

**REPORT
ON SCIENTIFIC ACTIVITIES
1985**

**POLISH ACADEMY OF SCIENCES
MEDICAL RESEARCH CENTRE**

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ON SCIENTIFIC ACTIVITIES
1985**

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RESEARCH REPORT

STUDIES ON THE FUNCTION OF THE NERVOUS SYSTEM AND ON MECHANISMS CONTROLLING BASIC FUNCTIONS OF THE ORGANISM

Department of Neurophysiology
Head: Prof. Witold Karczewski

FUNCTIONAL ORGANIZATION OF RESPIRATORY NEURONAL NETWORK — SOURCES AND MECHANISMS OF RESPIRATORY DRIVE

- a) The split-brainstem preparation and the functional organization of the respiratory oscillator.
- b) Studies on the contribution of raphe nuclei to the control of respiratory reflexes.
- c) Determination of laryngeal resistances during tidal breathing through the larynx in control conditions and following the progressive denervation of the airways.

a) The „split-brainstem“ preparation was applied in decerebrated animals and extended to further animal species — dogs and guinea-pigs. It was demonstrated that in dogs the mid-line section extending 15 mm rostral to the obex desynchronizes respiratory sub-centres. It was shown that due to the difference in the brainstem blood supply in the dog (compared to cats and rabbits) haemorrhages and brain oedema frequently follow the mid-line section.

The main effect of a mid-sagittal sections in the guinea-pig was an arrest of respiration, not reversible by chemical stimuli. The organization of the respiratory oscillator differs in various species. From desynchronization in rabbits, monkeys and dogs following mid-line separation, to the standstill of the respiratory activity in cats and guinea-pigs (the standstill in cats could be reversed by the chemical stimuli).

It has been shown that a progressive elimination of the medullary pathways to the phrenic and intercostal motoneurons makes these motoneurons more prone to „autonomic“ generation of phasic activity; the intensity of the segmental reflexes increases.

In the studies on the role of the dorsal motor nucleus of the vagus nerve it was shown that the pharmacological blockade of its neurones abolishes the Breuer-Hering reflex in rabbits, mimicking „central vagotomy“, followed by the decay of the activity in the recurrent laryngeal nerve.

In collaboration with dr Fedorko from the Department of Human Physiology of the Medical Academy, it has been demonstrated that the neurones of the dorsal group in the rabbit project ipsi- or contralaterally to phrenic motoneurons (in few cases both ipsi — and contralaterally).

(K. Budzińska, M. Głogowska, H. Gromysz, W.A. Karczewski, J.R. Romaniuk

with the technical assistance of Miss U. Jernajczyk, Mrs E. Jędrychowska and Mrs K. Semerau-Siemianowska).

b) Eight experiments with an electrical stimulation and pharmacological blockade of the raphe nucleus did not bring unequivocal results. It could not be excluded that raphe nucleus as a structure of particular localisation and topography might be functionally non-uniform and the essential region for the function studies was not seized as yet. The application of serotonergic agents at this region is planned in future experiments.

(K. Budzińska, J.R. Romaniuk with the technical assistance of Mrs K. Semerau-Siemianowska).

c) The resistance posed by the larynx was shown to depend upon its innervation. After deafferentation of the larynx the resistance went-up seven fold, whereas previous sensory denervation of the larynx did not affect the resistance. It has been shown that maximum airflow in the respiratory cycle is reduced by the laryngeal slit in the control conditions, whereas the paralysed larynx diminished flow by 60%. In line with this is the finding that the minute ventilation is enhanced while breathing with omission of the larynx 1.1 times in the control conditions and doubled after denervation of the airways.

(M. Szereda-Przestaszewska and B. Kamosińska with the technical assistance of Mrs E. Jędrychowska).

ROLE OF THE CHANGES IN ACID-BASE BALANCE IN THE CONTROL OF BREATHING

a) Elucidation of the effects of central and peripheral chemoreceptors on the dynamics of ketamine — induced apneustic pattern of breathing.

b) Working-out of a model describing the role of the chemoreceptive input in the control of phasic respiratory function.

c) Search into the role of the vagus nerves in an intracerebral vasospasm following subarachnoid haemorrhage.

a) In the anaesthetized, paralysed and artificially ventilated cats the apneustic breathing was induced with the successive, dose increasing, intravenous injections of ketamine. The effects of peripheral chemoreceptors on the pattern of apneustic breathing were searched for in normocapnic hypoxia and hypercapnic hyperoxia. It was shown that hypoxia and hypercapnia restore the normal pattern of breathing, shortening the inspiratory phase. These stimuli enhance the central inspiratory activity both in the control conditions and after ketamine. The mechanism of action of ketamine on the inspiratory oscillator seems to rely on the decrease in the rate of augmentation of the inspiratory activity generated within the medulla.

(P. Grieb, H. Gromysz, M. Pokorski with the technical assistance of Miss U. Jernajczyk).

b) The model of apneustic breathing with the use of fractional doses of ketamine has been worked out. It was shown that „ketamine apneusis“ is cha-

racterized by the prolonged, irregular inspirations, oscillatory in character; the expiratory time remains unchanged. The present results (see also point a)) suggest that the ketamine apneusis does not fit the Clark — von Euler's model. Studies on the interaction between the chemoreceptive input and the respiratory oscillator during ketamine apneusis might bring about information on the manner of the transition of tonic chemoreceptive input into the phasic output. (P. Grieb, M. Pokorski with the technical assistance of Miss U. Jernajczyk).

c) Studies were performed on the role of the cholinergic and adrenergic innervation of the blood vessels of the brain-stem in subarachnoid haemorrhage. It was shown that in SAH the activity of noradrenaline increases, whereas the activity of cholinesterase drops down. These changes got normalised after metylpregnisolone. Neurotomy of the greater petrosal nerve restores the cerebral circulation disturbed in the course of SAH and prevents the intracerebral vasospasm after subarachnoid blood injection. It was shown as well that the cervical vagotomy normalizes the intracerebral circulation affected by subarachnoid haemorrhage.

