *Swine in Biomedical Research*, M.E. Tubleson (ed.), Plenum Publ. Corp., Vol. 3: 1811, 1986.

V. Muscle and Sports Physiology

CELL MEDIATED IMMUNITY INDUCED BY THE MOUSE LDH VIRUS IN RATS OF THE WISTAR STRAIN. *A. Jandová, J. Bendl, M. Nedbalová, S. Trojan, M. Vávrová*, Second Clinic of Gynaecology and Obstetrics and ¹Institute of Physiology, First Faculty of Medicine, Charles University, Prague.

The levels of antigens against the mouse LDH virus reflect cell energy metabolism (1). The LDH virus affects the cell mediated immunity (2). The receptors responsible for this reaction are localized mainly in membranes of T₄ lymphocytes and macrophages. An increased immune reactions was found to occur spontaneously in 64.5 % of 31 healthy Wistar strain males and in 63.6 % of 33 healthy Wistar strain females. We coupled six fathers with high levels of antigens against the LDH virus. From a total of 87 newborns, there were 41.7 % of opositively reacting males and 33.3 % of positively reacting females, respectively. The results represent a starting point for further studies of the reaction of T4 lymphocytes and macrophages from Wistar rats in which the levels of antigens against the mouse LDH virus has been increased experimentally (e.g. by hypoxia).

1. *Jandová A., Molyčka K., Kobilková J., Čoupek J.*: Arch. Gynecol. Obstet. 245(14): 476–478, 1989.
2. *Riley V.*: Cancer Res. 34: 1752–1754, 1974.

REACTION TIME MEASUREMENTS IN ATHLETES. *M. Sleboda, E. Kužma, J. Lipková, V. Stulrajter, V. Psalman*, Faculty of Physical Education and Sports, Comenius University and ¹Slovak Academy of Sciences, Bratislava, Slovak Republic.

The purpose of this study was to measure the reaction time of athletes after receiving (1) a visual cue from a computer screen or (2) and auditory cue. Reaction time (RT) is the same it takes to initiate a specified action after receiving a designated signal (cue). This study includes 3 types of RT: (1) simple RT (2) disjunctive RT (for two or more cues) and (3) RT connected with dispersing of attention. We asked 24 athletes (only males were chosen) to respond as quickly as possible to visual cues on a colour monitor and auditory cues received from earphones. The subjects pressed a computer joystick and the duration between cue and response was measured by the computer. These subjects were secondary school students ranging in age from 11 to 13. Previous studies dealing with RT involving visual cues from a colour monitor showed that a duration of RT depends on the colour of the cue and background. (*) These data showed that: (1) the shortest RT is for yellow, orange and red colours, (2) the longest RT is for grey, green and blue colours if they are projected on a grey background. Our observations lead to the conclusion that RT is an important factor in all sports performances.

- (*) *Stulrajter V.*: Development of neuro-muscular abilities of athletes, Bratislava, Sport, 1989, 107 pp.

THE EFFECT OF MENTAL STRESS ON CIRCULATORY AND RESPIRATORY VARIABLES. *M. Al-Kubati, J. Siegelová, B. Fišer*, Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic.

Medical students (n=33) who passed their final oral examination (in pathological physiology) were examined 30 min before (assumed mental stress) and 30 min after the examination (relief). During 3 min lasting period of metronome-controlled breathing we measured and calculated: systolic and diastolic blood pressure (SBP, DBP; Peňáz method), cycle interval (I), baroreflex sensitivity (BRS; spectral analysis) together with flow volume curves and airway resistance (Flowscreen Jaeger). When compared to the postexamination values, the preexamination SBP and DBP were significantly higher ($p < 0.01$ and $p < 0.05$, respectively), and BRS values were significantly lower ($p < 0.01$ and $p < 0.05$, respectively; $BRS = 8.74 \pm 4.69$ vs. 7.11 ± 3.03 ms/mmHg). In contrast, both respiratory parameters were about the same. It is concluded that the cardiovascular system is more sensitive to moderate mental stress than the respiratory system.

PHYSIOLOGICAL ASPECTS OF LATERALITY. *J. Lipková, V. Stulrajter, A. Scholcová*, Faculty of Physical Education and Sports, Comenius University, Bratislava, Slovak Republic.

The aim of this study was to assess the role of the laterality during sport performance. This experiment included several functional indicators of the neuro-muscular system, namely: (1) a grip muscular force (HGF), (2) extensor and flexor muscle force of the legs (EFFL), (3) reaction time (RT), (4) reflex time (RefT). For the purpose of this study 24 male athletes were chosen. They were secondary school students ranging in age from 11 to 14 years. We tested them twice. The measurements were repeated after a 3-month period. This period consisted of compensatory training, mainly of stretching and strength exercises for nondominant extremities. The obtained results showed that: (a) At HGF – (1) Input test found a statistically significant difference for the dominant hand, (2) Output test confirmed the same difference. (B) At EFFL – (1) Input test showed statistical significant difference for the dominant leg, (2) Output test found no significant difference. (C) RT values were significantly shorter for the dominant hand. (D) RefT increased after the 3-month training period, but differences between the dominant and nondominant leg were higher. The results lead us to repeat the tests and to confirm the positive influence of stretching.

A CASE STUDY OF PERIODIC BREATHING IN RELAXATION. *A. Stančák*, Department of Physiology and Clinical Physiology, Third Medical Faculty, Charles University, Prague, Czech Republic.

In 13 experiments we investigated one male subject, aged 27 years, without neurological or respiratory pathology, who exhibited a periodic breathing pattern consisting of alternating periods of polypnoea and apnoea during relaxation. The polypnoeic part (20–60 s breathing at 1–4 Hz frequency) was accompanied by tachycardia (90–120 b.p.m.) and increased systolic blood pressure (140–180 mm Hg). Fast abdominal respiratory movements were modulated by a slower rhythm of 0.2 Hz frequency, and occasionally by a 0.1 Hz rhythm. During the apnoeic part which lasted 60–120 s, there were slow pendulum-like horizontal eye movements synchronous with heart beats, and rhythmical contractions of anterior abdominal muscles (0.25 Hz). Oxygen saturation in arterial blood decreased to 70 % at the beginning of polypnoea. We suggest that this unusual type of periodic breathing resulted from instability of the chemoreflex control system of respiration in a predisposed subject.

AN ANALYTICAL COMPUTER PROGRAM FOR TESTING TEMPERATURE CONDITIONS DURING PHYSICAL LOADING. *E. Kukurová, J. Antal, Z. Herdová*, Institute of Medical Physics and Biophysics, Medical Faculty, Comenius University, Bratislava, Slovak Republic.

Computer simulation of load-temperature testing responses is based on the Quasi Living Code Systems' theory – QLCS. It represents an important noninvasive diagnostic method used in modern medical practice. This computer simulation provides objective and complex information about a subject's health status and its assessment after a quantitative physical load performed on a temperature-ergometric device-Schiller. Application of the QLCS theory on Schiller's data thus obtained enabled a quantitative evaluation of the control-energetical processes, presented in the living organism during time-related changes of the physical load. We called this "The organism's load-temperature characteristic model – MZTCO". As a result, we were able to evaluate (1) experimental and stimulated load-temperature responses ZTKe, ZTKs; 2) to obtain ZTKe and ZTKs (complex processing) and 3) to evaluate the subject's health status.

