

# **Proceedings of the Czechoslovak Physiological Society**

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## I. Physiology of Cell Membranes. Neuromuscular Physiology

STIMULATION OF PLASMA MEMBRANE  $\text{Ca}^{2+}$  PUMP: ACIDIC PHOSPHOLIPID EFFECT OF DMSO AND POLYCATIONS. *J. Lehotský, L. Raeymaekers, R. Casteels*, Comenius University, Jesenius Faculty of Medicine, Martin and <sup>1</sup>Catholic University, Leuven, Belgium.

At least two reaction steps are involved in the activation of the purified plasma membrane  $\text{Ca}^{2+}$  pump by acidic phospholipids which depend on the type of phospholipid (1). We compared the effect of acidic phospholipids on  $\text{Ca}^{2+}$  ATPase (cycling activity) and p-nitrophenylphosphatase ( $\text{E}_2$ -form activity) catalyzed by the  $\text{Ca}^{2+}$  pump. PIP-like PS activated  $\text{Ca}^{2+}$  ATPase activity by modifying the ATP activation curve with an increasing  $V_{\text{max}}$  of the high affinity site. DMSO (10 %) stimulated  $\text{Ca}^{2+}$  ATPase activity in PC by a factor of 1.36. The stimulation was only weak in PS and activity in PIP was inhibited. The phospholipid head group also strongly affected DMSO stimulation of phosphatase activity. Positively charged neomycin exerted no effect on  $\text{Ca}^{2+}$  ATPase activity, reactivated by PC or PS, but the stimulatory action of PIP was suppressed. The relative enhancing effect of phosphatase activity by PS was not influenced. Both hydrolytic activities catalyzed by the  $\text{Ca}^{2+}$  pump were differentially affected by organic solvents and polycations with respect to the kind of phospholipid.

*J. Lehotský, Raeymaekers L., Missiaen L., Wuy R.: Biochim. Biophys. Acta 1105: 118-124, 1992.*

CHARACTERISTICS OF NaF-INDUCED  $\text{Ca}^{2+}$  INCREASE IN ISOLATED RAT BRAIN NERVE TERMINALS MEASURED WITH <sup>45</sup>Ca AND FURA-2. *J. Orlický, R. Bottliková*, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava.

Sodium fluoride (NaF), which is known to activate directly various guanine nucleotide binding proteins (G-proteins) in the plasma membrane, induced a dose-dependent  $\text{Ca}^{2+}$  increase in isolated rat brain nerve terminals. The aim of the present study was to investigate the relationship between the NaF-induced  $\text{Ca}^{2+}$  uptake and the changes of the free calcium concentration in intraterminals. It was shown that 10 mmol.l<sup>-1</sup> NaF is sufficient to increase the <sup>45</sup>Ca content in the synaptosomal preparations which was on the border of significance. This effect of NaF was dependent on an enhanced influx of <sup>45</sup>Ca from the external medium and was manifested by a transient increase of ( $\text{Ca}^{2+}$ )<sub>i</sub> in fura-2 loaded nerve terminals. The depolarization of the nerve terminals by elevated external concentrations of KCl (30 mmol.l<sup>-1</sup>) further increased, on the contrary, calmodulin (1 μmol.l<sup>-1</sup>) or reduced levels of external ( $\text{Ca}^{2+}$ )<sub>o</sub> (0.1 mol.l<sup>-1</sup>) decreased both the NaF-induced <sup>45</sup>Ca uptake and ( $\text{Ca}^{2+}$ )<sub>i</sub> oscillation. The results indicate that NaF stimulates the  $\text{Ca}^{2+}$  influx through activation of G-proteins associated with the calcium channel.

LARGE CONDUCTANCE  $\text{Cl}^-$  CHANNEL IN MYOBLASTS OF THE L6 RAT MUSCLE CELL LINE. *O. Hurnák, J. Zachar*, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava.

The existence of a voltage sensitive, high conductance chloride channel in undifferentiated cells (myoblasts) of the L6 rat muscle cell line is presented. The conductance of the channel in symmetrical 120 mmol/l choline chloride is 331±4 pS. The probability of the channel being in the open state decreases with the increase of the imposed voltage. Due to the inactivation at high membrane potentials (both negative and positive) from the equilibrium potential, the channel can only be resolved clearly by the pulse technique. The incidence of the channel in successful patch trials was higher (≈ 50 %) than is usually reported. The channel shows at least one definite substate and pronounced flickerings between the substate and the main open state. The channel might play a role in regulatory volume readjustment.

CONDUCTANCE OF THE CALCIUM RELEASING CHANNEL FROM THE SARCOPLASMIC RETICULUM OF THE CRAYFISH. *J. Benková, J. Zachar*, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava.

We have recently identified and purified a ryanodine receptor/ $\text{Ca}^{2+}$  channel in the sarcoplasmic reticulum of the crayfish (*Astacus fluviatilis*) (1). When incorporated into planar lipid bilayers, the single channel conductance of purified  $\text{Ca}^{2+}$  release channels was about 100 pS in symmetrical NaCl solutions. In order to find out whether the channel properties were preserved after the purification procedures, we examined single-channel conductances of the  $\text{Ca}^{2+}$  release channel in native sarcoplasmic reticulum (SR) membranes (heavy fractions of the SR) with  $\text{Ca}^{2+}$  as the permeating cation. The main single-channel conductance in 250/50 mmol/l Cs<sup>+</sup> (cis/trans) was 139±29 pS (n=12). It adds support for the assumption that the purified calcium release channel conductance represents the conductance of the native channel.

*1. Formelová J., Hurnák O., Zachar J.: Gen. Physiol. Biophys. 9: 445-453, 1990.*

VOLTAGE DEPENDENCE OF CALCIUM RELEASE DURING ACTIVATION OF CONTRACTION IN CRAYFISH SKELETAL MUSCLE FIBRES. *J. Poledna, A. Šimurdová*, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava.

There is a direct link between the time courses of calcium release and contraction. The presented analysis of calcium release in crayfish skeletal muscle fibres is based on the hypothesis (1), which expresses the way in which the number of switched-on crossbridges and elicited force are related to the calcium occupancy of troponin. The model describes calcium diffusion and binding to specific regulatory sites in a sarcomere. Calcium ions activate the interactions of contractile proteins and therefore the generation of force. Experimental records of isolated fibre tension under voltage clamp conditions were approximated by this model. This made it possible to determine the free and bound calcium distribution in the sarcomere and its time course. The steady state calcium concentration at terminal cisternae has a S-shaped voltage dependence with the saturation of about 10 μmol/l at positive membrane potentials.

*1. Poledna J.: Gen. Physiol. Biophys. 8: 81-90, 1989.*

Pb<sup>2+</sup> IONS BLOCK CALCIUM CURRENTS IN THE MUSCLE MEMBRANE SPIKING ON THE CALCIUM PRINCIPLE. *M. Henček, D. Zacharová, J. Pavelková*, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava.

