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Editor:
W. Dziedzic

Scientific Consultant:
H. Kaciuba-Uściłko, Professor of Physiology

Polish Academy of Sciences
Medical Research Centre
3 Dworkowa Str., 00-784 Warsaw - Poland
Telephones: 49 64 93, 49 69 73
Fax: 48-22 49 69 73

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European Journal of Lymphology (Europe)
Flebolinfologia Italiana (Italy)
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E. Orlewska, M.D., Ph.D.
Member of Polish Immunological Society

D. Sadowska-Szablisty, M.Sc.
Member of Polish Immunological Society

G. Szczęsny, M.D.

C. Szczęśniak, M.D.

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J, Kiljański, M.D.

D. Kosson, M.D.

A. Lipkowski, M.Chem., Ph.D., D.Sc., Assoc. Prof. of Endocrinology

Research Follow. Department of Anesthesia, Massachusetts General Hospital, Harvard Medical School, Boston, USA

Member of: International Association for Study of Pain
European Peptide Society
American Chemical Society
American Peptide Society
Polish Chemical Society
Polish Biochemical Society
Polish Pharmacological Society

Member of Editorial Boards of:
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Member of: European Society for Clinical Investigation
European Thyroid Association

M. Wrocławska, M.D.

RESEARCH REPORT

DEPARTMENT OF NEUROPHYSIOLOGY
Head: Assoc. Professor Mieczysław Pokorski
3 Dworkowa Str., 00-784 Warsaw
Telephone: 49 58 29

ROLE OF NEUROACTIVE SUBSTANCES AND PERIPHERAL CHEMIRECEPTORS IN SHAPING THE RESPIRATORY PATTERN

Assoc. Prof. Mieczysław Pokorski

The effect on respiration and the hypoxic respiratory responses of a phospholipase C blocker, phenylmethylsulfonyl fluoride (PMSF) were studied in anesthetized cats. PMSF was administered intravenously, intraperitoneally, or as pledgets laid straight on the carotid bodies. Chronic experiments were also done in which respiration was studied 7 days after intraperitoneal injection of PMSF. It was found that antagonism of phospholipase C that leads to the inhibition of generation of lipid-derived second messengers in the cell dampens the stimulatory ventilatory response to hypoxia. This response was entirely abolished in the chronic experiments. Electron microscopic studies of the carotid body ultrastructure showed advanced pathological changes and structural damage of the cells. These changes support the role of the phosphoinositide cascade in signal transduction in the carotid body (Pokorski and Strosznajder).

Concerning other neuroactive substances, the mechanisms of the inhibitory effects of a benzodiazepine antagonist, flumazenil, were studied in the cat. The preliminary results indicate that these inhibitory effects may be mediated by the cholinergic system, as atropine counteracts the respiratory depression (Pokorski).

In the other experimental series we have measured the respiratory resistance of the larynx in response to an intravenous administration of serotonin (5-hydroxytryptamine) in anaesthetized cats. It was shown that the prompt post-serotonin apnoea coincided with almost five-fold increase in the expiratory laryngeal resistance. In the resumed breathing that followed apnoea the inspiratory and expiratory laryngeal resistance increased by 30% and 70%, respectively. This response was independent of the laryngeal afferents.

The constriction of the larynx during the expiratory arrest of breathing was paralleled by the appearance of the expiratory activity in the superior laryngeal nerve (SLN). Its magnitude depended on preserved contralateral laryngeal afferentation.

In post-apnoeic breathing the respiratory activity of the superior laryngeal nerve reverted to its inspiratory/expiratory pattern and remained significantly augmented in cats with one SLN spared (Szereda-Przestaszewska and Wypych).

ANATOMICAL CONNECTIONS AND THE ROLE OF SOME RESPIRATION RELATED NEURONAL GROUPS IN MODIFICATION OF THE RESPIRATORY PATTERN; THE MECHANISMS OF THE CONTROL OF THE RESPIRATORY ACCESSORY MUSCLES

Dr. Krystyna Budzińska

Studies on rabbits concerning the pathogenesis of respiratory arrest in obstructive sleep apnea syndrome (OSAS) have shown disproportional activity of the phrenic and hypoglossal nerves that could be associated with the upper airway obstruction. The disproportion was increased when either the rate or volume of artificial ventilation were changed. Moreover, hypoxia enhanced disproportional changes in both nerves whereas ventilation with oxygen-enriched air diminished them. Preliminary clinical studies indicate that patients with OSAS breathing with oxygen-enriched air have shown a decreased sleep apnoea index. The longest apnoea episodes were eliminated. An increase in oxygen saturation and stabilization of the cardiovascular system were observed. Clinical studies will be continued (Jernajczyk).

The hypothesis was verified that changes in excitation of baroreceptors are responsible for disproportions between phrenic and hypoglossal nerve activity observed during blood pressure fluctuations. It was found that selective activation of baroreceptors by lowering air pressure around the rabbits neck diminishes the amplitude of the hypoglossal nerve disproportionally more than the activity of the phrenic nerve. The response was similar to the observed during a rise of systemic pressure and was eliminated after baroreceptor denervation. These findings confirm that changes in systemic pressure *via* baroreflex may lead to disproportions between the activity of the hypoglossal and phrenic nerves. Applying the bilateral carotid baro- and chemoreceptor denervation in this series of experiments we have compared hypoxic depression of the hypoglossal and phrenic nerves. It was found that after peripheral chemodenervation moderate hypoxia caused a three fold greated suppression of the hypoglossal nerve amplitude than that of phrenic nerve (Janczewski).

Previous electrophysiological studies suggested the role of the motor nucleus of the trigeminal nerve (nVmt) in regulation of respiration. We have looked, therefore, for the anatomical connection of the nVmt with phrenic motoneurones and/or with medullary structures where respiratory premotoneurones are present. A fluorescent

tracer - Fluoro-Ruby was injected to the phrenic nucleus at C_4-C_6 spinal segments. The retrograde transport of the tracer to the region of nVmt was not found in five of the experiments. In one case a faint projection in the nVmt was found when the tracer was applied to the C_4 segment. Injection of the Fluoro-Ruby into nVmt revealed diffuse connections with the medullary and pontine reticular formation. However, the connections within the region of phrenic motoneurones and medullary premotoneurones were not encountered. This study will be continued (Budzińska).

The investigations were undertaken to evaluate whether nitric oxide (NO), involvd in neural transmission, modulates the Hering-Breuer reflex and takes part in the phenomenon of short-term memory in the respiratory system. The results demonstrate that systemic application of the inhibitors of nitric oxide synthase (NOS) or the substrate for the nitric oxide synthesis cause changes in the respiratory amplitude and timing. The magnitude of the Hering-Breuer reflex remained unchanged in comparison with control conditions. The mechanism of short-term memory in the respiratory system activated by pontine and vagus nerve stimulation does not involve nitric oxide. Studies on the other experimental model of short-term memory in the respiratory system will be performed.

Since NOS was found in the region of the medulla where the terminals of afferent fibres of baroreceptors are localized, the role of NO in baroreflex was studied. Changes in the responses to the investigated stimuli before and after inhibition of NOS indicate that NO takes part in the modulation of the baroreceptor reflex (Budzińska and Wojtal).