THE USE OF A NEW METHOD IN THE ESTIMATION OF VENTILATORY PARAMETERS DURING PHYSICAL STRESS. T. Paul, L. Horanská, V. Srajer, V. Čermák, Department of Physiology and Clinical Physiology, Third Medical Faculty, Charles University and Institute of Sports Medicine, Prague, Czech Republic.

A new method for determination of oxygen consumption and minute ventilation during physical stress is described. It is based on calculation of the regression lines from the recovery period values of both parameters which are determined during each breathing cycle. The values obtained by this new extrapolated method are compared with those obtained by the classical method. The O_2 consumption measurements were compared in 44 adult subjects. Correlation between the two methods has been proved at the 0.001 level of significance. The mean value of classical measurements was $3954 \pm 839 \text{ ml} \cdot \text{min}^{-1}$, paired test $t=1.55$ (statistically nonsignificant). The mean value of extrapolated one was $3991 \pm 834 \text{ ml} \cdot \text{min}^{-1}$. Minute ventilation was compared in the same manner in 31 adult persons. Correlation was proved at the same level (0.001). The mean value of the classical measurements was $133.1 \pm 33.2 \text{ l} \cdot \text{min}^{-1}$, S.D., extrapolated was $135.3 \pm 32.7 \text{ l} \cdot \text{min}^{-1}$ paired test $t=1.91$ (statistically nonsignificant). Different types of regressions and the use of longer or shorter periods of sampled breathing cycles are also compared in the present work.

PHYSICAL ACTIVITY RELATED TO MORPHO-FUNCTIONAL VARIABLES IN PREPUBESCENTS. I. DIET AND BLOOD PARAMETERS. J. Vránová, J. Melichna, R. Džouhá, Z. Holeček, L. Havlíčková, Department of Physiology and Biomedical Centre, Faculty of Physical Education and Sports, Charles University, Prague, Czech Republic.

The effect of physical activity on somatic development of adolescents in relation to their physical performance and some health aspects concerning the prevention of modern life-style diseases was investigated in 12-year-old school boys ($n=54$). On the basis of the intensity of their weekly habitual physical activity (questionnaire) expressed as % of BMR, the subjects were divided into 3 groups (hypokinetic - 159 %, normokinetic - 180 % and hyperkinetic - 203 %, with a regular training program). Energy expenditure ($9.00-11.28 \text{ MJ} \cdot \text{d}^{-1}$) related to their food intake ($9.91-12.98 \text{ MJ} \cdot \text{d}^{-1}$) in all the tested groups, but a lower intake of iron ($10.0-13.8 \text{ mg}$) and vit. C ($36.9-46.9 \text{ mg}$) was observed (questionnaire). Haemodilution (haematocrit - 39 %) was found which was accompanied by a lower erythrocyte number (4.62 pl^{-1}) and serum triacylglycerol level ($0.54 \text{ mmol} \cdot \text{l}^{-1}$) in the trained boys.

PHYSICAL ACTIVITY RELATED TO MORPHO-FUNCTIONAL VARIABLES IN PREPUBESCENTS. II. ANTHROPOMETRICAL DATA AND NON-ROUTINE FUNCTIONAL TESTS. S. Bartůňková, E. Kohlíková, P. Vodička, L. Havlíčková, Department of Physiology, Faculty of Physical Education and Sports, Charles University, Prague, Czech Republic.

In 3 groups (hyper-, normo-, and hypokinetic) of 12-year-old boys anthropometrical indices and body fat were determined by using IBM methods. Reaction time (RT) to visual and/or acoustic stimulus and simple sensory motor skills (tapping, pursuit tracking test (PRT)) were also assessed. The function of the autonomic nervous system was evaluated by means of the orthostatic test. No differences among the tested groups in anthropometrical data as well as in visual RT were found. However, the hypothesis concerning the relationship between the level of habitual physical activity and the results of all other tests was confirmed, i.e. better sensorimotor abilities (PRT contact time - 39 vs 27 s) and acoustic RT (198 vs 218 ms) in trained children compared with the hypokinetic group were noted. A higher level of sympathetic excitation was found in all the tested children.

PHYSICAL ACTIVITY RELATED TO MORPHO-FUNCTIONAL VARIABLES IN PREPUBESCENTS. III. VITAL CAPACITY AND PHYSICAL PERFORMANCE TESTS. J. Heller, J. Novotný, L. Havlíčková, Biomedical Research Centre and ¹Department of Physiology, Faculty of Physical Education and Sports, Charles University, Prague, Czech Republic.

Three groups of 12-year-old boys differing in their habitual activity (hyper-: $n=24$, normo-: $n=13$, and hypokinetic: $n=17$) took part in the study. Vital capacity (VC) was determined at rest, then all the subjects completed a 30-second anaerobic all-out test (Wingate) and incremental maximal cycle ergometer test. Hyperkinetic boys had a higher ($p<0.05$) VC than normo- and hypokinetic ones (2.9 vs 2.4 and 2.5 l , respectively), and higher maximal anaerobic power (12 vs 10 and $9 \text{ W} \cdot \text{kg}^{-1}$) and anaerobic capacity (267 vs 232 and $221 \text{ J} \cdot \text{kg}^{-1}$, respectively). Hypokinetic boys differed from the other two groups by a lower W_{170} (2.0 vs 2.7 and $2.4 \text{ W} \cdot \text{kg}^{-1}$ in hyper- and normokinetic boys, respectively). $VO_{2\text{max}}$ in hypokinetic boys was significantly lower ($p<0.05$) than in hyperkinetic ones (46 vs $54 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$). Despite this, anaerobic ventilatory threshold ($76-77 \% VO_{2\text{max}}$) did not differ among the groups.

MUSCLE ATROPHY INDUCED BY NOCICEPTIVE STIMULATION IN RATS: WHY IS THE ATROPHY LOWER IN MALES THAN IN FEMALES? H. Knotková-Urbancová^{1,2}, P. Hník, P. Knotek², ¹Institute of Physiology, Academy of Sciences of the Czech Republic and ²Department for the Study of Pain, STAPRO, Pardubice, Czech Republic.

Unilateral fracture (F) of metatarsal bones (performed in rats under pentobarbital anaesthesia) causes muscle atrophy (MA) which is significantly lower in males than in females. We tested if this difference of soleus (SOL) and tibialis anterior, gastrocnemius (GA), extensor digitorum longus (EDL), and tibialis anterior (TA) muscles is influenced by 1. lower pain sensitivity (measured by tail-flick test) in males vs. females, and/or 2. anabolic influence of androgens on skeletal muscles in males. The tail-flick latency prior to F was significantly longer in males than in females (<0.001). Three and seven days after F, the difference of latency was not apparent. When male rats were castrated prior to F, muscle atrophy of extensors (SOL, GA) was significantly greater ($p<0.05$, $p<0.05$) than in control non-castrated males, and was similar to muscle atrophy in females. In flexors (EDL, TA) no significant difference in castrated vs. non-castrated males was apparent.

EXPRESSION OF MYOSIN HEAVY CHAINS IN DENERVATED ADULT RAT INTRAFUSAL FIBRES. T. Soukup, M. Novotová¹, L.-E. Thornell², Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic, ¹Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic and ²Department of Anatomy, University of Umea, Umea, Sweden.