(K. Głowicki, W. Janczewski, A. Kukwa (A.M., Warsaw), W. Zaleski (COMT, Warsaw), M. Ryba with the technical assistance of Mr S. Januszewski).

The MERA 400 computer was adapted to simultaneous analysis of four independent variables. Two microcomputers Fujitsu were installed; one for word processing, the other for statistical analysis. Their application in on-line experimental data processing is being prepared. The laboratory staff was trained in BASIC.

(L. Czerwosz and J. Kulesza).

See the LIST OF PUBLICATIONS:

11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 24, 35, 39, 40, 41, 42, 43, 44, 47, 48, 49, 50, 51, 52, 55, 64, 66, 68, 69, 80, 81, 82, 83, 84, 142, 144, 145, 146, 147, 148, 149, 158, 159, 160, 161, 162, 167, 168, 169, 184, 185, 186.

Department of Applied Physiology

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ADAPTATION TO PHYSICAL EXERCISE AND CHANGES IN ENVIRONMENT

1. Metabolism and its control

a) To elucidate contribution of changes in skeletal muscle sensitivity to insulin to mechanisms of impaired glucose tolerance under conditions of restricted physical activity and stress experiments were performed on rats with tenotomized muscles as well as on animals with prolonged hyperadrenaline-mia. Hyperadrenalinemia was produced in rats by s.c. implantation of adrenalinic tablets, releasing the hormone at a constant rate ($1.8 \mu\text{g}/\text{min}^{-1}$) for at least 24 hours.

Muscle sensitivity to insulin, determined in vitro, was expressed as insulin concentration in the incubation medium (from 1.0 to 10 000 $\mu\text{U}/\text{ml}^1$) inducing half of the maximum stimulation of lactate production or glycogen synthesis.

Both the tenotomized muscles and those taken from hyperadrenalinemic rats showed markedly decreased insulin sensitivity. In both cases this effect was reversed by adenosine antagonist: 8-phenyltheophylline, added to the incubation medium, thus suggesting a role of adenosine in the reduced muscle insulin sensitivity.

b) Continuing studies on the factors modifying working ability the effects of: i) prolonged hyperadrenalinemia, ii) food deprivation; iii) hyperthermia; and iiiii) deficit of thyroid hormones on work performance and exercise metabolism were investigated.

i) Hyperadrenalinemia, sustained in rats for 24 h (as described above) was found to produce marked depletion of liver and muscle glycogen, enhancement of muscle pyruvate and lactate contents, as well as a decrease in the contents of high energy phosphates (ATP, phosphocreatine). Duration of exercise until exhaustion was by approx. 50% shorter in hyperadrenalinemic rats in comparison with controls, which can be attributed to the depletion of body carbohydrate stores.

ii) It was found that food deprivation of dogs for 7 days, resulting in body weight loss of approximately 2 kg, causes a marked decrease in muscle glycogen with a concomitant increase in muscle lactate content and elevations in the plasma free fatty acid and ketone acid concentrations. During treadmill exercise of moderate intensity, performed by the food deprived dogs, the rate of muscle glycogen utilization was reduced and high energy phosphate contents decreased more than in control exercise. However, the performance time and the amount of total work performed were not affected by food deprivation.

iii) In the studies carried out on cooperation with the Institute of Physiology, University of Giessen (West Germany) chronically implanted heat exchangers were used to differentiate effects of local muscle hyperthermia from those of general hyperthermia on metabolic responses to a short-term physical exercise of moderate intensity in goats. Muscle hyperthermia at the relatively low core body temperature did not affect exercise metabolism while an increase in internal temperature without any changes in working muscle temperature caused an increase in respiratory heat loss with reduction of arterial PCO_2 and HCO_3 , and a significant elevation of blood lactate level.

In experiments carried out on dogs it was demonstrated that the progressive enhancement in body temperature during consecutive exercise bouts of the same intensity can be attributed to the increasing activity of the adrenergic system, and it is attenuated by blockade of beta-adrenergic receptors.

iiiii) Continuing studies on the role of thyroid hormones in thermoregulation and control of metabolism physiological responses to treadmill exercise of different intensities (from 40 to 70 W/kg⁻¹) were compared in thyroidectomized and intact-control dogs. The effects of thyroid hormone deficiency were particularly well pronounced during the exercise with the highest work load. They included: reduced muscle temperature and heart rate responses to exercise, decreased rates of muscle glycogen utilization, lactate production and enhanced depletion of high energy phosphates.

2. Physiological basis of physical training

In cooperation with the Department of Physiology, University of Kuopio (Finland) the effect of three month slalom training on physical performance capacity was investigated in 23 healthy subjects. Slalom training increased significantly the maximal work capacity (W_{max}) and the maximal blood lactate concentration (LA_{max}), but did not effect the maximal oxygen consumption (V_{O_2max}) measured during the bicycle ergometer tests. Slalom training lowered the anaerobic threshold in all subjects. The increase in physical performance capacity caused by the regular, short interval type slalom training seems to be due to an increase in the anaerobic rather than aerobic capacity.