FURTHER CHARACTERIZATION OF IMMUNOSUPPRESSIVE AND CYTOTOXIC PROPERTIES; APPLICABILITY OF THE DRUG IN NEUROTRANSPLANTATION AND IN TREATMENT OF CNS DISEASES

Assoc. Prof. Pawel Grieb

Two clinical trials with 2-CDA (cladribine, 2-chloro-2'-deoxyadenosine) in remitting-relapsing multiple sclerosis are in progress. The analysis of data from an open pilot trial with 11 patients showed that the drug given subcutaneously, 5 mg daily for 5 consecutive days, repeated monthly up to 6 treatments, is well tolerated and produces clinically insignificant side effects (mild marrow depression). Preliminary analysis of data collected during the first year of double-blind, randomized, placebo-controlled trial with 85 patients suggested that such a treatment produces some improvement of the neurological status (quantitated in the EDSS scale) and decreases the frequency of relapses.

SIGNAL TRANSDUCTION IN THE CAROTID BODY CHEMORECEPTORS

Assoc. Prof. Mieczysław Pokorski
Supported by the State Committee for Scientific Research: grant # 4 0295 91 01

The results of this project showed that the phosphoinositide cascade has a part in the molecular mechanisms of chemotransduction in the cat carotid body. Chemoexcitation by carotid body stimuli causes activation of phospholipase C. This enzyme degrades phosphatidylinositols, which leads to generation of lipid-derived second messengers that initiate the cellular responses. In this study the basic factors regulating the phospholipase C activity: G proteins, ATP, calcium and magnesium ions were investigated.

ARACHIDONIC ACID IN SIGNAL TRANSDUCTION IN CAT CAROTID BODIES

M.Sc. Robert Strosznajder
Supported by the State Committee for Scientific Research: grant # 6 P207 029 05

During the last year studies concerned the evaluation and comparision of arachidonic acid (AA) incorporation into phosphatidylinositol (PtdIns) and phosphatidylcholine (PtdCh) and also into some other phospholipids of cat carotid bodies. Significant differences in the activity of these processes were observed. AA was more actively incorporated into PtdIns as compared to PtdCh and other phospholipids. Acute hypoxia specifically inhibits AA incorporation into PtdIns.

Concomitantly the level of radioactivity of arachidonylo-CoA (AA-CoA) was determined. It was observed that acute hypoxia significantly enchances the level of radioactivity of AA-CoA. The data concerning AA release and incorporation into PtdIns were presented in a graphic form as an additional presentation at FEBS Meeting in Helsinki 1994.

LARYNGEAL CONTRIBUTION TO THE RESPIRATORY RESPONSE TO CO_2

Assoc. Prof. Małgorzata Szereda-Przestaszewska
Supported by the State Committee for Scientific Research: grant # 6 6361 92 01

Increased CO_2 concentration in the airflow passing through the larynx isolated from the lower airways decreased minute ventilation significantly more than inha-

lation of room air in the intact and subsequently vagotomized cats.

The depression of ventilation was due both to the decrease in tidal volume and respiratory rate. The latter resulted from the significant prolongation of the expiratory time, with no appreciable change in the inspiratory time.

The respiratory responses to intralaryngeal CO_2 of the intact and subsequently vagotomized cats were statistically indistinguishable.

Sensory denervation of the larynx excluded the hypoventilatory response from the larynx exposed to increased CO_2 in the expiratory airflow.

OBSTRUCTIVE SLEEP APNOEA AS A CONSEQUENCE OF POSITIVE FEEDBACK BETWEEN SKELETAL MUSCLE TONE AND PHASIC ACTIVITY OF THE NERVES SUPPLYING UPPER AIRWAY MUSCLES

Dr. Wiktor Janczewski

Supported by the State Committee for Scientific Research: grant # 6 P207 028 05

Obstructive sleep apnoeas and hypopnoeas are the most common abnormalities of breathing that occur during sleep. Inefficient activity in the nerves supplying upper airway muscle results in a complete occlusion of the upper airway (UA).

According to our hypothesis the loss of skeletal muscle tone, always occurring during sleep, suppresses the activity of nerves to UA muscles.

We recorded simultaneously EMG of the genioglossus muscle and neural activity of the hypoglossal (n. XII), facial (n. VII) and phrenic (n. Ph) nerves in 18 vagotomized rabbits. This enabled us to study the influence of the skeletal muscle tension on the activity of the nerves supplying UA muscles. Muscle tension was reduced or eliminated several times during each experiment by means of a short acting neuromuscular blocking agent Norcuron (vecuronium bromide - 0.03 mg/kg i.v.) or tubocurarine (d-tubocurarinum chloratum i.v. 0.5 mg/kg) In order to keep chemical drive constant animals were mechanically ventilated via tracheostomy tube inserted below the larynx.

It was found the relaxation of the UA and other skeletal muscles results in reduction of the hypoglossal and facial nerve activity. The amplitude phrenic nerve activity remained unchanged. To shorten the duration of the competitive block induced by tubocurarine, neostigmine methylsulphate (i.v. 0.1 mg/kg) was administered. Partial or total restoration of the muscle tension increased n. XII activity. Electrical stimulation of one hypoglossal nerve increased activity of the other one. This effect lasted for more than ten breaths after termination of stimulation.

We conclude that the loss of skeletal muscle tone reduces the activity of the nerves supplying UA muscles. Our data may explain why administration of ethanol,

benzodiazepines, barbiturates and other drugs diminishing muscle tone predisposes to snoring or total collapse of the oropharyngeal airway.

ALLO- AND XENOGENEIC HETEROTOPIC GRAFTS OF FETAL BRAINSTEM

Assoc. Prof. Mirosław Ryba

Supported by the State Committee for Scientific Research: grant # 6 P207 050 05

Twenty-eight blocks of neural tissues from caudal part of the 18-day rabbit fetuses were implanted into the caudate nucleus of the adult rats. Twenty-three graft recipients survived. Immunosuppression consisted if cyclosporine A for 2 days and 2-CDA for 15 days, starting at the day of grafting. The treatment was repeated starting on day 36 after grafting. The grafts were not actuely rejected. Cells in the grafted tissues matured normally, as evidenced by the increasing in time expression of neuron-specific enolase (NSE) and myelin basic protein (MBP), protein markers of differentiation and myelinization, respectively. Furthermore, the grafted tissue displayed electrical activity (action potentials, either phasic or tonic), which was recorded extracellularly.

DEPARTMENT OF NEUROCHEMISTRY
Head: Professor Jerzy W. Łazarewicz
3 Dworkowa Str., 00-784Warsaw
Telephone: 49 58 97

NMDA RECEPTOR-MEDIATED DESTABILIZATION OF CALCIUM HOMEOSTASIS IN BRAIN NEURONS: COMPENSATORY MECHANISMS AND CHANGES IN EXPRESSION OF β-APP PROTEIN

Prof. Jerzy W. Łazarewicz

The aim of *in vivo* studies, in which we have combined the technique of intracerebral microdialysis with the assay for dialysate concentration of calcium ions ($[Ca^{2+}]$) and ^{45}Ca efflux from the prelabeled endogenous Ca^{2+} pool, was to demonstrate the NMDA-induced mobilisation of intracellular Ca^{2+} in different regions of the adult rat brain. We have found that in contradiction to the rabbit hippocampus in the rat hippocampus, particularly in CA4/DG and to a lesser extent in the rat thalamus but not in the rat striatum, application of 5 mM NMDA in the dialysis medium leads to approximately tenfold increase in ^{45}Ca efflux with concomitant decrease in dialysates' $[Ca^{2+}]$ by 50% (collaboration with Dr. Henrik Hagberg, Göteborg, Sweden). This effect was dependent on NMDA concentration, it was prevented by MK-801 and strongly inhibited by antagonists of Na^+/Ca^{2+} exchanger. These results indicate that in the rat hippocampus an NMDA receptor-dependent influx of calcium to neurones is accompanied by an intracellular ^{45}Ca efflux from neurones, mostly via Na^+/Ca^{2+} exchange. This effect seems to reflect a complex mechanism of destabilization of Ca^{2+} homeostasis comprising also compensatory elements. Further studies aimed to identify intracellular ^{45}Ca pools involved are in progress.