The development of muscle spindles and expression of spindle specific myosin heavy chains (MHC) are dependent on the presence of sensory innervation. Mature spindles survive denervation, but it was not known whether they retain the expression of spindle specific MHCs. We have therefore studied the MHC expression in adult hind limb muscle spindles after section of the sciatic nerve. Our results have shown that 3-12 months after denervation, the intrafusal fibres expressed all MHCs present in normal adult spindles. However, the typical differences between nucleag bag₁ and nuclear bag₂ fibres were diminished and nuclear chain fibres reexpressed embryonic MHC. It can be concluded that adult intrafusal fibres express typical MHCs even in the long lasting absence of sensory and motor innervation, but the typical pattern of their MHC expression is less distinct than that in normal spindles.

VI. Methods

OPERATOR'S SUPPORT IN THE SECOND GENERATION COMPUTER CONTROLLED EXPERIMENTS. *J. Kaminskij, I. Krekule, J. Bureš*, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague.

The increasing processing power of personal computers is predominantly used to support a human operator. These routines improve the human-computer interface, e.g. graphical user interfaces (GUI, see Windows) and take over some tedious tasks of the operator. The latter type of routines can be well illustrated by the SW developed for Morris water tank experiments running on an IBM PC. It consists of the following routines: i) check of the TV camera and interface HW functioning, ii) set-up of the experiment according to a parametric file, iii) trace the chosen sequence of starting locations, iv) keep track of the chosen sequence of trials, v) guide acoustically the manual positioning of the target, vi) export of collected data into a statistical SW. The above SW is supported by a HW which controls various navigation cues such as light or acoustical beams, or manipulation with the target, or a software diaphragm etc. The described SW support made the PC system suitable even for complicated experiments, e.g. pharmacological screening.

APPARATUS FOR AUTOMATED APPETITIVELY REWARDED PLACE NAVIGATION IN A CIRCULAR OPEN FIELD. *F. Rech, A. Zahalka, L. Zinyuk¹, J. Bureš*, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and ¹Institute of Physiology, Ukrainian Academy of Sciences, Kiev.

Analysis of hippocampal place cells requires the generation of exploratory and goal directed behaviours. An apparatus developed for this purpose consists of an elevated (1 m) circular open field ($r=50$ cm) which can be rotated around its axis. The presence of a rat in a chosen target area is detected by a capacitive sensor the electrode of which is placed below the field floor. Detection elicits an electronically programmed sequence of events: 1. The sensor is disconnected. 2. A mechanical arm emerges from under the disk and delivers 50 μ l water to the target area. 3. After 10 s the arm returns under the disk. 4. The field rotates clockwise or anticlockwise by 135°. 5. After the movement is stopped, the sensor is reconnected to allow monitoring of the rat's return to the target. Since the position of the sensor did not change, the displaced rat has to approach the same place (relative to remote landmarks in the room). Rats learn the task rapidly and emit up to 50 target approaches during a 30 min session.

AN INTRODUCTORY TOXICOLOGICAL STUDY OF NEW COPPER COMPLEXES. *E. Szabová, D. Zeljenková, M. Melník*, Institute of Preventive and Clinical Medicine, Bratislava, Slovak Republic.

The importance of copper in health and disease is well known. Copper is involved in almost all steps of metabolism and shows a strong affinity for nucleic acid components. New substances composed of Cu/nif₂, Cu/nif₂/ron₂, Cu/sal₂, Cu/sal₂/ron₂ were synthesized by prof. M. Melník. The individual components of these substances have proved successful in the therapy of some forms of rheumatoid arthritis. The study represents an introductory toxicological study of the applicability of copper in practice. LD₅₀ of the above substances was established by testing acute oral toxicity in mice. Histological investigation did not show any significant pathomorphological changes of the observed organs (lungs, liver, spleen, kidneys, heart, skeleton muscle, stomach, fore-stomach, small and large intestine) in any examined group of animals of the tested compounds. Cu/nif₂ and Cu/nif₂/ron₂ were also studied with the aim to investigate their potential mutagenic effect. Cytogenetic analysis of the bone marrow of male and female ICR mice confirmed no mutagenic effect in studied concentrations.

G-Z: A SEMI-AUTOMATIC SYSTEM FOR EVALUATION OF GEOMETRICAL CHARACTERISTICS OF STRUCTURES OBSERVED IN A LIGHT MICROSCOPE. *P. Karen, L. Kubínová, J. Jirkovský¹, J. Mikulec¹, I. Krekule*, Institute of Physiology, Academy of Sciences of the Czech Republic, and ¹Vidis Ltd., Prague, Czech Republic.

Modern, design-based stereological methods estimating geometrical characteristics of a structure observed in the light microscope can be effectively implemented by semi-automatic systems including a TV camera, a PC enhanced by a TV frame grabber, a TV monitor and an interactive SW implementing stereological methods (e.g. Fenestra (GB), Grid (DK)). A G-Z system is described, the development of which was inexpensive, because it involved a simple frame grabber ZOB supporting only the necessary functions and because the SW became a combination of two already developed program packages: i) ZOB 2 – a menu driven SW of the grabber ZOB which manipulates with the observed image and ii) GSE – a graphical editor which generates coherent test systems (rasters) superimposed on the observed image as a graphic overlay. Development of the G-Z system is aimed at automatic operation.

A THREE DIMENSIONAL STUDY OF VILLI AND CAPILLARY BED IN THE PLACENTA OF HEALTHY WOMEN AND DIABETES MELLITUS PATIENTS BY USING C.S.L.M. *L. Kubínová, M. Jirkovská¹, P. Hach¹, R. Palovský, P. Karen, I. Krekule*, Institute of Physiology, Academy of Sciences of the Czech Republic and ¹Institute of Histology and Embryology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

A light confocal microscope (BIO-RAD MRC 600) was used for studying thick (30 μ m) slices of placenta tissue which were stained by haematoxylin-eosin. Profiles of both villi and blood vessels were segmented semi-automatically according to their grey values. Series of thick slices of selected villi and thin optical sections within them were studied. The volume of a given villus and its capillary bed were estimated by the stereological method of Cavalieri. The outer surface area of a villus and the inner surface area of the capillary bed were estimated by the spatial grid method. The recorded tissue was also reconstructed by a computer using both the surface and volume rendering from slices. The reconstruction was done by using an interactive visualization programme package AVS.

STOCHASTIC AND CHAOTIC MODELS APPLIED FOR DETECTION OF THE ONSET OF EEG BEREITSCHAFT POTENTIALS. *D. Popivanov, A. Mineva, J. Dushanova, I. Krekule¹*, Institute of Physiology, Bulgarian Academy of Sciences, Sofia and ¹Institute of Physiology, Academy of Sciences of the Czech Republic, Prague.

Trend-like EEG potential of maximal amplitude of 10 μ V which precedes voluntary movements, i.e. Bereitschaft potential (BP) is difficult to detect because of its high S/N, especially when its low amplitude onset is in question. We are reporting about the application of two models of EEG i) autoregressive function (AR) and ii) deterministic chaos (DC). Some parameters of these models (i.e. the minimal autoregression order or bifurcation parameter) were tracked along the recorded EEG and their changes (discrepancies) were detected and considered as to mark the onset of the BP. The detected changes of parameters coincided in both models with the onset of the BP, however, both models have also produced some false alarms. Therefore, a voting mechanism will be applied to design an automatic BP detection system based either on AR or DC models in the future.