An influence of 3 months' endurance training on thermoregulatory responses to exercise was tested in healthy male volunteers. After the training programme applied the sweating reaction was triggered markedly faster, and the internal body temperature increase was significantly lower in response to the same relative work load than before training.

Longitudinal studies on training effects in boys (aged from 15 to 18 years) revealed that endurance training significantly increases V_{O_2max} in comparison with untrained boys of comparable age not earlier than after 2 year-training programme. However, the maximal O_2 pulse, being a sensitive indicator of cardiovascular adaptation, was significantly higher in trained than untrained boys already within 1 year. Anaerobic capacity increased with age of the subjects independently of the level of their physical activity.

3. Exercise tolerance in cardiac patients

Continuing studies on work tolerance in patients with coronary heart disease cardiovascular responses to walking at low ambient temperature were followed up. The data demonstrated considerable impairment of the patients' working ability under these conditions as compared to the results of a standard exercise test, or walking with similar speed at high ambient temperature.

It was also shown that consumption of a single dose of alcohol (1 ml of 40% ethyl alcohol/kg⁻¹) before the standard exercise test eliminates, in majority of cases, subjective symptoms of myocardial ischemia (pain) without any favorable effects on ECG indices of ischemia.

4. Extracellular electrolytes in the kidney

The original method developed in this laboratory enabled assessment of electrolyte concentration in the interstitium of an in situ kidney from measure-

ments of tissue electrical admittance. Such measurements were so far performed in large animals (dog and rabbit). At present, the technique was adapted for use in the rat. By miniaturizing the electrodes and reducing their number to three, the tissue damage secondary to their insertion into the kidney was minimized. The electrode set-up used in the rat enables measurements of tissue admittance across the outer and inner medulla separately, as well as across the whole renal medulla.

Electrode placement did not significantly reduce the renal blood flow and had a variable but minor effect on the glomerular filtration rate. Stable admittance records over 2—3 hours were noted. A typical response to intravenous injection of furosemide, a loop diuretic, consisted of a rapid drop in admittance, indicating blockade of NaCl delivery from loops to the interstitium and a decrease in interstitial electrolyte concentration.

The importance of performing admittance measurements in the rat stems from the fact that this species is most commonly used for experimental studies of renal function.

See the LIST OF PUBLICATIONS:

9, 10, 22, 36, 46, 56, 77, 78, 79, 85, 86, 90, 91, 92, 93, 94, 103, 110, 130, 131, 132, 133, 170, 171, 172, 173, 193, 202, 203, 208, 209, 210.

Cardiovascular Laboratory

Head: Assoc. prof. Krystyna Cedro-Ceremużyńska

Several lines of investigations were continued throughout this year: 1) Studies carried out on the hypertrophic rat's heart have shown that propranolol exerts an inhibitory effect upon protein synthesis *in vitro*. The obtained results suggest that this effect, if present *in vivo*, might contribute to therapeutic effectiveness of this drug in hypertrophic cardiomyopathy; 2) Studies on the metabolism of inosine in the human heart have been continued by infusing inosine into the patients undergoing heart catheterisation for diagnostic purposes. Inosine metabolites in arterial and coronary sinus blood were determined by HPLC. This study, performed in collaboration with Erasmus University in Rotterdam, has just been completed and the results are being analysed; 3) It has been found that prostacyclin attenuates peroxidation of myocardial lipids which is significantly augmented by an excess of catecholamines. These results suggest that attenuation of an oxidative damage of the membranes might be the component of cytoprotective action of prostacyclin; 4) Prostacyclin was found to exert a cytoprotective effect upon cultured cerebral tissue damaged by anoxia. This property is not shared by prostacyclin analogue, Iloprost. 5) The mechanism of stress-induced myocardial damage was studied in conscious pigs exposed to immobilization stress. Enhancement of lipid peroxidation of myocardial membranes was concomittant with a decrease in mito-

chondrial ATP and ultrastructural damage. It is suggested that enhancement of peroxidative processes in the myocardial membranes might contribute to the mechanism of stress-induced myocardial injury.

See the LIST OF PUBLICATIONS:

21, 61, 62, 63, 87, 106, 156.

Laboratory of Experimental Surgery

Head: Assoc. prof. Jerzy Borkowski

1. An impact of transcutaneous nerve stimulation (TNS) on wound healing velocity.

The aim of this study was to assess whether TNS affects healing velocity of uncomplicated skin wounds. For estimation of wound healing process an impedance method was used — i.e. measurement of electric impedance of tissues for alternating current at selected frequencies 100 and 1000 Hz. As a measure of wound healing the percentage ratio of tissue resistance (MI) values in consecutive days of experiments was used.

The experiments were carried out on Wistar rats with uncomplicated skin wounds. Animals were stimulated once a day after producing a wound with the sharped current (100 Hz, duration of a single impulse 1 msec, energy 0,1—0,2 J). An acceleration of healing process (by 21%) was found in comparison with the control group (without TNS).

2. An assesement of efficacy of TNS in clinical and experimental studies based on methods applying radioisotopes as well as on the measurements of skin resistance.

The purpose of this study was to estimate an impact of TNS on velocity of washing out J^{125} from the stimulated place i.e. assesement of TNS effect on blood flow velocity in the examined skin area. Studies were carried out on 20 Wistar rats stimulated for 40 minutes by rectangular current (frequency 100 Hz, duration of a single impulse 1 msec, energy 0,1—0,2 J). Radioactivity of skin was measured by the scyntylation counter every 10 minutes till 90% of isotope was washed out.