In order to determine intracellular $[Ca^{2+}]$ inside deep brain structures *in vivo*, we have constructed a prototype of the optical fibre fluorimetric probe, attached to the existing Hitachi spectrofluorimeter. This device has passed favourably preliminary tests *in vitro*.

We have also initiated studies tended towards elucidation of the role of NMDA receptors and intracellular calcium in the mechanism of accumulation of potentially neurotoxic β amyloid (βA) protein in the brain. Proteolysis of the β amyloid protein precursor (β-APP), normally resulting in βA cleavage may became amyloidogenic under certain conditions. In the present studies we have used a Pulsinelli's 4VO

model of the forebrain ischemia in rats. Changes in the expression of different β-APP fragments in homogenates of the hippocampus collected at different postischemic periods were detected by immunoblotting. Biphasic changes in β-APP expression were found. Two hours after ischemia a decrease in expressions of the βA and of the C-terminal (cytosolic) β-APP fragments without significant changes in the immunoreactivity of the N-terminal (extracellular) fragment was noted. After one and seven days of ischemia an increase in the immunoreactivity of all β-APP fragments, particularly of the C-terminal fragment and of the 751/770 isoform marker, was found. These results, explaining previous controversy concerning the effect of ischemia on β-APP expression in brain, revealed stimulation of β-APP processing during the initial postischemic period, following by an enhanced β-APP expression afterwards. We have studied the role of NMDA receptors in β-APP proteolysis *in vitro* implying a superfusion of the hippocampal slices of adult rats with the medium containing NMDA. A release of β-APP fragments to superfusates was assessed immunochemically. Application of 1 mM and particularly 2.5 mM NMDA induced a selective significant increase in the immunoreactivity of the N-terminal fragment of β-APP and of a part of βA sequence, without any detectable release of the C-terminal fragments. This effect was reduced any antagonists of NMDA receptor, MK-801 and CPP, and in a calcium-free medium. Thus, these results demonstrate NMDA receptor-dependent, Ca^{2+}-mediated activation of β-APP processing in brain neurones. The exact mechanism of this phenomenon remains to be detected.

AMINOACID NEUROTRANSMITTERS IN BRAIN ISCHEMIA AND AGING. EFFECT OF GLUCOCORTICOIDES

Prof. Joanna Strosznajder

Aminoacid neurotransmitters: glutamate and GABA are the major excitatory or inhibitory mediators of chemical communication among neurons in the CNS. They play a crucial role in development of the CNS in learning and memory formation.

These neurotransmitter receptor systems regulate the function of the CNS on the molecular level througth the metabolic second messengers formation and ion fluxes. In the case of unregulation they may participate in neuronal degeneration or neuronal death.

In the last year we have evaluated the properties and function of GABA/Cl^- channel 4 and 30 days after global ischemia. The properties of Cl^- channels were changed in relation to the reperfusion time. The evaluation of kinetic parameters of chloride channels ligand [^{35}S] TBPS dissociation, indicated a decrease of the

openning time of chloride channel on the 4th day of reperfusion exclusively in hippocampus. However, 30 day after ischemia the changes were observed not only in hippocampus but also in cerebral cortex. The shorter half life of the fast phase of TBPS dissociation, indicating the shorter than in normoxic brain openning time of Cl channel, was found for GABA operated Cl^- channel and also for other population of Cl^- channels. Moreover, in the presence of 50-100 μM muscimol the Cl^- uptake into brain cortex synaptoneurosomes isolated 30 days after brain ischemia significantly decreases as compared to normoxic brain. After this prolonged reperfusion time (30 days) the binding of TBPS into synaptic plasma membrane isolated from the whole hippocampus and cerebral cortex of ischemic brain was slightly lower as compared to control. Moreover, the analysis of biphasic TBPS dissociation also demonstrated that the percentage population of Cl^- channels in the open state is not changed during reperfusion in hippocampus and brain cortex. These results indicate that ischemia-reperfusion injury altered the properties and function of Cl^- channel but it had not significant effect on the total amount of Cl^- channels. These changes may be responsible for the lower hyperpolarization ability of the $GABA_A$/Cl receptor complex. Looking for the mechanism of these modyfications a few factors which accompanied ischemia were taking into consideration that means the effect of polyunsaturated fatty acids, peroxidation reactions and the changes of pH value. It was found that all these factors affected properties and function of the $GABA_A$ receptor. The best correlation was found between the modification induced by arachidonic acid and reperfusion injury. It is known that the level of free fatty acids in the brain is regulated by deacylation - reacylation reactions. Disturbances of this regulation may be responsible for the accumulation of free fatty acids. In the last year the mechanism of previously reported glutamatergic-receptor dependent inhibition of arachidonic acid incorporation into membrane phospholipids was elucidated. It was found that the complex of receptor dependent biochemical events, leading to the activation of nitric oxide synthase and liberation of NO, which participate in this inhibition. The NO-mediated S-nitrosylation of AA-CoA ligase and acyl-transferase or NO dependent free radical injury may be responsible for the lower activity of these enzymes. The cGMP-dependent phosphorylation and NO-mediated ADP-rybosylation are not involved in NMDA-receptor dependent inhibition of arachidonic acid incorporation. It was observed by us that nitric oxide is liberated during NMDA-receptor activation with different dynamics and activity in different part of the brain. Brain aging activates nitric oxide synthase in hippocampus but concomitantly decreases the level of cGMP. These results indicate that aging significantly modify the signal transduction in glutamatergic receptor system including such mediators as NO and cGMP. Hydrocortisone has no effect on NOS activity and cGMP in hippocampus indicating that only constitutive form of NOS is activated. The results of our studies al-

lowed us to suggest that stimulation of $GABA_A$ receptor system, application of Mg^{2+} ions together with scavengers of free radicals and free fatty acids may be safe and useful method for the brain protection during ischemia-reperfusion injury and aging.

SIGNAL TRANSDUCTION AND GENE EXPRESSION IN BRAIN PATHOLOGY (ISCHEMIA AND *pt*-RABBIT MUTATION)

Assoc. Prof. Krystyna Domańska-Janik

Brain ischemia

Involvement of protein kinases in the initiation and prolonged of the pathological cascade of events leading to delayed, postischemic neuronal death was further investigated. Transient activation of calcium-phospholipid-dependent kinase (PKC) resulted in potentiation of the release of excitatory amino acids (EAA) from nerve endings. Similar effect was observed after direct PKC activation by phorbol esters in hippocampal slices *in vitro*. This effect was completely abolished by H-7, a potent PKC inhibitor treatment.

The other set of experiments revealed that synaptic transmission during and after ischemia can be further modified by the rapid and profound inhibition of the other neuron-specific kinase: calcium/calmodulin-dependent kinase II (CaM KII). The inhibition of catalytic activity was followed by the enzyme translocation toward membranes, its extensive (auto)phosphorylation and the substantial changes in its calcium dependency.

The recent data strongly suggest that synaptic transmission can be effectively modified by the opioid receptors. We have characterised (in collaboration with University of Arizona), the novel class of ligands interacting with κ-opioid receptor which is the putative inhibitor of presynaptic voltage-dependent calcium channels. The influence of these ligands on postischemic EAA release from nerve endings is under investigation.