The calcium currents recorded from internally perfused muscle fibre segments of the crayfish (vaseline - gap voltage clamp) can be split by means of the Hodgkin - Huxley equations into two or three components differing in the rate of activation and inactivation respectively (1). All three Ca conductances were suppressed after addition of Pb<sup>2+</sup> (50, 100, 200 and 300 μmol/l Pb(NO<sub>3</sub>)<sub>2</sub>) to the external saline of the following composition (in mmol/l): 13.5 Ca glutamate, 208 TMA<sup>+</sup>. The Pb<sup>2+</sup> effect was concentration and time dependent. At threshold concentrations 50-100 μmol/l the blocking effect was more pronounced on the fast inactivating Ca channel. The difference disappeared at higher concentrations. The time constants of activation τ<sub>m</sub> were not changed or only slightly prolonged. The time constant of inactivation τ<sub>h</sub> was significantly prolonged in the slowly inactivating channel only. Recovery was dependent on the time of action as well as on the concentration.

*1. Henček M., Zacharová D.: Gen. Physiol. Biophys. 10: 599-605, 1991.*

THE EFFECT OF  $Pb^{2+}$  IONS ON THE ELECTRICAL AND CONTRACTILE ACTIVITY OF MUSCLE FIBRES WITH CALCIUM ELECTROGENESIS. E. Lipskaja, D. Zacharová, J. Pavelková, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava.

Muscle fibres of crustaceans represent an useful model for studying the mode of action of substances influencing calcium channels, as the membrane generates active responses on the calcium principle and the excitation-contraction link is dependent on external calcium. We followed the effect of  $Pb^{2+}$  ions (25-200  $\mu\text{mol/l}$   $Pb(\text{NO}_3)_2$ ), which were shown to suppress the Ca currents in muscle fibre segments (1) on the contractile responses of single muscle fibres induced by massive electrical stimulation, by potassium depolarization (4-32 times the normal [K] and caffeine (6 mmol/l). Both single twitches and tetanic tension as well as potassium contractures were suppressed. The caffeine contractures were diminished. Local membrane responses to microelectrode stimulation were increased in proportion to [Pb] and the contraction was lowered. The strontium action potential (exchange of  $\text{Ca}^{2+}$  for  $\text{Sr}^{2+}$  ions) was blocked by  $Pb^{2+}$  ions.

1. Henček M., Zacharová D., Pavelková J.: Physiol. Res. 42: 1993 (in press).

THE EFFECT OF  $Zn^{2+}$  ON THE EXCITATION AND CONTRACTION OF SINGLE MUSCLE FIBRES OF THE CRAYFISH. J. Pavelková, D. Zacharová, M. Henček, E. Lipskaja, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava.

$Zn^{2+}$  ions are reported to act as charge carriers (1) in some muscle fibres with calcium electrogenesis, while they may act in others as blockers of calcium electrogenesis (2). We tried to solve this discrepancy in muscle fibres of the crayfish, which also spike on the calcium principle and possess an E-C link dependent on external calcium. The electrogenic effect of  $Zn^{2+}$  ions (6.5, 13.5 and 27 mmol/l) in a Ca free solution was very low in comparison with the effect of  $\text{Sr}^{2+}$  and  $\text{Ba}^{2+}$  ions. The blocking effect on contractile responses (200  $\mu\text{mol/l}$  to 10 mmol/l) was transient with a recovery to initial or facilitated amplitudes. A permanent contractile block ensued at higher ( $Zn^{2+}$ ) (10-27 mmol/l). The effect of  $Zn^{2+}$  ions on calcium currents was similar. It follows that the electrogenic effect of  $Zn^{2+}$  ions is feeble and the blocking effect of  $Zn^{2+}$  ions is the lowest out of the series of inorganic blockers ( $Pb^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ).

1. Fukuda J., Kawa K.: Science 196: 309-311, 1977.

2. Hagiwara S., Takahashi K.: J. Gen Physiol. 50: 383-601, 1967.

THE EFFECT OF DISODIUM CHROMOGLYCATE AND BENZO(A)PYRENE ON CALCIUM CHANNELS IN THE MEMBRANE OF CRAYFISH SKELETAL MUSCLE. A. Vlčeková, M. Henček, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava.

It is known that disodium chromoglycate (DSCG) inhibits the carcinogenic effect of benzo(a)pyrene (BaP) (1). The mechanism of this inhibition is not yet fully understood. Using the voltage clamp technique, we tried to determine the effect of both these compounds on the membrane conductance to calcium ions. DSCG at a concentration of 5 mmol/l significantly (by up to 60 %) increased the amplitude of the calcium current at all membrane potentials. On the other hand, BaP at a concentration of 4 mmol/l inhibited calcium flow through the membrane (at a depolarization to -20 mV by 40 %), and shifted both the threshold voltage of channel activation and the peak of the current-voltage curve, to more depolarized values. It also slowed down the time constant of activation  $\tau_m$  by up to 50 %. The results have shown that the effect of both compounds on calcium channels is to a significant extent opposite and, as DSCG significantly increases calcium flow through calcium channels, it can be assumed that calcium ions play a role in the protective effect of this compound.

1. Arnold F.: Lancet 337: 865-866, 1991.

CAFFEINE-INDUCED LOCAL OSCILLATIONS OF FROG SKELETAL MUSCLE SARCOMERS. A. Šimurdová, J. Poledna, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava.

Oscillations of individual sarcomeres in a skeletal, muscle cell provide a model for studying the repeated release of calcium from intracellular stores. We have used optical diffraction methods to characterize the transient release of calcium from the sarcoplasmic reticulum induced by subthreshold concentrations of caffeine in the range from 1 to 1.5 mmol/l. The intensity and structure of the first diffraction maximum were measured by a photodiode or linear 256 photodiode array (CCD). At rest, the distribution of light intensity at its maximum was usually composed of several peaks. The subpeaks represented two or more discrete length populations which form domains of serially contiguous sarcomeres of the same length (1). Caffeine added in a subthreshold concentration facilitated the local release of calcium. Spontaneously contracting sarcomeres form clusters and thus influence the intensity distribution in diffraction maxima. The power spectral density of intensity changes was analysed.

1. Judy M.M., Summerour V., LeConey T., Roa R.L., Templeton G.H.: Biophys. J. 37: 475-487, 1982.

ELECTRICAL CONSTANTS IN PHASIC AND TONIC INSECT MUSCLE FIBRES. S. Ivlev, E. Lipskaja, J. Pavelková, M. Novotová, Yu. E. Mandelstam, J.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia and <sup>1</sup>Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava.

Insect muscle fibres can be classed as either phasic or tonic according to their morphological and functional properties. The M. retractor unguis in the hindleg of the locust *Locusta migratoria* M. contains both phasic (P) and tonic (T) fibres (2). The fibre diameter and lengths are the same in both fibre types. They differ in their values of the resting potential (P-RP=56.4±0.9; T-RP=45.8±2.1 mV) and the frequency and values of EPSPs and MEPSPs. The passive electrical properties were investigated in identified (P) and (T) muscle fibres using square wave analysis (1). The following values were found in (P) and (T) muscle fibres:  $\lambda=1.40\pm0.20$  3.24±0.25 mm;  $R_i=64.7\pm32.7$ ; 38.15±2.15  $\Omega\text{cm}$ ;  $R_m=414.93\pm62.07$ ; 1800.45±89.4  $\Omega\text{cm}^2$ ;  $\tau_m=4.43\pm0.8$ ; 10.52±0.74 ms;  $C_m=12.06\pm2.2$ ; 6.14±0.43  $\mu\text{F}\cdot\text{cm}^{-2}$ ;  $r_i=501.9\pm134$ ; 237.5±64.7  $\text{k}\Omega\cdot\text{mm}^{-1}$ ;  $r_m=353.7\pm81.3$ ; 1179.3±138.4  $\text{k}\Omega\cdot\text{mm}$  respectively.