The determinations were carried out before, during and 1 hour after TNS — every 1 hour till the initial values were achieved. It was found that TNS causes washing out of J^{125} from the tissue 40% faster than in the control group (without TNS). Duration of the single impulse effect persisted for 3—6 hours. In the next series of investigations the measurements of skin resistance were made to evaluate an impact of TNS on skin blood flow.

Experiments were carried out on 20 Wistar rats. TNS was applied identically as in above described experiments with J^{125} . Tissue resistance for alternating current i.e. the impedance absolute value (MI) and phase value (AI) was mea-

sured before TNS, immediately after TNS cessation and then every 0,5 h till the initial values were obtained.

Percentage ratios of the values obtained at selected frequencies 100 and 1000 Hz were compared. Immediately after TNS a decrease in both MI and AI occurred, accompanied by the displacement of maximum AI to the higher frequencies. Values of MI and AI at succeeding measurements were returning to initial values within 3 h. Thus, the effectiveness of TNS for approx. 3 h of the treatment was proved by two methods.

3. A possible role of TNS in the therapy of pes diabeticum.

An impact of a single TNS on muscular blood flow through the ischemic area was examined. The isotope measurements of muscular blood perfusion were carried out in a group of 10 patients, using the intraarterial injection of labelled ^{99m}Tc microspheres. After control measurements performed to assess features of pathologic lesions, the study was repeated in few days following TNS.

A comparison of the scyntygraphic images obtained immediately after TNS with the control ones indicates an improvement of blood flow into the ischemic area after the applied stimulation. However further studies in which TNS was applied for a period of 3 weeks — 3 months failed to confirm an effect of TNS on healing of diabetic ulcers. Cessation of conventional surgical treatment was found to increase the existing pathological lesion area.

See the LIST OF PUBLICATIONS:

70, 71, 72, 73, 99, 100, 101, 102.

STUDIES ON THE STRUCTURE AND BIOLOGICAL PROPERTIES OF THE NERVOUS TISSUE

Department of Neuropathology

Head: Prof. Mirosław Mossakowski

PATHOMECHANISM OF HEPATOGENIC ENCEPHALOPATHY (HE) IN THE THIOACETAMIDE MODEL

Plasma and brain levels of arginine and its metabolites in the arginine — glutamate (— GABA) pathway, and the activities of two brain enzymes of this pathway: arginase and ornithine aminotransferase, were measured in rats in the metabolic and precomatous phase of HE. Plasma arginine decreased in the metabolic phase and increased in the precomatous phase, whereas ornithine and glutamate increased and urea decreased in both phases. Brain amino acid levels remained unchanged throughout HE, whereas both brain enzymes showed a gradual increase in their activities. The results are indicative of the in-

creased involvement of a certain pool of arginine as a precursor of amino acid neurotransmitters: glutamate and GABA. Studies on astrocytes derived from HE rats revealed a decrease of oxygen consumption in these cells in advanced HE, related to substrates deficit rather than intrinsic damage of the cell oxidation systems. Changes in substrates availability appear to be related to fluctuations in the pyruvate carboxylase activity — the key CO₂ fixing enzyme. Astrocytes in early HE are metabolically activated, which is manifested by increased potassium — stimulated inward calcium transport and enhanced GABA release. The latter process is understood to counteract an excess accumulation of this inhibitory neurotransmitter under conditions of decreased GABA transamination. The above described changes coincided with the decreased cerebral blood flow, both in the early and advanced HE. However, only the advanced stage was characterized by decreased cerebral oxygen consumption.

EFFECT OF SERA FROM PATIENTS WITH HEPOTOLENTICULAR DEGENERATION TREATED WITH D-PENICILLAMINE, ON THE MORPHOLOGICAL AND IMMUNOMORPHOLOGICAL PICTURE OF WILSONIAN GLIOPATHY IN ORGANOTYPIC NERVOUS TISSUE CULTURE

Despite a decreased copper level, the sera produced typical, though intensive glial abnormalities. The character and intensity of gliopathic changes were related to the ceruloplasmin activity in serum, which is known to determine the free to bound copper ratio. The altered glial cells preserved the antigenic properties of normal astrocytes. Studies on the autopsy material from Wilson's disease confirmed this conclusion: All abnormal forms of astrocytes, typical of this disease, revealed the presence of GFAP and S-100 protein.

PATHOMECHANISM OF ISCHEMIC DAMAGE OF THE CENTRAL NERVOUS SYSTEM

Complete cerebral ischemia was shown to produce severe structural damage in the cerebral cortex basal ganglia and medulla oblongata, progressing despite the recovery and normalization of bioelectric activity of the brain. The damage was characterized by degeneration of nerve cells and cerebral edema. Noteworthy were ultrastructural abnormalities of neuronal and astrocytic nuclei, suggesting disturbed intracellular electrolyte balance. Administration of PGI₂ during complete ischemia attenuated cerebral edema and damage of cytoplasmic structures in cortical motor neurons, but remained without any effect on the nuclear — both nucleoplasmic and nucleoskeletal — changes. PGI₂ was not effective towards the adrenocortical cell nuclei, which undergo changes in complete cerebral ischemia. However, this prostacyclin counteracted the late loss of CA₁ neurons following a short term cerebral ischemia in Mongolian gerbil. The dependence of the preventive activity on the moment of administration suggested a concerned vasoactive and cytoprotective mechanism

of action. The same pattern was observed with respect to indomethacin. A hypothesis has been put forward relating the molecular mechanism of action of the two compounds to the inhibition of calcium influx into the cells during postischemic neural excitation. A synthetic prostacyclin analogue ZK 36374 failed to exert a cytoprotective activity, a conclusion confirmed in studies on the organotypic nervous tissue culture subjected to anoxia. Early and late structural brain damage in the experimental model of clinical death in the rat, produced by noninvasive cardiac arrest, was analyzed in relation to other models. The analysis confirmed the utility of the present model for studying the pathomechanism of postischemic encephalopathy, the major advantage being the long-term survival of the animals.