VII. Metabolism

METABOLISM OF BRANCHED-CHAIN AMINO ACIDS AFTER PARTIAL HEPATECTOMY. *M. Hořejšek¹, I. Tůžer², F. Skopec³*, ¹Department of Physiology, ²Department of Pharmacology and ³Radioisotope Laboratory, Charles University, Medical Faculty, Hradec Králové, Czech Republic.

The pathogenesis of disturbances of branched-chain amino acids (BCAA; Val, Leu, Ile) metabolism in liver disease is unknown. The aim of the present study was to evaluate the oxidation of BCAA after partial hepatectomy (70 % of the liver was removed) or a sham operation. The oxidation of BCAA was evaluated by measuring the release of ¹⁴CO₂ from ¹⁴C-leucine (whole body oxidation) and keto (1-¹⁴C) isocaproate (in muscle, liver mitochondria and in isolated perfused liver). No significant difference of leucine and ketoisocaproate oxidation by whole body and muscle tissue respectively was observed 2 days after surgery. However, the ketoisocaproate oxidation in liver mitochondria and in isolated liver perfusion (per gram of tissue) was elevated in partially hepatectomized rats. The adaptive reaction of the body to hepatic resection involves stimulation of branched-chain keto acid oxidation in the regenerating liver (per gram of tissue).

REGULATION OF GLUCOSE TRANSPORT IN VARIOUS MUSCLE TYPES IN SELECTED RAT MODELS OF INSULIN RESISTANCE. *D. Raučínová, E. Šeböková, I. Klimeš*, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

The insulin resistance of various origin usually also involves alteration of glucose transport in the muscle tissue. We therefore measured the GLUT4 gene expression (slot blot technique) and GLUT4 protein levels (Western blot) in Wistar rats fed a high (63 cal %) sucrose (HS) diet, which induces hypertriglyceridaemia, insulin resistance, glucose intolerance, and in hereditary hypertriglyceridaemic, insulin resistant (HTG) and neonatally streptozotocin (90 mg.kg⁻¹ b.w. i.p.) treated (Neo STZ) diabetic rats. Feeding HS diet to Wistar rats doubled the GLUT4 gene expression (203.5 ± 4.5 % of the controls) in the quadriceps femoris muscle leaving the GLUT4 protein level unchanged. A similar increase of GLUT4 gene expression (181.0 ± 15.0 %) accompanied by a 54 % reduction of GLUT4 protein levels was noted in HTG animals fed the standard laboratory chow. On the contrary, in Neo STZ rats both the GLUT4 gene expression and protein levels were approximately lower by a half (44.0 ± 13.0 % and 0.61 ± 0.15 arbitrary units, respectively). Analyses of GLUT4 gene expression in the soleus and heart muscles corresponded to the results obtained in the HS fed and in the HTG rats, but not in the Neo STZ diabetic animals. The data indicate a tissue-specific and/or a situation dependent regulation of glucose transport in insulin resistance.

6'-DEOXO-BROMOCRIPTINE ALLEVIATES GLUCOSE TOLERANCE ABNORMALITIES IN THE GENETICALLY HYPERTENSIVE RATS OF THE KOLETSKY TYPE. *V. Golda, L. Cvak¹*, Institute of Experimental Neurosurgery, Faculty of Medicine, Charles University, Hradec Králové and ¹GALÉNA, Opava-Komárov, Czech Republic.

In genetically hypertensive obese rats (SHR-O) of the Koletsky (2) type and in their non-obese siblings (SHR), we found (1) a very high initial increase of glycaemia after glucose loading (30 % glucose 3 g/kg applied intragastrically after 14 h of fasting which was followed by a relative large decrease. Blood samples were obtained through a heparinized capillary from the retrobulbar plexus under light ether anaesthesia. Bromocriptine as well as 6'-deoxo-bromocriptine were administered for 10 days i.p. in the dose of 2 mg/kg/day as the maleate salt. 6'-deoxo-bromocriptine alleviates the mentioned glucose tolerance abnormalities, but these were sex and substrain dependent. The maximal alleviation was expressed in SHR males and in SHR-O females. Bromocriptine exhibited a minimal alleviation effect.

1. *Golda V., Petr R.*: *Physiol. Bohemoslov.* 39: 560, 1990.

2. *Koletska S.*: *Am. J. Pathol.* 80: 129-140, 1975.

THE ANAEROBIC THRESHOLD ACCORDING TO CONCONI CAN PROVIDE INFORMATION ABOUT THE MAXIMAL LACTATE STEADY STATE. *V. Bunc, P. Hofman*, Faculty of Physical Education and Sports, Charles University, Prague, Czech Republic.

The aim of this study was to examine whether the anaerobic threshold according to Conconi (CO) can determine the maximal lactate steady state (LA_{ssmax}). Firstly, 7 healthy female students (mean age 20.7 ± 1.6 years; VO_{2max}.kg⁻¹ = 49.3 ± 3.1 ml.kg⁻¹.min⁻¹) performed an incremental exercise test (10 W.min⁻¹) on a cycle ergometer. The CO was assessed by means of a computer model from the relation of HR on exercise intensity. Secondly, two endurance 20 min tests (ET) were performed. The first (ET₁) was performed at exercise intensity which was about 10 % lower than at the CO level, the second (ET₂) at exercise intensity about 10 % higher than at the CO level. The functional variables were evaluated each 5 min of exercise. The blood LA showed a "clear" steady state in ET₁. A non-steady state course of LA was found in ET₂. None of the subjects were able to maintain the exercise throughout the 20 min period in ET₂ (the mean time when the exercise had to be interrupted was 10.4 ± 3.7 min). The LA at the end of ET₂ was significantly higher than in ET₁, (p < 0.05) (7.0 ± 1.8 and 3.3 ± 2.2 mmol.l⁻¹) respectively). These results indicate that since LA_{ssmax} lies between ET₁ and ET₂ CO may be used for assessing LA_{ssmax}.

RELATIONSHIP OF NUTRITION AND FAT DISTRIBUTION IN MOTHER AND CHILD. *J. Pařízková, A. Alberti¹, D. Fruttini¹*, Laboratory of Health Promotion, Fourth Clinic Internal Medicine, First Medical School, Charles University, Prague, Czech Republic and ¹Instituto di Scienza dell'Alimentazione, Università degli Studi, Perugia, Italy.

Nutritional status (antropometric variables, body mass index, skinfold thicknesses) assessed in mothers and in their children was followed either in newborns or later at the preschool age. Significant relationships among nutritional variables and fat distribution of mothers and children were found. Fat distribution during pregnancy and in newborns correlated significantly in sons only. The weight gain during pregnancy correlated significantly with subcutaneous fat in newborn daughters. The energy intake and diet composition (i.e. the percentage of energy covered by proteins, fats and carbohydrates) during pregnancy correlated significantly with fat distribution and serum lipids in newborn sons only, i.e. these relationships of nutritional characteristics of mothers and their offsprings were sex-dependent.