1. Henček M., Zachar J.: Physiol. Bohemoslov. 14: 297-311, 1965.

2. Novotová M., Mandelstam Yu.E., Lipskaja E., Zacharová D., Uhrík B.: Zh. evolut. biochem. physiol. 3: 360-367, 1989.

CONCOMITANT MEASUREMENT OF SMALL AND RAPID ION CONCENTRATION CHANGES AND BIOPOTENTIALS USING DOUBLE-BARREL ION SELECTIVE COAXIAL MICROELECTRODES (ISCM). E. Ujec, Institute of Physiology, Czechoslovak Academy of Sciences, Prague.

A technique has been developed for concomitant measurements of biopotentials, and slow and rapid ionic changes. It is necessary to conform with two requirements, in order to record minute ionic changes: 1. A sufficiently sensitive sensor, i.e. double-barrel ion selective coaxial microelectrode (ISCM). 2. A differential D.C. amplifier, with circuits for frequency compensation of both ISCM channels. The amplifier makes it possible to record three parameters concomitantly in graphic form a) the concentration, b) the potential time course, c) the sum of preceding recordings. Measurements of rapid changes in extracellular ion concentrations ( $\text{Ca}^{2+}$ ) with a rise time less than 1 ms differentiating changes of several tens of micromoles were thus possible. The Schaffer-collaterals in hippocampal slices were stimulated electrically and recordings were made in the region of pyramidal cells.

## II. Physiology of Blood, Muscle Work and Sport

RELATION BETWEEN LEFT VENTRICULAR MASS AND INSTANTANEOUS SPATIAL CARDIAC VECTORS. *I. Ruttkay-Nedecký, E. Vanžurová*, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava.

Echocardiographically obtained signs of left ventricular hypertrophy were correlated with vectorcardiographical parameters in a group of 82 patients with congenital aortic stenosis. A significantly negative correlation was found between the spatial magnitude of the QRS vector at 10 and 20 ms after the QRS onset and interventricular septum thickness, left ventricular posterior wall thickness and left ventricular minor axis. A highest positive correlation was found between septum thickness as well as the posterior wall thickness and the spatial vector amplitude at 50 ms of the QRS complex and between the minor axis and the spatial magnitude at 60 ms QRS. The results speak in favour of the necessity to differentiate the electrocardiographical signs of left ventricular hypertrophy with respect to ventricular activation time.

POSTNATAL DEVELOPMENT OF BLOOD PRESSURE (BP) AND HEART RATE (HR) IN NEONATES WITH DIFFERENT NEONATAL CARE. *D. Andrásyová, E. Kellerová, H. Drobná*<sup>1</sup>, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, and <sup>1</sup>Neonatal Department of Gynaecology and Obstetrics, Derer's Hospital, Bratislava.

In order to evaluate the possible environmental influences on BP and HR development in early postnatal life we investigated full-term neonates at the age of 1-5 days in different neonatal care units. Three hundred and fifty five neonates from a neonatal unit (gestational age 37-42 weeks, birth weight 3393±446 g), 132 in a rooming-in regime with comparable gestational age (3392±460 g) and a group of 27 neonates at a neonatal unit with special care, of the same gestational age (3296±497 g). The postnatal development of HR differed during the first 5 days in the above mentioned groups. HR significantly decreased by 5 % only in the group from the neonatal unit. In the others, it oscillated around the value of the first day, being higher on the average. During the first postnatal days, BP increased by 10-15 % on the average in physiological neonates in the rooming-in regime or from the neonatal unit (with no difference between these two groups). In the group with special care, only an insignificant trend of BP increment was observed, in spite of the increased HR. It seems that HR is a more sensitive indicator of the environmental influences on neonates than BP.

INFLUENCE OF ENVIRONMENTAL FACTORS ON BLOOD PRESSURE IN PRESCHOOL CHILDREN. *V. Regecová, E. Kellerová*, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava.

The distribution of blood pressure (BP) values in 1544 urban children aged 3-7 years, in relation to life conditions (12 mainly socioeconomic factors ascertained by a parental questionnaire, the noise level of the dwelling place and of the kindergarten and residence duration in Bratislava) was investigated. In children living or visiting kindergartens in noisy parts of the city (environmental noise above 60 dB), the incidence of borderline (above 75 percentil, i.e. 115/70 mm Hg) and high (above 95 percentil, i.e. 120/75 mm Hg) BP values and mean diastolic BP were significantly higher as compared with children living in quiet localities. The duration of the urban environmental influence (over 3 years), age of the first attendance at the kindergarten, university education of the mother, previous grandparents care were also reflected in significantly higher mean BP values, as well as the incidence of values above 75 and 95 percentiles. BP was also affected by the number and order of the sibling in the family.

ORTHOSTATIC INTOLERANCE IN CHILDREN WITH COLLAPSES. *K. Javorka, J. Buchanec<sup>1</sup>, O. Chromá<sup>1</sup>*, Department of Physiology, Jesenius Medical Faculty and <sup>1</sup>Paediatrics Clinic, Faculty Hospital, Martin.

The authors followed up some cardiovascular responses during passive orthostasis - head-up tilt in 25 children (mean age 11.5±0.4 years) with orthostatic collapses in their case history. The results were compared to the values in a control group (22 children, the same mean age). Systemic blood pressure (BP), the heart rate and rheographic curves from the forearm and the finger were recorded first in the supine position and during the first and fifth minute of the head-up tilt position (75°). The heart rate and the diastolic BP was increased and the systolic BP was unchanged in the controls during the passive tilt. The relative stroke volume (RSV) in the finger was decreased during the first minute of tilting. It did not differ from the initial values in the 5th minute. In children with collapses, the symptoms of peripheral vasodilation (RSV values increased by 81.4±13.3 % in the 5th minute, the values of diastolic BP were less pronounced) and more marked tachycardiac reactions, compared with the controls, were found during the head-up tilt. The reactivity of the studied cardiovascular parameters in children with collapses was improved by autogenic training.

THE RELATIONSHIP BETWEEN PULSE PRESSURE AND PRE-PRECEDING CARDIAC INTERVAL DURING ORTHOSTASIS AND ISOMETRIC EXERCISE IN MAN. *B. Fišer, N. Honzíkoviá*, Department of Physiology, Faculty of Medicine, Masaryk University, Brno.