STUDIES ON THE EXTRAPYRAMIDAL SYNDROME PRODUCED BY COMBINATION OF TREATMENT WITH COBALT SALTS AND ISCHEMIA

An imbalance between the dopaminergic and GABA-ergic system was observed in various CNS structures. An increase of DA to HVA ratio is considered to indicate an inhibition of DA catabolism. Histofluorescence studies revealed a decrease of serotonin in the white matter. Morphological studies demonstrated topographic differences with respect to structural damage, with prevalence to white matter, in which edematous changes were confirmed by quantitative analysis.

STUDIES ON THE DEVELOPMENT AND MORPHOLOGICAL PROPERTIES OF SUBSTANTIA NIGRA IN TISSUE CULTURE

Structural differences between the organotypic and dissociated cell culture were emphasized. Histofluorescence studies revealed presence of dopamine in substantia nigra neurons in both models in organotypic culture, morphological differentiation into two cytological types as well as intranigral synaptic functions were found to be maintained. These morphological features render the cultures useful for model studies on selective dopaminergic neurotoxin. Administration of sera from patients with Parkinson's syndrome to the organotypic cultures produces non-specific degeneration of both types of neurons and disappearance of dopamine and is accompanied by moderate glial reaction. The absence of Levy's bodies in substantia nigra neurons points to the nonspecificity of this reaction.

STUDIES ON THE PERINEOPLASTIC PATHOLOGY OF THE CENTRAL NERVOUS SYSTEM

The growth of chemically-induced spinal cord gliomas, mostly oligodendrogliomas, does not impair myelin sheaths beyond the region of perineoplastic edema. Electron microscopic examination of the vessels revealed ultrastructu-

ral abnormalities of all the elements of vascular walls, suggesting retarded maturation and disturbed permeability. The maturation disturbances of the capillaries may be related to out of balance gliovascular interaction due to tissue edema.

IMMUNOPATHOLOGICAL AND IMMUNOMORPHOLOGICAL STUDIES IN DIFFERENT NERVOUS TISSUE CULTURES

Immune serum containing antibodies against specific glial antigen selectively damaged oligodendrocytes, the myelin sheaths being the major target. Neither neurons nor astrocytes were affected. In other studies, pituicytes showed negative immunostaining for glutamine synthetase, which is in a sharp contrast to other glial populations. This difference may be of biological significance.

See the LIST OF PUBLICATIONS:

1, 2, 3, 4, 65, 88, 89, 96, 97, 98, 111, 121, 125, 126, 127, 128, 129, 138, 139, 140, 141, 143, 154, 155, 157, 164, 175, 189, 190, 191, 192, 194, 197, 198, 207.

Department of Neurochemistry

Head: Assoc. prof. Jerzy Łazarewicz

THE ROLE OF MEMBRANE LIPID AND PROTEIN DISTURBANCES, RELATED TO SYNAPTIC DISFUNCTION, IN THE PATHOGENESIS OF CNS NEURONAL INJURY

In 1985 studies were carried out in two closely related fields and concerned metabolism of biologically active lipids and proteins of brain membranes and the function of synaptic endings isolated as synaptosomes.

The studies indicated that brain ischemia induced degradation of phosphatidylinositol (Poly-PI) mainly triphosphatidylinositol, exclusively located in synaptosomes. The accumulation of inositol phosphate metabolites suggested the activation of not only phospholipase C but also phospholipase A and lysophospholipases. The disturbance in Poly-PI were connected with the liberation of two second messengers as IP_3 and diacylglycerol (DG). Metabolism of DG by the route of diacylglycerol kinase was suppressed in synaptosomes isolated from ischemic brain. It seems that it can be actively metabolised by DG-lipase.

Further investigations on the pathomechanism of myelin disturbances in the neurological mutant „pt“ rabbit showed the reduction of myelin membrane by about 75% with the concomitantly higher amount of premyelin membrane. Experiments with labelled glucose indicated an inhibition of the radioactivity flow to myelin lipid and protein at the level of premyelin membrane.

The specific activity of myelin marker enzyme — CNP-ase was not changed in „pt“ rabbit but some changes in properties of the enzyme was observed. The organic compound of Hg^{2+} , depressed CNP-ase activity and this inhibition was reversed by liponic acid. Cholinergic denervation after septal lesion in the rat affected the content, composition and metabolism of gangliosides in hippocampus.

Studies on the participation of presynaptic elements in the pathology of neurons evoked by hyperoxia and application of excitotoxin N-methyl-aspartate (NMA) were carried out. Hyperoxia in vivo produced the free radical oxidation of lipid component of actomyosine — like protein from brain synaptosomes. In these conditions the lower content of protein -SH group and inactivation of Ca^{2+} Mg^{2+} ATP-ase were found.

Moreover, hyperoxia decreased a superprecipitation which is an in vitro model of physiological concentration of acto-myosin like protein.