***Pt* mutation in rabbits**

We have accomplished molecular characteristic of *pt*-rabbit mutation, the animal model of human Pelizaeus-Merzbacher disease (collaboration with Prof. J-M. Matthieu, Switzerland). Mutation influence PLP and its splicing variant DM-20 proteins. We have also sequenced rabbit Plp cDNA from normal animals. Rabbit Plp sequence, deduced from cDNA, differs from the human protein only at threonine[198]. The analysis of mutant cDNA revealed a transversion T→A in exon 2 of Plp gene. The point mutation which is placed at the end of the first potential

transmembrane domain, results in a substitution of histamine[36] by glutamine in PLP and DM-20 molecules. This transversion abolishes a restriction site which enabled us to screen each individual animal for *pt* trait. We have found a perfect correlation between the *pt* allele and the abnormal phenotype.

THE MECHANISMS OF DISTURBANCES IN NEUROTRANSMITTER TRANSPORT UNDER LEAD TOXICITY CONDITIONS

Prof. Urszula Rafalowska

Lead is widely distributed in the environment. This nonphysiological metal is known to be toxic and can affect organ systems in humans and animals. The main target for lead toxicity is the central nervous system. Both chronic and acute exposures to lead result in neuropathy and encephalopathy. It was postulated that Pb^{2+}/Ca^{2+} interactions might play an important role in the toxicity of Pb^{2+} in a neurotransmission process. It was also postulated that inhibitory effect of Pb^{2+} on voltage sensitive calcium channels, the inhibition of Na^{+}-K^{+}-ATPase by Pb2+ and the interaction of Pb with protein, may represent mechanisms underlying Pb toxicity.

Despite a considerable research effort, the subcellular mechanisms of Pb^{2+} action remain unclear.

Searching for other mechanisms, which can be connected with Pb^{2+}-effects an neurotransmission, we have considered the enhanced peroxidation process in synaptosomes in lead-toxicity conditions.

Three ways of poisoning were performed.

1) 200 mg $Pb(CH_3COO)_2$/l in drinking water was given to 3 week-old-rats for 3 months;
2) 15 mg $Pb(CH_3COO)_2$/kg b.w. was injected intraperitoneally for 7 days to rats weighing approx. 200 g;
3) Lead acetate was added to the medium containing synaptosomes.

Symaptosomes were isolated from hemispheres using a discontinuous Ficoll gradient according to Booth and Clark (*Biochem. J* 1978, 176, 365).

Our results indicated that the lead level in the synaptosomal fraction obtained from Pb^{2+} treated rats was much higher than in the control. Lead ions administrated *in vivo* and *in vitro* did not induce peroxidation itself, either in synaptosomes or in homogenates and microsomes of the brain, kidney and liver.

It also did not change Fe^{3+}-dependent peroxidation in synaptosomes and in brain homogenates (when μmolar concentrations of lead acetate were used), whereas it drastically increased peroxidation in homogenates or microsomes of liver and kidney. This finding suggested that lipid peroxidation may contribute to the toxic

action of Pb^{2+} in some tissues but not in the brain. Lack of effects of Pb^{2+} on peroxidation in synaptosomes and brain homogenates, demonstrated that this process cannot be responsible for the changes in neurotransmission that we ealier observed. It seems likely that regulatory mechanisms concerning the Pb2+ effects on peroxidation, operating in the animal body are stronger in the brain than in other tissues and are probably responsible for the protection of brain synaptosomes from peroxidation process.

ROLE OF NMDA RECEPTORS IN MECHANISM OF ARACHIDONIC ACID RELEASE IN BRAIN

Prof. Jerzy W. Łazarewicz
Supported by the State Committee for Scientific Research: grant # 4 0320 91 01

The method of microdialysis of the rabbit hippocampus *in vivo* has been utilised in studies on the effect of NMDA receptor stimulation on dialysate content of selected eicosanoids, metabolites of arachidonic acid, thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$, which are stabile products of biologically active thromboxane A_2 and prostacyclin. All pharmacological substances were applied in dialysis medium. Application of 1 mM NMDA for 20 min resulted in the five- and eight-fold increase in concentrations of thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$, respectively. An increase in NMDA concentration to 2.5 mM did not potentiate the maximal eicosanoid release, but significantly prolonged this increase. The release of eicosanoids induced by 1 mM NMDA was inhibited by 50% in the presence of 10 μM MK-801 and calcium-free media. Quinacrine (250 μM) inhibited this effect by 30%, whereas 10 μM indomethacin almost completely prevented it. An inhibitor of thromboxane synthase, 100 μM furegrelate reduced thromboxane B_2 release to only 25% of the control value, with concomitant increase in 6-keto prostaglandin $F_{1\alpha}$ production by 100%. These results indicate that stimulation of NMDA receptors induces a calcium-dependent production of thromboxane A_2 and prostacyclin in the rabbit hippocampus. It seems that apart from the extracellular calcium, also intracellular calcium stores play an important role in NMDA-induced activation of lipolysis. Thus, in the brain *in vivo* activation of NMDA receptors induced a release of arachidonic acid, which is a precursor of eicosanoids. This phenomenon, of possible pathophysiological importance, may be located in neurones, although transcellular mechanism of eicosanoid formation with participation of astroglia and capillaries should be also considered.

Utilising a method of microdialysis we have studied the effect of monosialoganglioside GM1, a substance of putative neuroprotective activity, on NMDA-

induced eicosanoid release in the rabbit hippocampus *in vivo*. It was shown that intramuscular injections of GM1, 30 mg/kg, for 3 days twice a day, decrease by 40% release of 6-keto prostaglandin $F_{1\alpha}$ evoked by 1 mM NMDA. The other results indicated that GM1 partially prevents NMDA-induced destabilization of Ca^{2+} homeostasis in the hippocampus and protects neurones from excitotoxic injury. These data indirectly confirm that calcium plays a key role in the mechanism of NMDA-induced lipolysis and neuronal injury, indicating a cytoprotective potential of gangliosides in the excitotoxic neuronal injury. However, further studies demonstrated that pretreatment of the Mongolian gerbils with GM1 significantly reduces 5-min forebrain ischemia-induced production of thromboxane B_2 in the cortex and hippocampus without concomitant neuroprotection of the CA1 neurones. Thus, in spite of GM1 modulation of arachidonic metabolism in the ischemic brain, a neuroprotective activity of the ganglioside seems to be insignificant in a drastic model of forebrain ischemia in gerbils.

ROLE OF NITRIC OXIDE IN PATHOMECHANISM OF BRAIN ISCHEMIA

Prof. Joanna Strosznajder

Supported by the State Committee for Scientific Research: grant # 6 P207 027 04

The last year studies concerned the evaluation of nitric oxide (NO) synthase activity and NO-mediated processes in brain subjected to ischemia reperfusion injury. Inhibitors, and some methods of molecular biology (mRNA isolation and Northern blotting), were introduced for identification of the NOS type. It was found that during ischemia and reperfusion time neuronal, constitutive form of NOS (cNOS) is activated. Using this technic the activation of gene for iNOS was observed. Further studies with RT-PCR will carried out. Moreover, it was observed that NO-mediated processes are responsible for the inhibition of some enzyme(s) involved in lipid mediator(s) metabolism, which may have additional implication in modulation of NMDA dependent signal transduction pathway.

MODULATION OF TRANSSYNAPTIC SIGNALING BY PLATELET ACTIVATING FACTOR

Assoc. Prof. Krystyna Domańska-Janik
Supported by the State Committee for Scientific Research: grant # 4 0319 91 01

The potential involvement of platelet-activating-factor (PAF, 1-0-alkyl 2-0-acetyl-sn-glycero-3-phosphorylcholine) in aggravation of ischemic brain injury has been recently postulated. We have demonstrated that several PAF-mediated biochemical responses in synaptoneurosomes *in vitro* reassemble these observed previously in ischemic brain and widely acknowledged as the potentially caused factors in this pathology.