A noninvasive continuous blood pressure record can be used for the assessment of heart failure (HF). A mathematically derived index based on the pulse pressure and the pre-preceding interval (D/T-1) significantly increases in HF patients (1). In the present study we determined D/T-1 in 10 healthy men during six experimental periods (orthostasis), two periods of isometric exercise and three periods of rest in-between. Each period lasted 2 minutes. No significant differences of D/T-1 were found among the measured periods. This response can be considered as physiological. A non-physiological response (an increase of D/T-1) may be expected in patients with latent HF. This will be the aim of our next study.

1. *Fišer, B., Honzíkoviá N., Semrád B.*: Physiol. Res. 41: 25-29, 1992.

BAROREFLEX HEART RATE SENSITIVITY STUDIED BY SPECTRAL ANALYSIS DURING CYCLING. *N. Honzíkoviá, B. Fišer, P. Konečná*, Department of Physiology, Faculty of Medicine, Masaryk University, Brno.

Spectral analysis of cardiac intervals (I) and systolic blood pressure (SBP) variability can be used for calculating the modulus at 0.1 Hz (M), an index of the baroreflex heart rate sensitivity (BRS) (1). We used this method in 10 young healthy subjects during pedalling. The blood pressure was recorded by Peñáz's noninvasive method at rest (R) and during 5-minute cycling (1 W/kg of body weight). Records were registered during the first (W1) and last (W2) 2min of bicycling. The following mean values (±S.D.) were obtained (R, W1, W2): I - 735±114, 529±38, 511±59, ms; SBP - 116.7±13.7, 177.4±22.0, 164.8±16.0 mm Hg; coherence between I and SBP at 0.1 Hz - 0.72±0.17, 0.79±0.12, 0.61±0.15; M - 10.9±6.0, 2.1±0.8, 1.9±1.3 ms/mm Hg. The persistence of high coherence (>0.5) during bicycling indicates that the BRS is mediated by the sympathetic nervous system.

1. *Mulder L.J.M.*: Assessment of cardiovascular reactivity by means of spectral analysis. Groningen 1988.

**STUDY OF THE CONDITIONS OF BLOOD O<sub>2</sub>-TRANSPORT IN PATIENTS OPERATED DURING EXTRA-CORPORAL BLOOD CIRCULATION.** A. Matášeje, I. Béder, M. Orgonášová, I. Pecháň<sup>1</sup>, Department of Physiology, Faculty of Medicine, Comenius University and <sup>1</sup>Institute of Cardiovascular Diseases, Bratislava.

Change of basic haematological variables and 2,3-diphosphoglycerate in erythrocytes were studied in 16 cardiac patients operated during extra-corporal blood circulation (ECC). According to the alteration of the variables studied, changes of O<sub>2</sub>-transport were observed. The variables were measured before, during and after the operation. After starting ECC, the haematocrit, haemoglobin and 2,3-DPG content in erythrocytes decreased in comparison with the initial values. After the operation, the 2,3-DPG content in erythrocytes increased by about 51 % on the 10th day (p<0.01). The results obtained can be used as a criterion for the quantification of blood transfusions ensuring a sufficient supply of tissues with O<sub>2</sub>.

**THE EFFECT OF L-CARNITINE LOADING ON THE PHYSICAL PERFORMANCE OF YOUNG TRAINED MEN.** J. Melichna, L. Havlíčková, J. Vránová, S. Otáhal, P. Vodička, B. Semiginovský, V. Horák, H. Rauchová, Z. Drahota, Departments of Physiology and Biomechanics, Faculty of Physical Education, Charles University, Prague, Institute of Animal Physiology and Genetics, Czechoslovak Academy of Sciences, Liběchov and Institute of Physiology, Czechoslovak Academy of Sciences, Prague.

L-carnitine (L-c) raises  $\dot{V}O_{2max}$  and endurance performance in athletes. Six trained young men formed a "placebo" group. Other 6 trained men, selected at random, received a 5-day lasting oral L-c load (3 g/day).  $\dot{V}O_{2max}$  was measured by the test *ad vita maxima*. Blood samples were analyzed for the lactate, glucose and FFA content. Strength parameters (*m. quadriceps femoris*) were determined by electrostimulation. The muscle fibre pattern and cytochromoxidase activity (*m. vastus lateralis*) were assessed by biopsy.  $\dot{V}O_{2max}$  and cytochromoxidase activity were the only parameters which were significantly increased (by +12.4 % and +54.9 % respectively) in subjects of the L-c group in comparison with those of the "placebo" group.

**SLEEP DEPRIVATION AND PSYCHOPHYSIOLOGICAL PERFORMANCE OF NONADAPTED SUBJECTS.** S. Bartůňková, J. Buchberger<sup>1</sup>, J. Kvapilík, D. Pavlů, Faculty of Physical Education and Sports, Charles University, Prague and <sup>1</sup>BIGA, Bern, Switzerland.

Adaptability of the human organism to night work is variable and individual. Sleep deprivation following a night shift disturbs the internal synchronisation and increases the probability of later reactions, errors and injuries in risk operations. We estimated the influence of night shifts on performance and sleep strategy by using mechanometry and continual heart rate registration. In 10 students without experience in night shifts we tested the reactivity (reaction time, tapping), sensomotoric coordination (Koerth rotation trial), postural stability (Romberg test), tremor (tremometry) and attention, memory and errors (number square test). Significant signs of cumulative fatigue were found only in a decreased tapping performance and a higher number of errors. The relationship between sleep duration and its quality were also assessed.

### III. Neurophysiology and Neuroontogenesis

**ELECTRICALLY EVOKED CATECHOLAMINE OVERFLOW IN THE STRIATUM MONITORED BY VOLTAMMETRY.** J. Pavlásek, C. Mašánová, P. Bielik, K. Murgaš<sup>1</sup>, Department of Neurophysiology, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava and <sup>1</sup>Department of Physiology, Faculty of Medicine, Kuwait University, Kuwait.

The voltammetric technique was used in anaesthetized rats for testing the influence of microstimulation (M) and of electroconvulsive stimulation (ECS) on the concentration changes of electro-oxidizable mediators (catechol substances) in the extracellular space of the corpus striatum (S). M applied in S (10 s, 10 Hz) significantly increased the catechol oxidative current (CA.OC), which ranged from 117 to 173 % of the control (136±20, n=5, p≤0.01). The CA.OC peak returned to the baseline within two minutes after M. A comparable result was observed when M episodes were repeated at 5-minute intervals. ECS greatly enhanced CA.OC. The CA.OC peak rose to 1360±380 % (n=3) of the control, the baseline was attained two minutes later. The mechanisms inducing transmitter overflow with consequent changes of receptor functions are considered, and the influence of ECS in S discussed in the context with its beneficial effects in psychopathic patients.

**PROTECTIVE EFFECT OF NEW 1,4-DIHYDROPYRIDINES ON LIPID PEROXIDATION IN THE POSTISCHAEMIC RAT BRAIN.** P. Noskovič, M. Fabiánová, Drug Research Institute, Modra.