SMALL LABORATORY BIOREACTORS: APPLICATION TO THE STUDY OF TOXICOLOGICAL ASPECTS IN XENOBIOTICS METABOLISM. *S. Hynie, V. Bencko¹, J. Martínek², H. Farghalli*, Department of Pharmacology, ¹Hygiene, ²Embryology and Histology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

Living organisms are being increasingly subjected to various foreign chemicals, whether they are drugs, food additives or pollutants. The usual responses to xenobiotics include pharmacological, toxic, immunologic and carcinogenic effects. Specifically, there are important compounds which are activated by various biotransformation mechanisms. Since the liver is one of the most important organs contributing to these reactions, we have prepared a hepatocyte bioreactor and have followed such reactions which might participate in the biotransformation of various pollutants, including polychlorinated biphenyls. As model drugs we tested the biotransformation of several standard xenobiotics (7-ethoxycoumarin, hexobarbital, 1-chloro-2,4-dinitrobenzene) and at the same time we followed the integrity of the cells histologically.

XENOBIOTIC METABOLISM IN IMMOBILIZED PERFUSED HEPATOCYTES: A CONVENIENT MODEL FOR PHASE I AND II BIOTRANSFORMATION STUDIES. *H. Farghali, V. Bencko, L. Kameniková, V. Křen, M. Mráz, S. Hynie*, First Faculty of Medicine, Charles University and ¹Institute for Postgraduate Education, Prague, Czech Republic.

The immobilized and perfused hepatocyte cellular model (bioreactor) represents a valid and a very convenient technique for studying xenobiotic activation or inactivation with unparalleled advantages over traditional cellular models. The biotransformation competence of hepatocytes in the bioreactor was confirmed by using 7-ethoxycoumarin (EC), hexobarbital (HX) and aminopyridine (AP) as substrates for phase I, and umbelliferone, 1-chloro-2,4-dinitrobenzene (CDNB), and 4-nitrophenol as substrates for phase II biotransformation reactions. The perfused hepatocyte bioreactor eliminated HX at a higher rate and exhibited higher glutathione transferase activity than hepatocytes in the suspension. The glucuronidation of EC was also more effective in the hepatocyte bioreactor than in the suspension of isolated hepatocytes.

BINDING CHARACTERISTICS OF NUCLEAR RECEPTORS FOR RETINOIC ACID IN THE RAT LIVER. *J. Brtko*, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Since retinoic acid receptors, thyroid hormone receptors and the vitamin D receptor belong to the same subfamily of the steroid – thyroid hormone receptor family of nuclear receptors which recognize similar hormone responsive elements with the consensus motif AGGTCA, the present study was undertaken to establish a novel *in vitro* method for quantitative determination of the nuclear retinoic acid receptors in rat liver. The binding characteristics of retinoic acid receptors in relation to various physico-chemical conditions: the pH, time and the temperature dependence, were evaluated. Three different binding sites for all trans-retinoic acids were found in nuclear proteins of the rat liver. One of them represents a high affinity and a low capacity retinoic acid specific binding site with the equilibrium association constant K_{a1} $0.687 \pm 0.294 \times 10^9$ l/mol and the maximal binding capacity B_{max1} 0.644 ± 0.397 pmol/mg. The other exhibit low affinity and high capacity retinoic acid binding sites with the K_{a2} 2.694×10^6 l/mol; B_{max2} 225 pmol/mg and K_{a3} 0.131×10^6 l/mol; B_{max3} 1125 pmol/mg, respectively.

PASSAGE OF AMINO ACIDS THROUGH THE SHEEP RUMEN EPITHELIUM. *Z. Faixová, J. Várady*, University of Veterinary Medicine, Košice, Slovak Republic.

Passage of lysine and leucine through the rumen epithelium of sheep was determined *in vitro* in the mucoso-serous direction after 60-min incubation. In the first part of the experiment, the passage of lysine in various concentrations was measured, when lysine was the only amino acid in the test solution. In the second part of experiment, leucine was added to each lysine concentration to a final leucine to lysine ratio of 1, 3 and 5. When the amount of lysine on the serous side after incubation was compared in both parts of the experiment, it was found that leucine added to the test solution affected the passage of lysine through the rumen epithelium. Leucine concentrations of 5, 15 and 20 μ M decreased the passage of 5 M lysine by 55, 68 and 62 %, respectively ($P < 0.01$). Leucine concentrations of 25 and 75 μ M decreased the passage of 25 μ M lysine by 58 and 38 %, respectively.

POTENTIAL CHANGES IN EMBRYONIC NEPHRONS OF THE CHICK IN RELATION TO THE HISTOCHEMICALLY DETECTED SODIUM PUMP. *Z. Zemanová, E. Ujec*, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague.

Predominantly indirect data are available on ion and water reabsorption in embryonic nephrons. However, an analysis of the role of transport properties of individual segments of the developing nephron is still lacking, as well as evidence for the involvement of these nephrons in avian embryo salt and water homeostasis. To solve this question, the chick embryo mesonephrons was chosen for the study of nephron development by electrophysiology (transtubular potential, TTP) and histochemistry ((Na, K-ATPase). We have proved, that the TTP increases along the nephron proximo-distally. This indicates that the proximal portions of the mesonephrons manifest the properties of loose epithelia, whereas the distal portions have presumably the properties of tight epithelia, as in the mammalian nephron. In accordance with this result, histochemically detected Na,K-ATPase exhibits higher activity in the distal portions of the nephron. These findings indicate that the distal nephron might take part in Na⁺ reabsorption.

VIII. Neurophysiology

INFLUENCE OF HYPOBARIC HYPOXIA ON CORTICAL EPILEPTIC AFTERDISCHARGES IN IMMATURE RATS. *D. Marešová, P. Mareš¹*, Institute of Physiology, First Faculty of Medicine and ¹Department of Pathophysiology, Third Faculty of Medicine, Charles University, Prague, Czech Republic.

The role of hypoxia in epileptogenesis in the immature brain was studied by means of cortical epileptic afterdischarges (ADs) in rat pups aged 12, 18 and 25 days. The animals were exposed for one hour to hypobaric hypoxia equal to LD10. Hypoxia did not interfere with the elicitation of ADs in 18 and 25-day-old rats, but it surprisingly blocked the ADs regeneration in the youngest group. The duration of ADs and postictal depression may reflect mechanisms arresting seizures. The first AD was significantly prolonged by hypoxia in both 18 and 25-day-old rats. The postictal depression present only in 25-day-old rats, was partly blocked by hypoxia in this age group. A progressive increase in the duration of ADs with stimulations repeated at 10-min intervals seen in control rats failed to appear in rats exposed to hypoxia. Hypoxia was found to induce mixed pro- and anticonvulsant effects in our experiments.

EFFECT OF TRANSMITTERS ON EXTRACELLULAR pH BASELINE IN THE RAT SPINAL CORD. *P. Jendelová, A. Chvátal, E. Šyková*, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague.