In synaptoneurosomes prepared from rat hippocampus 10 mM PAF concentration could trigger an observable elevation of intracellular calcium as measured by fluorescence Fura-2A probe. Similar elevation of synaptoneurosomal $[Ca^{2+}]_i$ was evoked by 1 mM glutamate treatment. As an effect of calcium entry after PAF application, translocation of protein kinase C (PKC) towards plasmatic membranes was demonstrated by ^{3}H-labelled phorbol-binding method. It was followed by an increase of 50 kD proteolytic fragment of the enzyme (PKM) recognized on Western blots with anti-PKC antibody. Incubation of synaptoneurosomes in the presence of calcium chelators abolished these effects of PAF and significantly decreased the content of PKC in the membranes.

Furthermore, PAF treatment markedly attenuated the receptor- and postreceptor-activated cAMP accumulation in synaptoneurosomes. The decrease of cAMP level seems to be secondary to the PAF-induced calcium entry with subsequent activation of cAMP-specific phosphodiesterase, since it was completely blocked by IBMX - a potent inhibitor of this enzyme.

Our observations indicate that PAF in concentration reported for ischemic brain can elevate $[Ca^{2+}]_i$ and potentiate calcium-dependent intracellular signalling in synaptoneurosomes *in vitro*, including PKC translocation/activation and proteolysis, followed by IBMX-sensitive inhibition of cAMP production.

The effect of PAF antagonist - BN52021 on [^{3}H]-D-Aspartate (D-Asp) release was investigated in rat hippocampal slices during and after incubation (20 min) in ischemia-like conditions. Ischemia did not influence the spontaneous D-Asp outflow whereas K^+-evoked, calcium-dependent release has been markedly enhanced in reoxygenated, postischemic slices. These slices revealed also a substantial translocation/activation of protein kinase C (PKC). BN52021 blocked both ischemia-induced effects. Moreover, PKC inhibitor - H7 attenuated post-ischemic, K^+-evoked, D-Asp release when β-PDBu, the PKC activator, stimulated normoxic slices to enhanced response.

Assuming that PKC is activated by ischemia in PAF-dependent manner and that this activation proceeds to enhanced glutamate exocitosis, we speculate on the envolvement of PAF receptor stimulation in the pathology of cerebral ischemia.

ENERGY METABOLISM IN BRAINS NERVE ENDINGS UNDER LEAD TOXICITY CONDITIONS

Prof. Urszula Rafałowska

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The mechanisms of lead toxicity in the CNS are not clear. It was postulated that Pb^{2+}/Ca^{2+} interactions may play an important role in toxicity of Pb^{2+} in neurotransmission process. Our investigations suggested existance of several mechanisms of lead toxicity, related to the individual neurotransmitter. In this work the lead effect on the level of energetic parameters in synaptosomes was investigated. Two models of toxicity were performed.

1) 200 mg $Pb(CH_3COO)_2$/l in drinking water was given to 3 week-old-rats for 3 months;
2) 15 mg $Pb(CH_3COO)_2$/kg b.w. was injected intraperitoneally for 7 days to rats weighing approx. 200 g;

Symaptosomes were isolated from hemispheres using a discontinuous Ficoll gradient according to Booth and Clark (*Biochem. J* 1978, 176, 365).

The levels of energetic parameters in synaptosomes were measured using the enzymatic-spectrophotometric methods. In the chronic model (3 month of poisoning) it was found the ATP-level decreased by about 30%, creatine phosphate increased 3 times. Creatine and AMP concentrations were unchanged but ADP increased slightly. In the acute model of toxicity ADP level increased drastically by about 95% and creatine phosphate increased 2 times. Creatine decreased slightly but ADP and AMP did not change.

These results have shown that the energetic processes can be disturbed by Pb^{2+} depending to lead dose and time of exposure.

In the acute model we observed protection effect manifested by increased energetic metabolism i.e. enhanced ATP and creatine phosphate levels. In the chronic model when lead acted for a longer time we observed destruction of energetic metabolism.

It seems, that disturbances in the energetic state of synaptosomes can be reason of observed disturbances in neurotransmission.

PHENOTYPIC DIFFERENCES AND MYELIN PROTEIN GENES EXPRESSION IN *pt*-RABBIT MUTANTS

M.Sc. Joanna Sypecka

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Paralytic tremor (*pt*), a hereditary neurological disorder of rabbits is a recessive, X-linked point mutation in exon 2 of Plp gene. Affected animals, although strictly controlled for their *pt* trait, differ significantly in their phenotype. The aim of the present study was to investigate whether there is any difference in the expression of the most typical markers of myelin in mutants which differ in their phenotypes. For our studies we have chosen 4 groups of animals aged 22; 42; 65 and 80 days; each group consisted of mutants expressing different phenotypes and age-matched controls. We have stated that degree of both the CNS hypomyelination and the underexpression of myelin specific proteins (PLP; MBP; MAG and CNP) observed in the brain homogenates, correlates with the severity of the neurological symptoms and is the highest in the most affected animals. This study revealed most significant differences in PLP expression between examined phenotypes, whereas contents of others investigated myelin markers seemed to be less affected.

In additional study we have investigated developmental expression of the glycolipid markers of oligodendrocyte (OL) maturation (i.e. 01; 04; POA antigens) by semiquantitative ELISA method. It was shown that from initially equal number of progenitors bearing proligodendroblast (POA) antigen in *pt* brain only a minute fraction of these cells could pass the more advanced maturation stages to the phenotypes expressing 04 and 01 antigens. However, this small fraction of differentiated OLs can produce myelin with normal, except of PLP and DM20, concentration of the other myelin-specific biochemical components. The mechanism(s) responsible for the ability of certain population of OLs to overcome PLP-mutation-guided inhibition of differentiation are not known at present.

DEPARTMENT OF NEUROPATHOLOGY
Head: Professor Andrzej Kapuściński
3 Dworkowa Str., 00-784 Warsaw
Telephone: 49 54 10

THE ROLE OF VASCULAR AND VASOGENIC FACTORS IN THE EVOLUTION OF POSTISCHEMIC ENCEPHALOPATHY

Prof. Mirosław J. Mossakowski

As in previous years, investigations were conducted on two experimental models of ischemia of the central nervous system: global cerebral ischemia resulting from the arrest of cardiac and respiratory activity in rats and short-lasting ischemia of the forebrain in Mongolian gerbils, due to bilateral ligation of the carotid arteries.

Investigation of the experimental material was supplemented by evaluation of human material from cases of cardiac arrest.

In the first model attention was focused on two problems: estimation of the state of microcirculation in the brain and an attempt of analysis of the postischemic changes in the tissue which might be a ground for the advancing encephalopathic syndrome.