Ischaemia leads to the generation of free radicals subsequent destruction of cell membranes. The aim of our study was to investigate the effect of new 1,4-dihydropyridines (DHP) and Nimodipine (a calcium entry blocker with a preferential effect on cerebral vessels (1) on lipid peroxidation (TBA-RS) in the ischaemic rat brain. The tested compounds (10 µg.kg<sup>-1</sup>.min<sup>-1</sup>) were administered intravenously during a 30-minute period (10 min before, 7 min during and 13 min after ischaemia). It was found that VULM 999 and nimodipine significantly decreased the concentration of TBS-RS in the brain cortex, striatum and hippocampus and had a protective effect on lipid peroxidation in the postischaemic rat brain. It means that the raised cytosolic calcium concentration can be one of the most important factors involved in these pathological processes.

I. Kazda S., Hoffmeister F.: Naunyn-Schmiedebergs Arch. Pharmacol. Suppl. 307: R43, 1979.

**PROTECTIVE EFFECT OF HYPOTHERMIA ON THE ISCHAEMIC DAMAGE OF CHOLINERGIC FUNCTIONS IN THE RABBIT SPINAL CORD.** Ž. Malatová, I. Vanický, J. Gálik, M. Maršala, Institute of Neurobiology, Slovak Academy of Sciences, Košice.

Occlusion of the rabbit abdominal aorta for 40 min causes severe ischaemic damage of the lumbosacral (LS) spinal cord with paralysis of the hind extremities. Neurological, neurophysiological and histochemical changes were accompanied with pronounced losses of choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities. The protective effect of local hypothermia, induced by epidural perfusion of a cooled physiological solution along the LS spinal cord 4 min before and during the occlusion, was studied. The spinal cord temperature decreased to 21-18 °C. After 24 h normothermic recirculation, the animals survived without any signs of neurological impairment with practically full recovery of spinal cord evoked potentials. ChAT and AChE activities in the gray matter were preserved at the control level and the histochemistry of AChE did not exhibit any pathological changes. Hypothermia applied under these experimental conditions effectively protected the spinal cord against ischaemic injury.

**RAT NEONATAL CORTEX AFTER X-IRRADIATION ON THE FIRST POSTNATAL DAY.** Z. *Simonová*, H. *Lassmann*<sup>1</sup>, E. *Syková*, Laboratory of Cellular Neurophysiology, Institute of Experimental Medicine, Czechoslovak Academy of Sciences, Prague and <sup>1</sup>Research Unit of Experimental Neuropathology, Austrian Academy of Sciences, Vienna.

The cortices of newborn rats, which were X-irradiated on the first postnatal day, were studied by light microscopy and immunochemistry. The somatosensory cortex of one hemisphere was X-irradiated (4000 r, area of irradiation 3 mm in diameter and investigated 3, 4, 8 and 11 days after irradiation (PI). Three and four days after PI we found vacuolization of the tissue and nuclear changes in nerve and glial cells. This cell degeneration and DNA fragmentation resulted in radiation necrosis. There was also blood brain barrier damage (IgG and albumin leakage), diffuse uptake of serum proteins (IgG, albumin) in the cytoplasm of nerve and glial cells, infiltration of macrophages and an increased number of GFAP positive astrocytes. Eight and eleven days after PI the cell density was reduced with persisting IgG and albumin leakage, minor infiltration by macrophages, and extensive scar formation by astrocytes. These findings are taken as evidence that X-ray irradiation has caused incomplete parenchymal necrosis.

**ONTOGENY OF NEOPHOBIA AND CONTEXT MEMORY.** J. *Mystliveček*, J. *Hassmannová*<sup>1</sup>, Institute of Pathophysiology, Medical Faculty, Charles University, Plzeň and <sup>1</sup>Institute of Physiology and Clinical Physiology, Third Faculty of Medicine, Charles University, Prague.

The ontogeny of neophobia was studied in rats of the Long Evans strain fasting for 24 hours, which were given food in a new context (open field) from 3 weeks to 3 months of age with identical numbers of males and females in each group. In 4 younger age groups, the pups were weaned at 21 days, the 4 other groups at 28 days (there were 2 groups at the age of 5 weeks which had been weaned differentially). Minimum neophobia was found in both genders at the age of 3 weeks, but it was more pronounced in females. There were two neophobia peaks in females: in 5-week pups weaned at the age of 3 weeks, another one at the age of 3 months. Neophobia in males increased at the age of 5 weeks which remained at approximately the same level. Optimum memory of the context without neophobia symptoms was found at the age of 4 weeks. Neither neophobia nor context memory, similarly as other forms of learning and memory, develop monotonously.

**CRITICAL PERIOD IN THE DEVELOPMENT OF RAT PACINIAN CORPUSCLES.** J. *Zelená*, I. *Jirmanová*, Institute of Physiology, Czechoslovak Academy of Sciences, Prague.

A quantitative study was made on crural Pacinian corpuscles after crushing the sciatic nerve in one to 15-day-old rats. After nerve crush in newborn rats, all corpuscles rapidly degenerated, and only 20 % of their normal number regenerated following reinnervation of the calf: the regenerated corpuscles were reduced in size and had multiple terminals and multiple inner cores, all enclosed in a thin capsule (1). After crushing the nerve in 5-day-old rats, the regenerated corpuscles had again multiple axon terminals with multiple inner cores and their number was decreased to 40 % of the control. The critical period terminated 7 days after birth. From this time onwards, over 90 % of Pacinian corpuscles survived after nerve crush and were subsequently reinnervated. However, their inner cores were composed of the original and newly formed lamellae, induced by multiple regenerated terminals.

1. *Zelená J.*: Neuroscience 6: 1675–1686, 1981.

## IV. Physiology of Cardiac and Vascular Cells

**POSITIVE COOPERATIVITY BETWEEN THE BINDING OF ALCURONIUM AND N-METHYLSCOPOLAMINE TO MUSCARINIC RECEPTORS IN THE RAT HEART: BINDING AT EQUILIBRIUM.** S. *Tuček*, J. *Proška*, Institute of Physiology, Czechoslovak Academy of Sciences, Prague.

We have recently found that, during 3 h incubations, the neuromuscular blocker alcuronium (Alc) increased the binding of N-(<sup>3</sup>H)methylscopolamine ((<sup>3</sup>H)NMS) to cardiac muscarinic receptors at low concentrations while it diminished it at high concentrations (1). This suggested that there was positive cooperativity between the two ligands at low, and competition at high concentrations of Alc. We have found that, at high Alc concentrations, the binding of (<sup>3</sup>H)NMS increased if the incubation time is prolonged, and that the binding of (<sup>3</sup>H)NMS is not diminished by Alc if the high concentration of Alc is only added after (<sup>3</sup>H)NMS had been permitted to bind. These observations suggest that Alc and (<sup>3</sup>H)NMS do not compete for identical binding sites.

1. *Tuček S., Musílková J., Nedoma J., Proška J., Shelkownikov S., Vorlíček J.*: Molec. Pharmacol. 38: 674–680, 1990.

**POSITIVE COOPERATIVITY BETWEEN THE BINDING OF ALCURONIUM AND N-METHYLSCOPOLAMINE TO MUSCARINIC RECEPTORS IN THE RAT HEART: KINETIC OBSERVATIONS.** J. *Proška*, S. *Tuček*, Institute of Physiology, Czechoslovak Academy of Sciences, Prague.