N-methyl-aspartate given into hippocampus produced an elevation of taurine and phosphoethanolamine concentrations in extracellular space. This receptor mediated effect depends on the presence of calcium ions and does not seem to be localised in the presynaptic part of nerve terminals.

See the LIST OF PUBLICATIONS:

6, 31, 32, 33, 45, 104, 105, 107, 109, 113, 114, 115, 152, 176, 177, 178, 179, 180, 195, 199, 200, 206.

Department of Neurosurgery

Head: Prof. dr Eugeniusz Mempel

BIOELECTRICAL BRAIN ACTIVITY AND MEMORY FUNCTION AFTER THE LESION OR ELECTRICAL STIMULATION OF THE SELECTED BRAIN STRUCTURES

1. The effect of Diazepam on the somatosensory evoked potentials (SEP).

The effect of Diazepam on SEP was studied in 15 patients with extrapyramidal disorders treated with stereotaxic lesions of the thalamic nuclei VL and Vim. The Diazepam application allowed to eliminate the artefacts caused by the increased muscular tension. The preoperative cortical SEP after the Diazepam injection (i.v. 10 ml of Relanium) showed the unchanged short-latency components (specific) of the potential, however the later components showed the decreased amplitude, often without the amplitude N63.

The intraoperative thalamic SEP showed a decreased amplitude with almost the flat curve including the disappearance of the specific component of the potential.

The postoperative contralateral cortical SEP in relation to the operated hemisphere were significantly changed, especially the amplitude was diminis-

Laboratory of Ultrastructure of the Nervous System

Head: Prof. Jerzy Borowicz

STUDIES OF THE INFLUENCE OF REPEATED HYPERTHERMIA ON THE ULTRASTRUCTURE OF THE HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM IN RABBITS

In previous studies it was revealed that 3 h exposure to heat (38—39°C) evokes activation of the neurosecretory system and increase in body temperature that does not return to the normal state for 24 hours after hyperthermia. In the final stage of these studies changes occurring after the hyperthermic-shock repeated three times daily were studied. The majority of rabbits bear with changes badly, some of them died, mostly on the third day of hyperthermia. Basic ultrastructural changes consisted of activation of the secretory neurons of the nucleus supraopticus and nucleus paraventricularis as well as increased neurosecretion in the hypophyseal neural lobe. In addition, in the neural lobe degenerative changes were observed both in axonal fibers and pituicytes (most pronounced after the third exposure to hyperthermia.). Thus hyperthermia repeated several times in 24 hours causes prolonged thermal stress leading simultaneously to several degenerative changes in the neurohemal organ.

See the LIST OF PUBLICATIONS:

37, 112, 151, 181, 182, 183.

STUDIES ON TRANSPLANTATION AND EXPERIMENTAL SURGERY

Department of Experimental Surgery and Transplantation

Head: Prof. Waldemar Olszewski

REGULATION OF THE IMMUNE PROTEIN TRANSPORT BLOOD-LYMPH

The parameters of active lymph flow in afferent lymph vessels of leg were examined in 9 healthy volunteers, aged 18—22 years. The purpose of these studies was to examine whether the initial changes following a rapid increase

in the venous pressure, as a decrease of lymph flow, amplitude and frequency of lymphatic pulse wave, subside after a prolonged venous stasis (4 h). The other purpose was to elucidate whether the prolonged venous stasis are followed by an increased capillary filtration rate which was not noted in 2 h — lasting observations. It was found that: a) an elevation of the venous pressure by 50 mmHg was followed during the first 30 min by an increase of the mean lymph pressure by 8—18 mmHg and after about 2 h a decrease to normal values (0—5 mmHg) occurred; b) after 2 h of venous stasis a decrease of the amplitude and lymphatic pulse wave frequency, noted during the first 30 min of venous stasis, was not observed; c) an increase of lymph flow and output appeared after 2 h of venous stasis. The decrease of lymph transport in the acute venous hypertension may be caused by a drop in lymph production subsequent to the decrease of capillary filtration rate. After 2 h of increased venous pressure the vasoconstrictive reaction in the arterial and venous system seems to subside. The protein content of lymph during 2—4 h of the venous stasis differed from that observed after 1 h. Lymph serum ratio decreased gradually for proteins with the low molecular weight (α_1 -acid glycoprotein, albumine), and slightly increased, particularly after 4 h for proteins with high molecular weight (β_1 -macroglobulin, IgM). These observations suggest that a prolonged acute venous stasis leads to increased capillary permeability.

Preliminary studies on the influence of anesthetics on transport of humoral and cellular factors from blood to lymph were carried out in 6 healthy volunteers. It was found that an injection of xylocaine causes an accumulation of erythrocytes and granulocytes in lymph which was most probably due to mechanical damage of skin and subcutaneous tissue by injection of fluid under pressure. Granulocytes and monocytes from xylocaine treated tissue were not able to adhere to plastic surface probably due to a direct effect of the anesthetic on the cell membrane. Formation of spontaneous rosettes between Langerhans cells and lymphocytes was not observed which points to changes in lymphocyte and macrophage membranes.

Penetration of antibiotics — temocillin and BRL 36650 penicillin to peripheral lymph was examined in healthy volunteers. Temocillin concentration in lymph was always lower than in serum with the half-life of 4.4 h in lymph and 4.9 in serum.

The ratio of area under the concentration curve in lymph and serum was 0.52. The high ability of temocillin to bind to proteins (85%) caused a slow increase in its concentration in lymph.