The first group consisted of investigation of the vascular spasm within the microcirculation elements occurring in the period of recirculation after 10-min global cerebral ischemia. The dynamics of changes were characterized and their pathomorphology was described using the scan- and transmission electron microscope. A relation between morphological changes in the vessels depending on the degree of contraction and the abundance and course of smooth muscles in their walls was demonstrated. The pattern of endothelial changes leading to a reduction of the vascular lumen, expressed in the form of irregular foldings of luminal endothelial surfaces was thought to be connected with disturbance of the function of own contractile apparatus of endothalial cells (Wiśniewski et al.). The second problem consisted in observation of blood platelets interaction with endothelium of the microcirculation vessels, manifested in formation of thrombocytic agglomerations within their lumina. This phenomenon was observed in various postischemic periods up to 12 months and concerned vessels of different caliber, both arteries and veins. The state of the platelets forming the aggregates seemed to indicate an active process of their production even in late periods after ischemia. These changes imply permanent disturbances of the vascular endothelium, as indicated by preliminary studies of the behaviour of adhesive substances on their surface. The presence of thrombocytic aggregates in the late postischemic encephalopathy and extravascular

accumulation of platelets may, in turn suggest their connection with tissue deposition of β amyloid (Pluta et al.). The third problem connected with the microcirculation state in the postischemic period was the estimation of an active transport of phenylalanine from the vascular system to the brain. In these investigations a considerable decrease was noted in the transport of amino acid to the brain, indicating disturbances in the mechanism of active transport, most pronounced in two phases of the postischemic period, falling to the period of 15-20 min and seventh day of recirculation. It should be stressed that the defect in the active transport mechanisms was found concurrent in time with damage of the blood-brain barrier for high-molecular substances described in former studies (Kapuściński).

Investigation on the tissue consequences of global cerebral ischemia concerned the behaviour of serotonin in the hippocampal nerve endings in the early postischemic period. By means of immunocytochemical techniques with the application of colloidal gold, adapted for electron microscopy, it was demonstrated that the serotonin content greatly increased in the surroundings of the microvessels in the early hours after ischemia, with subsequent reduction of its content. The striking rise of serotonin concentration in the surroundings of the microvessels, suggested possibility of its participation in the contraction of the microvessels, observed in this phase of postresuscitation. The changes in serotonin content may increase of the excitotoxic action of glutamate, leading to the damage of selectively vulnerable neurons of the hippocampal region (Gajkowska and Mossakowski). Another line were investigations on the tissue accumulation of the protein precursor of β-amyloid. Immunocytochemical studies demonstrated the presence of β-amyloid precursor both extra- and intracellularly. Perivascular extracellular deposits appeared as early as 3 h after ischemia and were present up to the 7th day when, intraneuronal deposits dominated. The deposits showed a preference for the structures of the brain hemispheres and cerebellum. The positive reaction was obtained with antibodies to β-amyloid and to N-terminal and the C-terminal fragment of the β-amyloid precursor, this suggesting accumulation of either its whole molecule or all its fragments. The results obtained indicate that global ischemia leads to extracellular accumulation of the protein precursor of β-amyloid. This may suggest, both its role in the pathogenesis of postischemic tissue lesions as well as participation of ischemic factors in the mechanism of Alzheimer's disease (Pluta et al.). Similar changes were found in preliminary studies of clinical material in cases of cardiac arrest in humans (Maślińska et al.).

In the second experimental model a distinct dependence was demonstrated of the extensiveness and intensity of pyramidal neuronal injury in the CA1 sector of Ammon's horn on the body temperature of the animals at the period of ischemia and after blood recirculation. These relations were found in experimental groups differing from one another in the time of occlusion of the carotid vessels. The

immunocytochemical expression of GFAP, showing a marked dependence on the intensity of neuronal lesions, indirectly also depended on changes in body temperature of the animals in the ischemic and postischemic periods (Gadamski and Szumańska). Investigation of the receptors of membrane glycoconjugates in the cell elements of nerve tissue and in vascular walls by means of the lectin histochemical techniques revealed pronounced abnormalities in the brains of Mongolian gerbils subjected to short-lasting forebrain ischemia. These changes affected the nerve cells both exhibiting and not exhibiting features of structural injury as well as astroglial and oligodendroglial cells and vascular wall elements. Noteworthy was variability of the reaction with the particular lectins showing affinity to various saccharides residua of the particular membrane glycoconjugates. Abnormalities of various dynamics, pointed to changes in the properties of the cellular and vascular membrane structures, no doubt, affecting their transport functions (Szumańska and Gadamski).

Similar investigations with the use of lectins which reveal glucose, galactose, mannose, neuraminic acid and fucose receptors were performed on clinical material from cases of cardiac arrest. Profound changes of the histochemical reaction were revealed here, depending on the type of cells, degree of advancement of tissue lesions and time of survival after cardiac arrest. Most important changes concerned astroglial elements, microglia and the vascular network (Zelman and Szumańska).

PARTICIPATION OF THE WHITE MATTER LESIONS IN THE PATHOLOGY OF DEGENERATIVE PROCESSES OF SENILE AGE

Assoc. Prof. Irmina B. Zelman

Myelin damage in Alzheimer's disease (AD), vasculogenic dementia and non-demented old-age cases: myelin changes observed in the white matter of the frontal lobe were divided into two groups: diffuse (myelin pallor) and focal mainly perivascular. The latter were significantly related with the patients age and intensity of cerebral atherosclerosis. Moreover, they were influenced by the intensity of cortical neurofibrillary degenerations. Myelin pallor was not influenced by atherosclerotic as well as Alzheimer's type degenerative changes. It seems that in the pathomechanism of central myelin damage participate secondary degenerations being a consequence of cytoskeleton pathology and cell body destruction. Amyloid deposition is only a factor predisposing to the development of neurofibrillary changes.

Immunostaining pattern of tau-1 and ubiquitin reactivity in the temporal white matter was different in subjects with AD and aged non-demented cases. Tau-1 immunoreactivity was observed in all AD brains, mainly in loosely dispersed neuropil threads, whereas in majority of non-demented cases the temporal white matter was

immunonegative for tau-1. Ubiquitin reactivity, characterized by the dot-like structures evenly distributed in temporal white matter was found in both AD and non-demented cases. It was concluded that different tau-1 and ubiquitin immunoreactivity reflects variable pathological changes identified in nerve fibres. Tau-1 immunolabeled neuropil threads correspond to neurofibrillary changes of Alzheimer's type, different from those in cerebral cortex by lack of ubiquitin immunostaining. Non-filamentous ubiquitin immunopositive structures represent presumably nonspecific nerve fibre degeneration related to various pathological events both in aging and AD.

Morphometric examination of astrocytes and microglia in the hippocampal formation (in collaboration with Department of Anatomy, Medical Academy, Gdańsk) revealed striking differences in astro- and microglia reactivity in AD and senile non-demented subjects. Significant increase in density of astrocytes was disclosed in AD in the whole hippocampal ormation, most pronounced in the CA1 sector, dentate gyrus and subiculum. Astrocytes were hypertrophic and often found in clusters in places corresponding to beta-amyloid deposition. Unlike the increase of astrocytes a marked loss of microglial cells was observed in all areas of the Ammon's horn, most significant in the dentate gyrus and CA4 sector. Only in layer II of the entorhinal cortex significant increase of microglial cells density was found.

The astroglia proliferation and hypertrophy is the response of this glial cell population to the presence of amyloid deposits, instead microglial reaction could not be directly related to the Alzheimer pathology.

Tellurium myelinopathy: ultrastructural studies in rats intoxicated IP with sodium tellurite revealed peripheral myelin lesions not only in developing but also in adult animals. Myelin changes in PNS were dose-dependent and related to the damage of Schwann cells which were more sensitive to the drug action than oligodendrocytes in the CNS. Thus, in the latter less pronounced myelin abnormalities were disclosed. It was shown that squalene, putative agnt engaged in pathomechanism of tellurium-induced myelinopathy, given IP to rats causes in the CNS histopathological myelin changes, similar though less severe to those developing after sodium tellurite intoxication.

QUIN neurotoxicity: neuroprotective effect of nimodipine (calcium entry blocker) against QUIN neurotoxicity was evidenced in organotypic culture of rat hippocampus with the use of oxylate-pyroantimonate-electron microscopic technique. Nimodipine prevented QUIN induced mitochondrial calcium overload in degenerating pyramidal neurons and postsynaptic dendrites, directly supporting the concept that protective action of Nimodipine is related to the inhibition of selective calcium influx into the vulnerable cells, thus preventing metabolic disturbances which ultimately lead to neuronal death.