Alcuronium (Alc) dose-dependently slows down the association of N-(<sup>3</sup>H)methylscopolamine ((<sup>3</sup>H)NMS) with and its dissociation from muscarinic receptor.  $K_d$  values calculated as ratios of dissociation/association rate constants are more diminished by Alc than the  $K_d$  values obtained from equilibrium binding experiments. Previous binding of Alc strongly interferes with subsequent binding of (<sup>3</sup>H)NMS, while the addition of Alc after (<sup>3</sup>H)NMS had been bound does not diminish the binding of (<sup>3</sup>H)NMS. It is suggested that the binding sites for Alc are in close vicinity to those of NMS and that Alc sterically hinders the access of NMS to its binding sites and virtually completely blocks its release from the NMS binding sites.

**LOCALIZATION OF THE BINDING SITE FOR ALCURONIUM ON RAT CARDIAC MUSCARINIC RECEPTORS.** J. *Jakubík*, S. *Tuček*, Institute of Physiology, Czechoslovak Academy of Sciences, Prague.

Alcuronium (Alc) has been shown earlier to exert a positive cooperative effect on the binding of N-(<sup>3</sup>H)methylscopolamine ((<sup>3</sup>H)NMS) to cardiac muscarinic receptors (1). Nothing is known about the allosteric sites to which Alc binds. Tyrosine and aspartate residues are known to play an important role in the binding of ligands to the "classical" muscarinic sites. We performed experiments in which cardiac muscarinic receptors were exposed to reagents known to bind covalantly to tyrosine and aspartate residues. Covalent modification of receptors by these reagents prevented the binding of (<sup>3</sup>H)NMS. Alc diminished (slowed down) this effect of protein modifiers. We propose that Alc binds in the close vicinity of (<sup>3</sup>H)NMS binding sites and that it sterically protects them against the modifying reagents.

1. *Tuček S., Musílková J., Nedoma J., Proška J., Shelkownikov S., Vorlíček J.*: Molec. Pharmacol. 38: 674–680, 1990.

THE EFFECTS OF M-CHOLINERGIC AND ADENOSINE RECEPTOR ACTIVATION ON PHOSPHORYLATION IN VENTRICULAR MYOCYTES ARE PERTUSSIS-TOXIN (PTX) SENSITIVE. P. Bokník, J. Neumann<sup>1</sup>, J. Segínko, J. Kyselovič<sup>2</sup>, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University, Bratislava, <sup>1</sup>Department of Pharmacology, University-Krhis, Eppendorf, Hamburg, FRG and <sup>2</sup>Institute of Pharmacology, Faculty of Medicine, Comenius University, Bratislava.

Acetylcholine and adenosine exert negative inotropic effects on ventricular preparations in the presence of isoprenaline (Iso) by affecting phospholamban phosphorylation (PLB-P). Ventricular myocytes (VM) were isolated from PTX (180 µg/kg) pretreated and control guinea-pig hearts. PLB-P was measured by electrophoresis and autoradiography. Iso (0.1 µM, 1 µM) concentration dependently increased PLB-P to 158 % of the controls, respectively. Carbachol (C) (1 µM) or N<sup>6</sup>-(R-Phenyl-isopropyl)-adenosine (PIA, 1 µM) in the presence of Iso (0.1 µM) attenuated the increase of PLB-P by 126 % and 124 %, respectively. In PTX-treated VM Iso (0.1 µM) increased PLB-P to 144 % of the controls and the effect of C and PIA was completely abolished (n=7-9). We conclude that the dephosphorylation of PLB is mediated via PTX-sensitive G-proteins.

4-AMINOPYRIDINE BLOCK OF TRANSIENT OUTWARD CURRENT IN RAT VENTRICULAR MYOCYTES IS FREQUENCY- AND VOLTAGE-DEPENDENT. M. Šimurdová, G. Christé, J. Šimurda, J. Moudr, Faculty of Medicine, Masaryk University, Brno and INSERM U121, Bron, France.

The effect of frequency and voltage of depolarizing pulses on the degree of 4-aminopyridine (4-AP) block of transient outward current (I<sub>to</sub>) was described in nerves as well as in multicellular cardiac muscle preparations. Similar effects could not be found in isolated rabbit cardiomyocytes (1). We studied I<sub>to</sub> in enzymatically isolated rat ventricular myocytes using the whole cell patch clamp technique with the aim to clarify this discrepancy. Steady state block of I<sub>to</sub> induced by 1 mM 4-AP was complete (100 %) at 0.125 Hz (regular stimulation by 300 ms, 150 mV depolarizing pulses from a holding voltage of -90 mV) but it was partly relieved (to 70 %) at 1.5 Hz. The degree of unblocking decreased with increasing concentration of 4-AP which offers an explanation for the absence of I<sub>to</sub> unblocking effect at the 2 mM concentration (1). We conclude that the 4-AP block of I<sub>to</sub> in cardiac cells is frequency- and voltage-dependent at concentrations below a critical level which depends on the sensitivity of the preparation to the drug.

1. Zygmunt A.C., Gibbons W.R.: *Circ. Res.* 68: 424-437, 1991.

ISOPROTERENOL MODIFIES I<sub>K1</sub>-SENSITIVITY TO BA<sup>2+</sup> CATIONS IN GUINEA-PIG CARDIAC MYOCYTES. E. Lacinová, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava.

An inwardly rectifying K<sup>+</sup> current (I<sub>K1</sub>) has been measured in guinea-pig cardiac myocytes employing the whole-cell patch clamp technique. I<sub>K1</sub> is known to be Ba<sup>2+</sup>-sensitive. Isoproterenol-dependent regulation has not yet been shown. In our experiments there was no effect of isoproterenol on I<sub>K1</sub>, however, the blocking effect of Ba<sup>2+</sup> was significantly reduced on isoproterenol-treated myocytes. When I<sub>K1</sub> was suppressed by Ba<sup>2+</sup> first, isoproterenol significantly enhanced the inward current. Because the current was activated by hyperpolarizing pulses from holding potentials -40 mV to -80 mV, the observed current cannot be contaminated by a Ca<sup>2+</sup> current. This current was not suppressed by a protein kinase A inhibitor, H9, included in the internal solution, nor was its time course similar to any known Cl<sup>-</sup> current. The most probable explanation is that isoproterenol significantly modifies I<sub>K1</sub>-sensitivity to Ba<sup>2+</sup> cations.

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INHIBITION OF CALCIUM CURRENTS BY CLOMIPRAMINE AND MAPROTIline. I. Zahradník, A. Zahradníková, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava.

Cardiotoxic side effects of antidepressant drugs represent a serious therapeutical problem. The effects of clomipramine and maprotiline, members of the tricyclic antidepressant family, were tested on L-type calcium currents in enzymatically isolated rat ventricular myocytes using the whole cell patch clamp technique. Currents were recorded from cells equilibrated in 20 µM TTX-containing Tyrode solution and with a K<sup>+</sup>-free internal solution. The membrane potential was held at -50 mV and voltage pulses were delivered at 3 s intervals. Both clomipramine and maprotiline decreased peak I<sub>Ca</sub> after extracellular application with a K<sub>D</sub> of 10 µM and 40 µM, respectively. Significant blocking effects were observed at therapeutical doses. A slight decrease in the rate of inactivation and no changes in the voltage dependence of I-V curves were observed. Blocking effects could only be reversed after short, about 15 s, exposures. Recovery from exposures longer than 1 min was very slow, indicating impairment of Ca channel metabolic regulation in addition to the direct action.