Activity-related alkaline-acid changes in extracellular pH (pH_e) affect neuronal excitability and synaptic transmission. Effects of excitatory and inhibitory amino acids (EAA, IAA) on the pH_e baseline in dorsal horns of the isolated spinal cord were studied in 3 to 14-day-old rats by means of H⁺-selective microelectrodes. The application of GABA (10^{-3} M) in the superfusing solution produced an alkaline shift (AS) in pH_e baseline by about 0.3–0.5 pH units, which was blocked by picrotoxin (10^{-3} M). GABA had no effect on the pH_e baseline in a HCO₃-free superfusing solution, which was buffered with HEPES. The application of glutamate receptor agonists (aspartate, glutamate, NMDA, AMPA or kainate) produced AS by about 0.2–0.6 pH units. AS produced by NMDA (10^{-6} M) was blocked by MK801 and by ketamine, while AS induced by kainate of AMPA (10^{-6} M) was blocked by CNQX. AS evoked by EAA was not affected by Ca²⁺ channel blockers (Mg²⁺) and occurred even when gliogenesis in the spinal cord was blocked by X-irradiation of rat pups on the first postnatal day. The AS produced by electrical stimulation of afferent input in 3 to 8-day-old rats was partially blocked by picrotoxin as well as by antagonists of glutamate receptors CNQX and MK801. We conclude that the observed alkaline changes are of postsynaptic origin. The AS apparently result from HCO₃-efflux (e.g. GABA) or H⁺ influx through ligand-gated ion channels.

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

The extracellular potassium ion concentration ($[K^+]_e$) was measured in the brain hemispheres of control rats using double-barrel potassium ion selective microelectrodes (K^+ -ISM). During the penetration of K^+ -ISM from the brain surface changes in $[K^+]_e$ were recorded when the K^+ -ISM tip was kept at a definite depth under the surface for a longer time. After several minutes, when no change in $[K^+]_e$ appeared, this value was called the "resting level of $[K^+]_e$ ". There were different "resting levels of $[K^+]_e$ " found in specific brain structures. An elevated $[K^+]_e$ was found around 4 $mmol \cdot l^{-1}$ in the hippocampus. The $[K^+]_e$ distribution of the thalamus depends on the site of measurement as follows: lateral or posterolateral thalamus – about 4; ventromedial nucleus – 4 to 5; ventroposteromedial (VPM) and ventroposterolateral (VPL) nuclei – 6 to 6.5. The $[K^+]_e$ values were 3.3 to 3.4 in the zona incerta and nucleus subthalamicus. In animal models with chronic pain (after longlasting deafferentation) elevated values of "resting $[K^+]_e$ " were found, particularly in the hemisphere contralateral to the site of deafferentation, i.e.: VPM and VPL nuclei, where the values were up to 11 contralaterally versus 5 to 6 ipsilaterally. These results suggest that K^+ activity in the extracellular space of non-stimulated nervous tissue depends on the spontaneous neuronal activity of the specific region.

THE DEVELOPMENT OF SPONTANEOUS ACTIVITY IN THE GENERATOR OF EMBRYONIC MOTILITY UNDER THE CHRONIC INFLUENCE OF *N*-NITRO-L-ARGININE METHYLESTER. *J. Sedláček*, Institute of Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

N-nitro-L-arginine methylester (20 mg/kg e.w., acute administration) depresses the spontaneous motility (1). This communication deals with the chronic effect of this drug in chick embryos. The drug was applied continuously from day 4 to day 12 of incubation in a average dose of 2.10 mg/kg e.w./24 h. On day 17 of incubation, the frequency of spontaneous movements was tested. 1. The resting activity (60 min) was diminished from 36.4 ± 2.10 movement/min to 20.5 ± 2.04 (56.3 %, $p < 0.001$). 2. The sensitivity to some centrally acting drugs (metrazol, NMDA, ketamine, glycine) was significantly changed. These findings have shown that the role of the NO-ergic system, including NO-synthase, is important in the development of spontaneous activity of the central generator of embryonic motility in chick embryos.

1. *Sedláček J.*: Phys. Res. 43: 3P, 1994.

INFLUENCE OF GABA UPTAKE BLOCKER NNC 05-0711 ON PENTYLENETETRAZOL-INDUCED MOTOR SEIZURES IN DEVELOPING RATS. *R. Haugvicová¹, H. Kubová^{1,2}, P. Mares^{1,2}*, ¹Institute of Physiology, Academy of Sciences of the Czech Republic and ²Third Faculty of Medicine, Charles University, Prague, Czech Republic.

One of the possible ways how to enhance GABAergic inhibition and thus achieve anticonvulsant action is to inhibit the reuptake of GABA. NNC 05-0711 (Novo Nordisk), a derivative of nipecotic acid, was tested against pentylenetetrazol-induced motor seizures in rat pups 7, 12, 18 and 25 days old. NNC in doses of 10 and 20 mg/kg i.p. abolished minimal, clonic seizures in 18 and 25 days old rats, i.e. in such age groups, where this seizure pattern was regularly elicited under control conditions. Furthermore, tonic phase of major, generalized tonic-clonic seizures was suppressed in 12- and 18-day-old animals. The specific action against minimal seizures is unique among antiepileptic drugs.

NADPH-DIAPHORASE POSITIVE NEURONES IN CENTRAL AUDITORY STRUCTURES OF THE GUINEA-PIG. *R. Druga, J. Syka¹*, Institute of Anatomy, First Faculty of Medicine, Charles University and ¹Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague.

The distribution of NADPH-diaphorase positive neurones in central auditory structures was studied in the guinea-pig. NADPH-diaphorase, which is also NO-synthase, identifies neurones, which are a possible source of NO. After perfusion with aldehyde fixation, frozen sections (40 μm) were incubated (1). In the caudal part of the brain stem, NADPH-d positive neurones were present in the ventral cochlear nucleus only. Large amount of positive neurones were found in the inferior colliculus, mainly in the dorsal and external cortices and in the intercollicular commissura. In the medial geniculate body, positive neurones were distributed in the nc. suprageniculatus and nc. medialis. Positively labelled neurones were localized in all fields of the auditory cortex with a slight prevalence in the infragranular layers.

1. *Scherer-Singler U., Vincent S.R., Kimura H., McGeer E.G.*: J. Neurosci. Meth. 9+: 229–234, 1983.

BEHAVIOURAL TESTING OF AUDITORY THRESHOLDS IN THE RAT WITH INSTRUMENTAL CONDITIONING. *N. Rybalko, J. Syka, M. Jílek, G. Brožek¹*, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic and ¹Institute of Physiology, Second Faculty of Medicine, Charles University, Prague, Czech Republic.

Food deprived rats were placed in a Skinner box equipped with two levers of an automatic feeder (1). Pressing the starting lever was followed after a variable interval (range 0.5–5 s controlled by a computer program) by a tone (duration 1 s, frequency 1–32 kHz, intensity in the range 0–50 dB SPL). When the rat pressed the second lever during the tone presentation, it was rewarded by a pellet. In case of the second lever was pressed before or after the tone presentation, the rat was not rewarded and a 5 s punishment pause followed. The intensity of the tone was controlled by the experimenter. The threshold was assessed as the intensity of the sound, which resulted in 50 % correct responses.

1. *Brožek G., Syka J., Rybalko N., Popelář J., Jílek M.*: Physiol. Res. 43: 4P, 1994.

ACTIVITY OF INFERIOR COLLICULUS NEURONES IN THE GUINEA-PIG UNDER URETHANE AND PENTOBARBITAL ANAESTHESIA. *J. Astl, J. Popelář, J. Syka*, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague.