DYNAMICS OF CLINICAL COURSE AND NEUROPATHOLOGICAL AND RADIOLOGICAL ABNORMALITIES IN DEMENTIA SYNDROMES WITH AN EARLY ONSET OF THE DISEASE

Prof. Mirosław J. Mossakowski

Clinical studies of patients with dementia syndrome have been continued. The investigations were carried out in collaboration with the Department of Radiology, Warsaw Medical School. The CT studies concerned focal changes in the cerebral white matter and the occurrence of hypodense areas in the periventricular regions (leukoaraiosis) in patients with Alzheimer disease (AD), with dementia of vascular origin (Multi-infarct-dementia - MID) and mixed processes. These observations were confronted with a control group of subjects showing no symptoms of dementia. Special attention was turned to the coexistance of signs and symptoms of atherosclerosis. This was based both on anamnesis and clinical examination. Risk factors of atherosclerosis such as diabetes, hypertension and coronary disease were carefully estimated. Neurological findings were correlated with the radiological changes and with depth of dementia, estimated according to Folsteins MMS and Reisberger's GDS scales. The results were statistically elaborated. Changes in the white matter appeared mainly in cases where dementia of Alzheimer's degeneration type was accompanied by atherosclerosis. These changes were more frequent in cases where dementia was more advanced, but this tendency was not statistically significant.

Another subject in investigation was the occurrence of nicotinism in demented patients considered as a factor reducing the risk of AD development. Contrary to the literature reports, in the presently examined population no influence of this "protective factor" was detected. It seems that the supposition on the protective role of smoking may be true in the group of women exclusively. It would rather seem that it may be due to less frequent nicotinism in women of the age of the patients examined, owing more to cultural habits, and not to the hypothetic stimulation of the nicotin receptors.

In 1994 investigations with the use of magnetic resonance (MRI) were started in a group of patients previously examined with use of the computer tomography imaging. Evaluation of changes within the cortex and white matter in MRI, as much more precise, will be in the future an important verification of up to date conclusions based on CT. Neuropsychological studies were continued in correlation with radiological evaluation.

The first case of Lewy bodies disease in Poland was described. It is a particular disease of degenerative nature, considered as a variant of AD.

DYSEMBRYOPLASIA AS THE MORPHOLOGICAL BASIS FOR TEMPORAL EPILEPSY

Prof. Halina Kroh

The study was performed on biopsy material from Neurosurgery Department of Medical School, Warsaw, in 1989-1994, obtained from 98 patients suffering from temporal epilepsy, among whom were 33 patients with intractable epilepsy and 65 with epileptic fits due to neoplasms of temporal lobes exclusively. In the group of patients with intractable temporal epilepsy of the duration of 2-36 yr (average 16.6), the hippocampal sclerosis was found in 24 patients, perivascular lymphocytic infiltrations in 8 cases, some small malformations and vascular changes in 7 cases, ectopic neurons in the white matter and dysplastic cortical changes in 1 case. Among patients with neoplastic tumors, 56 complained of temporal epilepsy lasting of 1 mo.-36 yr (average 6.7 yr). In case of glial tumors ectopic neurons in the white matter and in some patients the disorder of cortical lamination were present. Neoplastic invasion of hippocampus was found in 6 cases, whereas hippocampal sclerosis occurred in 3 patients.

The separate group of 6 cases classified as dysembryoplastic neuroepithelial tumors (DNT) with a 1-27 yr long course of epilepsy was characterized in each case by a few small foci of gliomas in the cortex, beside main mass of non-defined glial type and associated with ectopic neurons in the white matter in 3 cases and cortical lamination disorganization of various degree also in 3 cases. This group of DNT tumors presents the features of dysembryoplastic disorder and neoplastic process. Dysembryoplastic cortical disorders are present also in the area surrounding typical foci of gliomas as well as in the patients with intractable epilepsy. The above findings may suggest common participation of dysembryoplastic changes in various groups of epileptic patients.

INTERRELATED DISTURBANCES OF INTRA- AND EXTRA-SYNAPTOSOMAL TRANSPORT OF VARIOUS NEUROTRANSMITTERS AND THE PATHOMECHANISM OF HEPATIC ENCEPHALOPATHY

Prof. Jan Albrecht

The effects of moderate hyperammonemia and *in vitro* treatment eith ammonia on the uptake of different neurotransmitters into synaptic vesicles and on the synaptic vesicular H^+-ATPase activity - an enzyme that provides energy for the uptake were investigated. Ammonia added *in vitro* at concentrations simulating acute hyper-

ammonemia *in vivo* stimulated the uptake of glutamic acid (GLU), but not the GABA or domine uptake. This stimulation is considered to contribute to increased GLU exocytosis usually accompanying acute, but not moderate hyperammonemic conditions. Increased GLU uptake may be causally related to the increase of H^+-ATPase also noted in ammonia treatment. None of the parameters under study was affected by moderate hyperammonemia in two different experimental models.

Experimental hepatic encephalopathy (HE) in the thioacetamide model has been shown to depress the release of newly labelled dopamine from striatal slices in response to stimulation of the NMDA class of GLU receptors. Impaired dopamine release is hypothesized to play a role in HE-induced motor disturbances.

Studies have been continued on the role of taurine (TAU), the putative CNS osmoregulator, in hyperammonemic brain edema. The effects of HE in the thioacetamide model and simple hyperammonemia (HA) in the ammonium acetate model on TAU release from cerebral cortical (c.c.) and basal ganglia (b.g.) slices were measured. HE-induced increase of water content in c.c. coincided with an increased spontaneous TAU release from c.c. slices. However, TAU release from c.c. induced by a hypoosmolar medium was not affected by HE indicating undistrurbed osmoregulation. TAU content in c.c. was not altered by HE: its increased release was partly compensated by an increased blood-to-brain transport of the amino acid, as manifested by an increased "brain uptake index" *in vivo*. In the HA model in b.g. slices an increased spontaneous release of TAU coincided with a decreased stimulated release indicating an impaired osmoregulatory capacity. The decreased osmoregulatory response may be related to the HA-induced decrease of TAU content in b.g.

MECHANISM OF AMMONIA-INDUCED TAURINE (TAU) RELEASE FROM MÜLLER GLIA CULTURED *IN VITRO*

Prof. Jan Albrecht

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The mechanism of ammonia-induced TAU release from cultured Müller cells has been shown to differ in many aspects from the release evoked by potassium, a generic release stimulus. Potassium-induced release was completely chloride- and calcium-dependent and was abolished in a hypertonic medium, in concordance with the osmoregulatory role of TAU. Ammonia-induced release turned out to be calcium-independent, only partly chloride-dependent and insensitive to hypertonicity. The enhanced magnitude of stimulation of the release by increasing ammonia concentrations was correlated with the elevation of cAMP levels, and the release was

abolished by an inhibitor of cAMP-dependent protein kinase. Hypothetically, the mechanism of ammonia-induced TAU release may consist in the activation of a TAU carrier (or a TAU channel) at the cell membrane, *via* its phosphorylation. This mechanism would comply with the neuromodulatory rather than osmoregulatory role of TAU in Müller glia.