BRL 36650 penicillin was injected in doses 1 g and 4 g. The half-time of the elimination from lymph was 1.6 and 1.7 h respectively, whereas that from serum was 1.2 and 1.6 h, depending on the dose. The ratio of the areas under the concentration curve in lymph and serum was 0.71 and 0.64, respectively. An increase of administered dose did not bring about the expected faster equilibration rate of the concentrations between intra- and extravascular compartments.

Spontaneous migration of lymphocytes through the lymphoid tissues

The circulating dendritic cells were characterized in peripheral human lymph and blood. It was found that blood contained about 0.5% OKT 6 positive cells. The proliferative response of blood lymphocytes in MLC was higher when lymph cells, enriched in OKT6⁺ cells, were used as stimulators, compared to blood monocytes. Radiation did not influence the stimulatory properties of OKT6⁺ lymph cells. Local hyperthermia of tissues from which lymph cells were collected increased the stimulatory properties of OKT6⁺ cells. Similarly, MLC reaction was higher when performed in culture at 39°C than 37°C. Blocking of T6 and M1 receptors on Langerhans cells did not decrease their stimulatory properties in MLC, however it attenuated the response of autologous lymphocytes to PHA.

An optimal time of cytotoxic test for effector liver cells towards K562 and YAC cell line was established. In 1 h and 2 h tests the cytotoxic effect was not observed, while after 4 h of incubation it was 4% for K562 and 18% for YAC1 cells. After 18 h of incubation the percentage of cytotoxicity was 29% for K562 and 61% for YAC1. The study showed that for the K562 cells the 18 h test seems to be optimal while YAC1 cells tested for 18 h revealed a high spontaneous lysis (55%) which makes the test unreliable.

A protocol of stimulation of cytotoxic cells from rat liver and blood in culture was established. Two stimulators were used: IL-2 supernatant of MLA 144 cell line and OK432 — attenuated *Streptococcus pyogenes* strain. A drop by 50% of the number of effector cells in 20 h cultures stimulated and nonstimulated was noted. There were no differences between cytotoxicity of stimulated and nonstimulated cells in 18 h test with K562, either from blood or from liver. However, the presence of IL2 in the 18 h test caused an increase of natural cytotoxicity. It was also found that liver cells, besides NK (natural killer) contained NC (natural cytotoxic) cell subpopulation, which lysed the WEHI 164 cells.

Prolongation of allograft survival time

Further characterization of cell population in donor blood transfused to recipient, responsible for prolongation of graft survival time was performed. Rat recipients (Wistar-AgB₅) of heterotopic heart graft received 1 ml of whole donor (August-AgB₂) blood on days -21, -14 and -7 before grafting. The mean survival time in untreated rats was 6.9±0.3 days, while after 1 transfusion 7 days before grafting it increased to 13.2±5.1 days. After 2 transfusions (-14, -7 days) it was 16.6±8.5 days while after 3 transfusions (-21, -14, -7 days) 14±0 days. It was found that the level of nonspecific spontaneous suppression increased with the number of transfusions and it was 3%, 30% and 43%, respectively. The level of autosuppression was 36% and 5% in rats after 2 and 3 transfusions. The specific suppressor cell activity was not observed in MLC; in con-

trast, a stimulatory effect was noted. In rats after 1, 2 and 3 transfusions 27%, 17% and 9% of stimulation in MLC was noted.

The appearance of Ia antigens in rat recipients (LEW-RT1^l) of orthotopic graft of left lung lobe donor (BN-RT1ⁿ) was examined. In control rats there were no Ia antigens on the surface of bronchial epithelium and vascular endothelium. After grafting appearance of Ia antigens on the surface of bronchial epithelium was observed. On day 4 and 5 all epithelium was Ia⁺. Labelling with ER13 and OX6 antibodies specific for RT1.D and RT1.B regions, respectively, showed that those antigens were expressed on the surface of the bronchial epithelium. With the use of HIS 19 antibody specific for Ia antigen of LEW strain, it could be shown that the newly expressed Ia antigens were of donor type (HIS 19⁺). In the group of rats treated with CsA, on day 5 after grafting a lower level of Ia expression on the surface of bronchial epithelium was observed. The intensity of labelling with ER13 and OX6 suggested a significant reduction of the expression of Ia from RT1.B region compared to RT1.D. In rats treated with CsA 100 days after grafting a stronger expression of Ia was observed than on day 5, however, significantly lower than in untreated rats. Rats treated with 2 doses of CsA showed a 30% drop in the perfusion of lung lobe 100 days after grafting.

In experiments on the influence of operative trauma on the immune response of T cells to autologous non T-cells (monocytes, lymphocytes B, „null“ cells) — the so called AMLR (autologous mixed lymphocytes reaction) was examined. The study was performed in 9 patients undergoing cholecystectomy. The preoperative level of the responsiveness in AMLR was 3882 ± 2644 cpm in a 6 days and 6098 ± 3506 cpm in a 7 days culture ($p < 0.05$). After the operation a significant decrease of the response on day 1 was observed, to 1168 ± 1360 cpm ($p < 0.05$) and 1960 ± 1738 cpm ($p < 0.01$), respectively. In a 7 days culture the response was below the preoperative values on the 3rd and 5th postoperative day, 2394 ± 2026 cpm ($p < 0.02$) and 3343 ± 2802 cpm ($p < 0.05$), respectively. The recovery was observed on day 7.