THE EFFECT OF DIDS ON CARDIAC SARCOPLASMIC RETICULUM Ca-RELEASE CHANNELS IS VOLTAGE-DEPENDENT. A. Zahradníková, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava.

Ca-release channels of the sarcoplasmic reticulum can be activated by the isothiocyanate-containing stilbene derivative DIDS. Its effect on the cardiac SR Ca-release channel was studied using the BLM technique. Vesicles from the microsomal fraction of canine ventricular myocardium, prepared by homogenization and differential centrifugation, were incorporated into bilayers. Single channel currents through the Ca-release channel were measured in 250:50 mM (cis:trans) Cs methanesulfonate. After application of 1 mM DIDS, the channel open probability increased 30 times at 0 mV, but only 3 times at -50 mV. Activation by DIDS reached a steady state within a few minutes. Voltage steps from 0 to -50 mV and back induced rapid deactivation/activation of the channel. These results can be interpreted as a covalent modification of the channel gating mechanism that induces voltage dependence.

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PROTEIN PROFILE OF CARDIAC MUSCLE IN MALE AND FEMALE RATS; EFFECT OF CHRONIC HYPOXIA. V. Pelouch, B. Ošádal, M. Milerová, J. Procházka, Institute of Physiology, Czechoslovak Academy of Sciences and Centre of Pediatric Cardiology, University Hospital Motol, Prague.

We have shown previously that cardiac resistance to acute anoxia is significantly higher in females than in males both in normoxic and chronically hypoxic rats. This fact may be at least partly influenced by the differences in protein composition. The purpose of this study was, therefore, to compare the cardiac protein profile in both sexes. Male and female Wistar rats (70-days-old) were exposed to intermittent high altitude hypoxia (IHA) in a barochamber (8 h a day, 5 days a week, stepwise up to 7000 m, number of exposures 26). The protein composition of both ventricles was already significantly different in normoxic animals; the proportion of collagen was significantly higher in females. IHA induced right ventricular (RV) hypertrophy, significantly lower in females compared with males. An increase of the total protein concentration and quantitative as well as qualitative changes of various protein fractions occurred in both sexes. Whereas the elevation of collagen and total collagenous proteins (all extracellular matrix proteins) was significantly higher in the RV of hypoxic males as compared with the hypoxic female myocardium, where elevation of sarcoplasmic and contractile proteins predominated. Our results support the hypothesis that protein profiling of cardiac muscle is sex dependent both under control and hypoxic conditions.

**SYSTOLIC MECHANICAL PERFORMANCE OF THE HETEROTOPICALLY TRANSPLANTED RAT HEART.**  
*F. Kolář, C. MacNaughton<sup>1</sup>, F. Papoušek, B. Korecky<sup>1</sup>*, Institute of Physiology, Czechoslovak Academy of Sciences, Prague and <sup>1</sup>Department of Physiology, University of Ottawa, Canada.

The heart of outbred male Wistar rats were transplanted with a part of the lungs into the abdomens of recipients of the same strain by attaching the stump of the aorta end to side to the abdominal aorta of the recipient. The animals were treated with cyclosporine A in a daily dose of 15 mg.kg<sup>-1</sup> for 7 days. Within 3, 14 and 28 days after surgery the mass of the left ventricle (LV) of the transplant decreased to 84.2, 54.2 and 42.8 % of the recipient I.V, respectively. Simultaneously, the LV geometry became more spherical. The developed pressure, maximum rate of pressure development and the slope of systolic stress-strain relationship were unaffected in the atrophic ventricles; the rate of relaxation, however, significantly decreased. Generally, the same results were obtained using inbred Lewis rats without cyclosporine treatment. The data indicate that mechanical performance of the transplanted hearts was maintained during the development of atrophy; the cardiotoxic effect of cyclosporine was not manifested under these conditions.

**ACUTE COOLING ENHANCES ENDOTHELIUM-DEPENDENT RELAXATION IN THE RABBIT THORACIC AORTA.**  
*J. Török, J. Kolárik*, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava.

The purpose of the present study was to determine the effect of acute moderate cooling on endothelium-dependent relaxation of isolated rings of the rabbit thoracic aorta. Aortic rings were precontracted with noradrenaline (10<sup>-6</sup> mol/l) or phenylephrine (10<sup>-6</sup> mol/l), and dose-response relationships to acetylcholine and sodium nitroprusside were performed in the presence of indomethacin (10<sup>-5</sup> mol/l). Lowering the bath temperature from 37 °C to 24 °C decreases the basal tension of aortic rings. Cumulative addition of acetylcholine (10<sup>-9</sup>–10<sup>-6</sup> mol/l) induced a concentration-dependent relaxation at 37 °C which was enhanced by cooling to 24 °C. The concentration-dependent relaxation to nitroprusside was similarly affected by the changes in bath temperature. These results are consistent with the idea that enhancement of endothelium-dependent relaxation of the aorta during cooling is probably due to a decreased inactivation of the endothelium-derived relaxing factor.

## V. Metabolism, Endocrinology and Physiology of Reproduction

**AIMS AND POSSIBILITIES OF ISOTOPIC METHODS IN EXPERIMENTAL TOXICOLOGY. IN VIVO STUDIES.**  
*M. Vojtíšek, D. Bittnerová, M. Cikrt, V. Bencko, Z. Bradna, J. Švihálková*, National Institute of Public Health, Prague.

The aim of the use of isotopic methods was to observe the fate of a toxic metal after its entry into a rat's body. Isotopic methods enable *in vivo* whole body measurements of the retention of a metal given together with its radionuclide. The method was used for studying the influence of Sinecal (fibre) on the retention and excretion of cadmium after a single oral dose of 5 mg CdCl<sub>2</sub>/rat (female, Wistar strain, 200-250 g b.w.) together with 370 kBq of Cd<sup>115m</sup>. Control animals: Larsen pellet diet, test animals: 50 % pellets + 50 % Sinecal by weight. The experiment lasted 96 h. In the test group, the retention curves indicated slower cadmium excretion up to 72 h after administration. In other tests, the differences between oral or intratracheal dosage of Mn<sup>2+</sup> were determined by assessing the retention curves. Furthermore, a concentration dependence of single or subchronic Cd<sup>2+</sup> doses could be detected by this method.

**AIMS AND POSSIBILITIES OF ISOTOPIC METHODS IN EXPERIMENTAL TOXICOLOGY. IN VITRO STUDIES.**  
*D. Bittnerová, M. Vojtíšek, M. Cikrt, V. Bencko, Z. Bradna, J. Švihálková*, National Institute of Public Health, Prague.