The aim of the study was to compare the basic parameters of neuronal responses in the inferior colliculus in the guinea-pig to acoustical stimulation under urethane anaesthesia (0.125 mg/kg intraperitoneally) and under pentobarbital anaesthesia (25 mg/kg intraperitoneally, premedication with phentanyl 50 mg/kg intramuscularly and droperidol 200 mg/kg intramuscularly). Extracellular unit activity in the inferior colliculus was recorded by means of glass micropipettes, tones of 50 ms duration were used for acoustical stimulation. In guinea-pigs under urethane anaesthesia, 64 % of neurones responded with a sustained type of response. Under pentobarbital anaesthesia, the occurrence of phasic and sustained types of the response was almost equal. Thresholds of responses at characteristic frequencies were significantly higher in urethane anaesthesia and the latencies were also longer. The distribution of individual types of responses to binaural stimulation was almost identical in both types of anaesthesia.

EFFECT OF STOBADINE ON LIPID PEROXIDATION AND PHOSPHOLIPID COMPOSITION AFTER SPINAL CORD ISCHAEMIA. *N. Lukáčová, M. Chavko, G. Halát*, Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

The influence of stobadine, a drug with the pyridoindol structure, was compared with thiopental and pentobarbital for its capability to inhibit stimulated peroxidation in spinal cord homogenates *in vitro*. The antioxidative capacity of the drug exceeded that of barbiturates by more than 100 fold. Stobadine was shown to inhibit the increase in TBA-RS formation in homogenates of the rabbit spinal cord subjected to 20 min ischaemia to the level comparable with the controls. Administration of the drug ($6 \text{ mg}\cdot\text{kg}^{-1}$) to animals 5 min before 20 min ischaemia had no effect on the level of lipid peroxidation products, however, it slowed down stimulated Fe^{2+} -dependent peroxidation after *in vitro* incubation of the homogenates and ameliorated the decrease in the concentration of phosphatidylserine and ethanolamine plasmalogens as compared with the non-treated animals. The administration of stobadine 2 min before the release of aorta occlusion increased the antiradical capacity in spinal cord homogenates and had an ameliorating effect on the phospholipid composition.

GABA_B, BUT NOT GABA_A SYSTEM PLAYS A ROLE IN POSTICTAL DEPRESSION IN RATS. *P. Mareš, V. Makal*, Institute of Physiology, Academy of Sciences of the Czech Republic and Third Faculty of Medicine, Charles University, Prague.

The impossibility of eliciting a second seizure shortly after the end of the first seizure could be taken as a measure of postictal depression. Epileptic afterdischarges (ADs) induced by electrical stimulation of the neocortex in 25-day-old rats with implanted electrodes were used: stimulation was repeated twice at an interval of 1 min, then drugs were administered and 10 min later the two stimulations were repeated. Ten animals were used for each drug. Control rats could not generate ADs one min after the end of the previous one. Drugs interfering with the GABA_A supramolecular complex (subconvulsant doses of bicuculline, picrotoxin, a benzodiazepine inverse agonist Ro 19-4603) did not change the postictal depression. On the contrary, it was partly blocked by CGS 35348, a GABA_B antagonist.

DEVELOPMENT OF EXPLORATORY ACTIVITY IN LABORATORY RATS. *A. Mikulecká, P. Mareš*, Institute of Physiology, Academy of Sciences of the Czech Republic and Department of Pathological Physiology, Third Faculty of Medicine, Charles University, Prague, Czech Republic.

Development of spontaneous behaviour was studied in rats 7, 9, 12, 15, 18, 25, 36, 42, 60 and 90 days old. Two groups were used: 1) animals tested just once, $N=20$ for each age; 2) other 20 male rats were repeatedly tested on all the above days. Frequency and duration of behavioural patterns in an open-field test were followed at 5 min intervals. Up to 25 days of age the results of the two groups were nearly the same. Later, with the development of functional activities, such as walking, rearing, grooming, significant differences appeared. Spontaneous activity of repeatedly tested animals was lower than that of naive rats up to 60 days of age. Developmental profiles of spontaneous activity also differed. Maximum activity was reached earlier (from the age of 25 days) and its decrease appeared later (after postnatal day 60) in the first group in comparison with repeatedly tested rats. The observed differences may be ascribed to experience with the experimental situation.

EFFECT OF QUINOLINATE LESIONS ON GAMMA-GLUTAMYL TRANSPEPTIDASE (GGT) ISOFORMS IN CAPILLARIES AND SYNAPTOSOMAL MEMBRANES OF THE RAT ENTORHINO-HIPPOCAMPAL COMPLEX. *L. Dvořáková, V. Lisý, F. Štastný*, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague.

Quinolate (QUIN) is an endogenous excitotoxic agonist of the NMDA type of glutamate receptor. Brain damage caused by intracerebroventricular injection of QUIN was indicated by changes in the activity of GGT in the hippocampus (Hip) and entorhinal cortex (EC) of 12, 30 and 50-day-old rats. In the oldest animals the QUIN lesion caused the largest GGT activity decline (Hip 85 %, EC 88 %). This effect consisted in remarkable suppression of GGT activity in the capillaries (Hip 60 %, EC 81 %), while no significant changes were found in synaptic membranes. The membranes contained five GGT isoforms differing in concanavalin A affinity, while only 2 of them were present in the capillaries. The lesion affected neither K_m^{app} ($0.69-0.79 \text{ mmol/l}$) nor the number of isoforms. Thus, elevated concentrations of QUIN in the cerebrospinal fluid may damage the blood-brain barrier endothelium but not synaptosomal membranes. (Supported by AS grants No. 71139 and 71160).

HIPPOCAMPAL GRANULAR LAYER LESIONS: REPLACEMENT OF LOST NEURONES BY IMPLANTATION OF A SUSPENSION OF EMBRYONAL NERVE TISSUE. *J. Pokorný*, Institute of Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

Granule cells of the dentate gyrus were eliminated by means of fluid injection into the infragranular cleavage plane of adult rats. The fluid injection was combined with a hippocampal neuronal suspension aquired from 17-day-old embryonic donors. One group of animals received a suspension with nerve cells stained with the fluorescent dye bisbenzimidazole (Hoechst 33342). Survival and organization of the grafted cells was investigated 30–60 days later. Extensive accumulation of neuronal tissue in Nissl stained sections was found in the location of the grafts. In well positioned grafts a new, irregular granule cell layer reappeared. Using epi-fluorescent illumination we could confirm that only the cells within the graft were labelled. It is possible to conclude that the cells found in the grafted area came from the donor tissue. They can partly restore the morphological picture of the lesioned neuronal structure.

COMPLETE ANOSMIA AND RELATED DEGENERATIVE CHANGES AFTER TOTAL OLFACTORY BULBECTOMY. *E. Račeková, I. Vanický, G. Sekerková, T. Žigová*, Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

A group of Wistar rats was tested in a food finding task and then the olfactory bulbs of these trained animals were bilaterally ablated. After 6 days of postoperative recovery the rats were retested in the same task. The significant failure to retrieve the hidden food was observed in seven animals out of ten. Complete bilateral olfactory bulbectomy, affecting at least the anterior third of the olfactory peduncle, induced anosmia confirmed by histological observations. After retesting, the animals were sacrificed under deep anaesthesia by intracardial perfusion of 4 % paraformaldehyde. The brains were frozen, serially cut ($40 \mu\text{m}$) and processed by Gallyas' silver impregnation procedure. Degenerative changes of the projections from the olfactory bulb also confirmed our behavioural observations.