In experiments on activation of circulating cells after surgery the level of OKT6⁺, OKT9⁺, and OKT10⁺ cells was determined in 9 patients after cholecystectomy. A preoperative level of OKT6⁺ cells in PBM cells of patients was $1.62 \pm 2.21\%$, OKT9⁺ — $1.25 \pm 1.67\%$, OKT10⁺ — $1.62 \pm 1.92\%$. After the operation an increase of these cells was observed. On the 5th postoperative day it was $2.94 \pm 2.7\%$, $3.12 \pm 1.73\%$ and $4.57 \pm 2.7\%$, respectively. On day 7th it was still above the preoperative level.

Influence of acute and prolonged hyperthermia on humoral and cellular immunity

It was shown that local hyperthermia of leg for 2—3 h in water bath at 44°C, causes an increase in a) the mitogenic response of lymphocytes from hyperthermic lymph and b) the stimulatory properties of hyperthermic lymphocytes towards allogenic blood lymphocytes. An increase in the number of

macrophage-like cells, from 5% to 10—12% was observed. These results suggest that the increase of stimulatory and responsive properties of lymph cells from hyperthermic skin are connected with the increase of the number of macrophage-like cells.

See the LIST OF PUBLICATIONS:

5, 7, 8, 38, 53, 54, 57, 58, 59, 60, 117, 118, 134, 135, 136, 137, 163, 165, 166, 174, 196.

OTHER RESEARCH WORKS

Mental Health Department

Head: dr Zygryd Juczyński

1. Psychophysiological and social determinants of life activity after myocardial infarction.

The achievement of pre-sickness activity is considered as a measure of cardiological rehabilitation's effectiveness. The subjects of the study were 100 men, aged 30—55 years, after first, uncomplicated heart infarct. The aim of the study was to establish factors determining and prognosticating the extent of changes in life activity after the myocardial infarction.

One year after the infarction thirty percent of the patients showed a substantial decrease of thier life-activity (family, professional, sociable, cultural and physical). Using the „ex post facto” model of inquiry 9 principal factors were identified as well as 8 secondary factors indicating activity changes after the infarction. Furthermore, relying on the „regressiqn-correlation” model 8 predicting factors were established. The following variables appeared to be predictors: 1. the way of infarction occurrence; 2. the kind of professional activity; 3. the feeling of satisfaction with life; 4. health status before the infarction; 5. subjective prognosis concerning health status; 6. indicator of the purpose in the life; 7. pattern of physiological reaction and 8. factor „J” of type A behavior pattern.

2. Follow-up study of alcohol-dependent men.

Based on the follow-up study of 40 year-old male alcoholics, two main groups of subjects were separated. Those included to the first group showed remission of alcohol related problems, subjects of the second group have still abused alcohol. The latter group could be divided into the following sub-groups: 1. patients with criminal behavior and frequent imprisonment; 2. patients with alcohol-related personality and behavior disorders, who also drink various chemicals containing ethyl alcohol.

3. Social non-adjustment: biological and psychosocial determinants.

The subjects of the study were 845 unemployed adults and two comparative groups: first group — all employed, second — receiving treatment on account of alcohol dependence syndrom.

Twenty three unemployed men were treated in the Psychiatric and Alcoholism Treatment Centre. Two thirds of the unemployed abused alcohol and 3/4 of them manifested the alcohol dependence syndrom. Above half of them have committed various delinquencies. Evident health disorders, limiting work ability were found in 14% of cases, slight — in 28%, minimal in 47% of cases. No relation was found between environmental factors of earlier development and present relation towards employment. Determinants, differentiating the groups have been related to the attitude toward life and manifested values.

See the LIST OF PUBLICATIONS:

74, 75, 76, 95, 150, 204, 205.

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29—30 March 1985, Genova, Italy

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Olszewski W., Grochowicz P., Grzelak J.

Satellite Symp. of the Tenth Intern. Meeting of the Intern.

Soc. for Neurochemistry „Biochemistry of Glial Cells“

12—15 May 1985, Liege, Belgium

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X Intern. Meeting of The Internat. Soc. for Neurochemistry

20—24 May 1985, Riva del Garda, Italy

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IV Intern. Symp. of Intracranial Pressure (ICP and Mechanisms of Brain Damage)

9—13 June 1985, Glasgow, U.K.

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V Intern. Symp. of the Med. Sci. Soc. „Motor Control 85“

10—15 June 1985, Varna, Bulgaria

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„Biochemistry of Exercise“ 6th Intern. Symp.

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XIX Annual Congr. of The Europ. Soc. for Clin. Respiratory Physiology „The Lung in growth and ageing“

24—28 June 1985, Hague, Netherlands

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Second Nordic Neurochemistry Meeting
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26—28 August 1985, Zalegerszeg, Hungary
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X Intern. Congr. of Lymphology
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The Neurobiology of the Control of Breathing
4—6 September 1985, Stockholm, Sweden
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ISHR, VI Congress Intern. Soc. for Heart Research of the Europ. Section
8—11 September 1985, Stockholm, Sweden
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7th European Immunology Meeting
8—13 September 1985, Jerusalem, Israel
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Adriatic Congr. of Breathing Regulation
14—21 September 1985, Dubrownik, Jugoslavia
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INSERM Intern. Symp. and NATO Workshop
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14—18 October 1985, Strasbourg, France
Strosznajder J.

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16—18 October, 1985, Brandenburg, DDR
Albrecht J., Dąmbska M.

XVIII Danube Symp. for Neuropathology

16—20 October 1985, Innsbruck, Austria

Kroh H., Maślińska D., Mossakowski M., Renkawek K., Śmiałek M., Weinrauder-Semkow H., Zelman I.

„Surgical Treatment of Epilepsy“

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Mempel E.

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