Individual organ retention measurements of a metal were carried out by means of radionuclides *in vitro*. In the first set of experiments the influence of Sinecal in the diet of rats was observed on the cadmium organ content. A higher liver and kidney cadmium retention was detected when compared to the controls (pellet diet only). A significant increase of manganese in the rat brain was ascertained after intratracheal application as compared to oral application of an equal manganese dose in a second group tests. This method was also applied for manganese and cadmium interaction studies (oral dosage of metals). The most interesting finding concerned an apparent increase of brain manganese concentration after subchronic cadmium pretreatment. Relevant organ radionuclide metal detections in these experiments closely correlated with *in vivo* measurements.

**EXPRESSION OF PHOSPHOPROTEIN STATHMIN IN THE REGENERATING LIVER.**  
*J. Koppel, P. Rehák, A. Sobel*, Institute of Animal Physiology, Slovak Academy of Sciences, Košice, Czechoslovakia and INSERM U 153, Paris, France.

Stathmin is a 19 kDa phosphoprotein presumably involved in cell regulations as an intracellular relay for extracellular signals activating diverse second messenger pathways. We have undertaken a detailed analysis, using Western blot and 2D PAGE, of the amount and phosphorylation of stathmin in liver of rats after two-thirds partial hepatectomy. Stathmin began to exhibit a significant increase at 24 h, reached its peak between 48 and 72 h and then gradually returned to control levels 7 days after hepatectomy. Immunoblot quantification showed a 10fold increase of stathmin concentration at 48 h regenerating liver. The time course of stathmin concentrations in regenerating liver suggested that it was a delayed consequence of hepatectomy. Hepatectomy did not apparently change the phosphorylation state of stathmin as only one stathmin spot corresponding to the phosphorylated form (P1) was detected. Nevertheless, it supports the notion of a similarity between the developing and regenerating liver as the comparable distribution of stathmin forms which exists in neonatal livers. It is suggested that stathmin plays a specific role in regulations of liver cell growth.

**MILK FAT CONTENT AND GROWTH OF RAT PUPS.**  
*Z. Rybošová, Š. Mozeš, S. Kuchár*, Institute of Animal Physiology, Slovak Academy of Sciences, Košice.

The relationship between the milk fat content and growth of rat pups from birth to the 20th day was investigated. The content of milk fat was determined by the crematocrit method. A wide range of the milk fat content with a great variability in each individual dam was observed during lactation. The pups fed on higher fat milk during the first decade of life grew more rapidly than pups fed on lower fat milk. A significant positive correlation (P<0.001) was found between the fat content of milk and the weight gains of pups only in the first decade. It indicates that the fat content of milk is important for the growth of pups mainly in the early postnatal period of life.

**INFLUENCE OF LITTER SIZE ON THE RNA CONTENT OF VMH NEURONES IN PREWEANED RATS.** *S. Mozeš, S. Kuchár, V. Nováková*, Institute of Animal Physiology, Slovak Academy of Sciences, Košice.

The weight gains and total RNA content of ventromedial (VMH) and lateral (LHA) hypothalamic cells of rat pups in normal litters (8 pups/nest) and in reduced litters (2 pups/nest) were investigated. A significant decrease of weight gains was found in pups reared in the reduced litters during the first 5 days. The RNA content in VMH cells of pups reared in reduced litters was significantly increased on the 15th and 30th day, but was unchanged in LHA cells. The results show that reducing a rat litter to 2 pups caused short-term undernutrition leading to permanent changes of the RNA content in VMH neurones.

**CHANGES OF BODY FAT AND RNA CONTENT IN VMH NEURONES IN NEONATALLY UNDERNOURISHED RATS.** *S. Kuchár, S. Mozeš, Z. Rybošová*, Institute of Animal Physiology, Slovak Academy of Sciences, Košice.

The RNA content in the neurones of the ventromedial hypothalamus (VMH) and the lateral hypothalamic area (LHA) and the epididymal, retroperitoneal and liver fat content of 150-day-old male rats subjected to neonatal undernutrition were investigated. The data show that pups undernourished at an early developmental stage had a significantly increased RNA content in VMH neurones and a significantly decreased epididymal, retroperitoneal and liver fat content, in comparison with control animals.

**SUBDIABETOGENIC STREPTOZOCIN TREATMENT IMPAIRS PREIMPLANTATION DEVELOPMENT OF THE MOUSE EMBRYO.** *J. Veselá, P. Reháč, J. Koppel*, Institute of Animal Physiology, Slovak Academy of Sciences, Košice.

To estimate the significance of insulin in the regulation of preimplantation embryo growth, female mice received a single subdiabetogenic dose of streptozocin 65 mg/kg 8 days or 14 days before mating, respectively. Mean glycaemia levels and the number of embryos per mouse did not differ significantly between the streptozocin treated and control groups. Morphological analysis of preimplantation embryos collected on day 3 of pregnancy revealed significant changes in the distribution pattern of preimplantation embryo stages recovered from streptozocin treated females. Continuous insulin treatment of streptozocin treated mice improved the impaired development of preimplantation embryos only in short lasting experiments. After the long subdiabetic period (14 days), the number of degenerated embryos was markedly increased in both streptozocin treated groups. It can be concluded that the subdiabetic state in female mice impaired preimplantation embryo development. This might be an effect of delayed oocyte maturation and/or impairment of oocyte fertilization.

**MECHANISM OF STIMULATORY ACTION OF FOLLICULAR FLUID ON PROGESTERONE FORMATION BY GRANULOSA CELLS.** *A. Danišová, G. Šrenkelová, Z. Víršik, J. Kolena*, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava.

In this study we investigated the mechanism of the stimulatory action of follicular fluid on the luteinization process of granulosa cells. Immature granulosa cells (GC), isolated from 1-3 mm follicles of porcine ovaries, were cultivated for 72-96 h in the presence of FSH. LH and follicular fluid were isolated from large (6-8 mm) follicles (LFF). Gonadotropins stimulated progesterone production by GC which was related to the production of cAMP and cGMP. A higher stimulatory effect on the secretion of progesterone, as compared with gonadotropins, was observed in the presence of LFF. LFF increased the formation of cGMP but, in contrast to gonadotropins, it did not affect cAMP formation. The addition of LFF to GC stimulated LH/hCG receptor induction without alteration of membrane lipid fluidity. The results indicate that the stimulatory effect of LFF on GC is mediated by a cGMP-regulatory pathway.

**THE EFFECT OF ADMINISTRATION OF ESTRADIOL ON BODY GROWTH OF YOUNG MALE RATS.** *S. Čikoš, S. Kuchár, J. Koppel*, Institute of Animal Physiology, Slovak Academy of Sciences, Košice.

The significance of reduced food intake in inhibition of body growth of estradiol treated young male rats was investigated. The results show that untreated male rats, restrictedly fed according to estradiol treated (at 32, 37 and 42 days of age) *ad libitum* fed animals, had significantly higher body weight, length of the tibia and thickness of the growth plate of the tibia at the end of the experiment (at 47 days of age) in comparison to the estradiol treated male rats. Furthermore, the rate of longitudinal growth of the tibia in restrictedly fed animals measured between 69 and 24 hours prior to killing was significantly higher than that of estradiol treated animals. These results show that the inhibitory effect of estradiol on body growth of young male rats is not only the result of decreased food intake.