EFFECT OF CIS-DICHLORODIAMINEPLATINUM II (CIS-DDP) ON CELL CYCLE KINETICS OF C6 GLIOMA CELLS IN CULTURE. F. Kocourek, G. Mazzini¹, V. Lisá, V. Mareš², Rita de Renzis¹, Cecilia Davegna¹, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, ¹Centro di Studio per l'Istochimica, C.N.R., Pavia, Italy and ²Department of Zoology, Kuwait University, Kuwait.

The cell cycle effects of a clinically used cytostatic drug cis-DDP have not yet been fully clarified. A block of cell cycling in late S/G2 phases was described earlier (1) in cultured C6 glioma cells after cis-DDP administration. The aim of this study was to find whether the cells are able to overcome this blockade. Cells treated with cis-DDP (5 µg/ml, 90 min) were examined by flow cytophotometry for the DNA content (stained with propidium iodide) and DNA synthesis (labelled by bromodeoxyuridine for 30 min). We found a subpopulation of polyploid cells. In addition we discovered that this subpopulation originates from the cells which overcome the blockade at S/G2. This effect of cis-DDP is independent of the phase of the cell cycle in which the cells were treated by cis-DDP.

1. Mareš V., Giordano P.A., Mazzini G., Lisá V., Pellicciari C., Scherini E., Bottioli G., Drobník J.: *Histochem J.* 19: 187-194, 1987.

COPPER INHIBITS RECEPTORS FOR EXCITATORY AMINO-ACIDS IN CULTURED HIPPOCAMPAL NEURONES. H. Zemková, V. Vlachová, L. Vyklícký Jr., Institute of Physiology, Academy of Sciences of the Czech Republic, Prague.

Copper is one of the most important trace elements in the brain of vertebrates. Cu²⁺ is released during synaptic transmission and its accumulation causes serious diseases of the CNS. The aim of our study was to test the effects of Cu²⁺ on membrane current responses induced by activation of receptors for excitatory amino acids in cultured hippocampal neurones. We found that AMPA/kainate as well as N-methyl-D-aspartate (NMDA) subtype of glutamate receptors are inhibited with micromolar concentrations of Cu²⁺. In contrast to the blockade of NMDA responses by Mg²⁺, the inhibition induced by Cu²⁺ is voltage-independent. The kinetic study of Cu²⁺ association rate with NMDA receptor indicates that the onset of Cu²⁺ inhibition of the agonist-activated receptors is about 20 times faster than the inhibition of non-activated receptors. The dissociation of Cu²⁺ from the NMDA receptor is very slow (biphasic time-course with time constants 150 ms and 600 ms) and independent of receptor activation. Our results indicate that Cu²⁺ plays an important role in the regulation of glutamate receptor activity and may represent one of the mechanisms involved in the pathogenesis of Wilson's disease.

MATHEMATICAL MODELLING OF COPPER INHIBITION OF N-METHYL-D-ASPARTATE (NMDA) TYPE OF GLUTAMATE RECEPTOR. V. Vlachová, H. Zemková, L. Vyklícký Jr., Institute of Physiology, Academy of Sciences of the Czech Republic, Prague.

The whole-cell patch-clamp technique was used to measure membrane currents induced by fast agonist application in cultured hippocampal neurones. This technique provided quantitative data suitable for modelling the action of inhibitors on the NMDA receptor-channel complex. Our aim was to characterize inhibitory effects of Cu²⁺ on NMDA receptors using measurements of kinetic reactions. The results have shown that Cu²⁺ is a powerful inhibitor of responses induced by NMDA. The onset of Cu²⁺ inhibition was dependent on both the agonist and Cu²⁺ concentration. Dissociation of Cu²⁺ from the NMDA receptor was slow (~150 ms and ~600 ms) and independent of receptor activation. Using a computer simulating program we designed a kinetic model describing Cu²⁺ inhibitory action at the NMDA receptors. The proposed model is based on the assumption that two molecules of the agonist are required for channel activation. The analysis of our data suggest that Cu²⁺ can bind either to the agonist unoccupied receptor or to the receptor with one molecule of agonist bound, preventing receptor activation. Proposed kinetic model of Cu²⁺ action on NMDA receptors is consistent with our experimental results concentration-dependent Cu²⁺ inhibition of the NMDA responses, an increase of apparent NMDA receptor desensitization, pseudocompetitive type of inhibition and the use-dependence of Cu²⁺ inhibition.

GLYCINE RESISTANT NMDA RECEPTOR DESENSITIZATION IS MEDIATED BY G-PROTEINS. R. Tureček, L. Vyklícký Sr., L. Vyklícký Jr., Institute of Physiology, Academy of Sciences of the Czech Republic, Prague.

N-methyl-D-aspartate (NMDA) subtype of glutamate receptors are assumed to be involved in many important brain functions. Evidence has been presented that intracellular Ca²⁺ induces reversible activation and irreversible glycine-resistant NMDA receptor desensitization (1). The aim of our study was to analyze the mechanisms involved in controlling the glycine-resistant NMDA receptor desensitization. Experiments were performed on cultured rat hippocampal neurones using the patch-clamp technique. In control experiments, rapid application of 100 µM NMDA, 10 µM glycine and 0.2 mM Ca²⁺ induced responses which were desensitized by 35.6 ± 7.5 % (n=9). A conditioning train of NMDA applications in the presence of 2 mM Ca²⁺ increased the desensitization by 12.0 ± 3.4 % (n=9). The degree of conditioning train-induced NMDA receptor desensitization was significantly decreased by intracellular application of drugs which activate G-proteins. In the presence of 5 mM CsF and 0.5 mM AlCl₃, 0.3 mM GTPγS or 0.3 mM GTP the increase in conditioning train-induced NMDA receptor desensitization was 3.6 ± 2.1 % (n=6), 0.1 ± 2.6 % (n=5) and 1.7 ± 2.1 % (n=5), respectively. In contrast, intracellular application of 0.3 mM ATPγS or 1.5 mM ATP did not prevent conditioning train-induced NMDA receptor desensitization. These findings suggest that G-proteins control desensitization of the NMDA receptor-channel complex.

1. Vyklícký L.: *J. Physiol.* 470: 575-600, 1993.

CONSTRUCTION AND FUNCTIONAL EXPRESSION OF A SINGLE-SITE ACETYLCHOLINE RECEPTOR. F. Vyskočil, J. Krůšek, A. Habartová, X.M. Yu¹, Z.W. Hall¹, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and ¹Department of Physiology, University of California, San Francisco, USA.

In COS cells, the surface expression of AChR with a δ subunit mutated at position 180, 189 or both 180 and 189 is similar to "wild" AChR, as shown by both bungarotoxin (BuTX) binding and channel currents induced by ACh. BuTX binding to surface AChR is significantly less protected by ACh and carbachol after the mutations. The protection ability of both agonists is absent in α-δ heterodimers with 180 and 180+189 mutations, indicating the absence of the agonist binding site. Curare protection of BuTX binding is not affected by these mutations. Current recordings demonstrate that δ mutations shift the dose-response curve towards high ACh concentrations. The Hill coefficient decreased from 2 (two binding sites) at the "wild" receptor to 1 (one binding site) after double mutation.