THE ASSESSMENT OF CYTOTOXICITY OF 2-CHLORODEOXYADENOSINE (2-CDA) AND 2-BROMODEOXYADENOSINE (2-BDA) TOWARD ANAPLASTIC GLIOMAS

Prof. Mirosław J. Mossakowski
Supported by the State Committee for Scientific Resarch: grant # 4 4344 92 03

Organotypic cell cultures, and dissociated cell cultures were grown from biopsies of human anaplastic gliomas. In organotypic cultures it was shown with the use of electron microscopy, that exposure to 1-3 μM 2-CDA or 2-BDA for up to 10 days causes progressive destruction of mitochondria and apoptotic disintegration of nuclei. These toxic effects were not found in organotypic cultures of rat hipocampus and cerebellum. It has also been found that these toxic effects are more pronounced in smaller, more anaplastic cells. The data suggest that undifferentiated glioblastoma cells may be sesceptible to cytotoxic effects of 2-CDA and 2-BDA,but upon differentiation they become resistant to the drug. Cytograms of differentiated cultures show that cells with low protein content are clonogenic, and in some cases 24-48 h exposure to 0.1-1 μM 2-CDA causes S-phase block and apoptosis.

ELABORATION OF EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE

Assoc. Prof. Ryszard Pluta
Supported by the State Committee for Scientific Resarch: grant # 6 P207 051 05

The aim of this study was to synthesize and formulate human β-amyloid peptide for chronic infusion studies in animal model. To stabilize the monomeric form of β-amyloid peptide and increase its stability in solution, we formed a complex of the peptide with β-cyclodextrin. The complex of β-amyloid peptide with β-cyclodextrin is highly soluble in water and stabilizes the monomeric form of the peptide in solution. The complex exhibits all biochemical and immunological properties of natu-

ral human β-amyloid peptide in bio-phase. Therefore, the β-amyloid peptide-β-cyclodextrin complex can be used as a pharmacological tool for better understanding of the β-amyloid peptide role in Alzheimer's disease.

Additionally for the first time, evidence has been provided that transient global cerebral ischemia can not only lead to increased cellular β-amyloid protein precursor expression but that it also induces an increase in extracellular β-amyloid protein precursor deposits containing the N-, C-terminal, and β-amyloid peptide epitopes of b-amyloid protein precursor.

LABORATORY OF DEVELOPMENTAL NEUROPATHOLOGY
Head: Professor Maria Dąmbska
3 Ludwika Pasteura Str., 02-093 Warsaw
Telephone: 22 96 27

EVALUATION OF SELECTED CNS STRUCTURE MATURATION AND OF EXO- AND ENDOGENIC LESIONS OCCURRING DURING THIS PERIOD

Prof. Maria Dąmbska

Morphological and morphometric study of dorsal vagal nucleus of the human medulla oblongata between midgestation and one year of life demonstrated a linear increase of all parameters characterizing the pre- and post-natal process of maturation. Particularly the number of large cells increased and their segregation into four groups according to their cytoarchitectonic types became evident.

The maturation of human hippocampus between 26-40 weeks if development was morphologically examined. An individual rate of this process in all hippocampal sectors was observed. It was particularly slow in CA1 sector.

The brains of two siblings with progressive spongy poliodystrophy were neuropathologically examined. The cases were the youngest between described so far, and the changes were particularly severe demonstrating a relationship between the process of development and the degree and topography of degenerative lesions.

IMMUNOCYTOCHEMICAL AND ULTRASTRUCTURAL ASPECTS OF CHANGES CAUSED BY GENETIC AND TOXIC DISORDERS IN THE DEVELOPING NERVOUS SYSTEM

Assoc. Prof. Danuta Maślińska

In the brain of rabbits treated with neurotoxic doses of vincristine, three new pathomechanisms of nerve fibre degeneration have been observed:

- degeneration of the axons of "dark neurons";
- neurotoxic dystrophy of the nerve cell bodies, followed by atrophy of their axons;
- apoptosis of oligodendroglial cells, followed by myelin sheath degeneration of some nerve fibres.

It has been also demonstrated that in the peripheral nervous system affected by vincristine neurotoxicity, degeneration of myelin sheath begins in Schmidt-Lanterman incisures.

In studies performed on the autopsied brains of individuals affected by cardiac arrest, overexpression of amyloid beta protein was found in numerous neurons and extracellular amyloid deposits scattered throughout the brain gray matter. Those deposits underwent remodelling and/or dissolution during survival after resuscitation.

In developing CNS of control and Down syndrome (DS) fetuses and children, epitops of amyloid beta protein were found only in some maturing neurons (spinal cord) of 20 weeks old DS fetuses.

LABORATORY OF THE ULTRASTRUCTURE OF THE NERVOUS SYSTEM
Head: Professor Jerzy Borowicz
3 Dworkowa Str., 00-784 Warsaw
Telephone: 49 54 20

EVALUATION OF THE INFLUENCE OF ISCHEMIA ON THE PICTURE AND COURSE OF REPARATIVE PROCESSES IN CHOSEN BRAIN STRUCTURES (HYPOTHALAMUS, BRAIN STEM, CEREBRAL CORTEX) WITH THE HELP OF MORPHOLOGICAL, CYTOCHEMICAL AND IMMUNOCYTOCHEMICAL TECHNIQUES

Prof. Jerzy Borowicz

During the accounted research period the remote results of total circulatory arrest were investigated. It has been shown that reparative processes occurring on the boundaries of the damaged perivascular zones amount of collagen fibrils in order to join the separated areas. Perivascular phagocytes have been located in these zone. Their presence depended on the time survival after reanimation and their number increased with this lenght of time. These phagocytes are multipotential cells and they participate in the processes of phagocytosis and enzymatic degradation of the necrotic elements of the neuropil following an incident of clinical death. The study of the zones well removed from the capillary vessels was concerned with the microglia. Attention was paid to the differentiation of the cytoplasmatic processes of the microglia, changes of the cytoplasmatic skeleton and the possibility of the migration of microglia within the neuropil. The results of the study revealed that the changes in the brains of animals that underwent cardiac arrest with subsequent reanimation increase as the time passes, which is further supported by the mobilisation of the protective system represented perivascular phagocytes and active dendrified microglia.

Electron microscopic immunocytochemical studies were carried out according to the immuno-gold method.

Studies of the hypothalamo-neurophyseal system concerning localisation of 5-HT and revealed presence of this neurotransmitter in very few pre- and postsynaptic parts of neurons of the paraventricular nucleus, while the neurons of the synaptic nucleus and the neurohypophysis did not reveal any 5-HT characteristic immunogold staining. It is therefore supposed that the serotoninergic system does not significantly affect synthesis and release of the posterior pituitary hormones.

Apart from the planned work ultrastructural examination of the thalamic arcuate nucleus of ovariectomized animals following application of estradiol and progesterone were carried out. Rapid reactions of this nucleus were observed only after progesterone injections. It is supposed that the arcuate nucleus may participate in the regulation of LH-RH release from the axonal endings in the median eminence.

Laboratory also participated actively in other research projects of the Institute.

DEPARTMENT OF NEUROSURGERY
Head: Professor Zbigniew Czernicki
16 Barska Str., 02-315 Warsaw
Telephone: 22 36 43

EVALUATION OF DISTURBANCES IN THE DYNAMICS OF INTRACRANIAL HOMEOSTASIS BY STUDYING VOLUME-PRESSURE RELATIONS OF CEREBRAL CIRCULATION AND COGNITIVE FUNCTION

Prof. Zbigniew Czernicki

In the reported period surgical activities of the clinic increased considerably. The number of operations grew by 25%, regular operations of cerebral aneurysms and operative neurotraumatological duties for the expanded region of town were started. Undoubtfully it contributed to the intensification of clinical research. Since it is necessary to collect material for further statistical elaboration in case of clinical studies not all of them can be presented in the form of printed publications.

In 1994 organizational effeorts of the clinic were aimed at the preparation of international symposium on intracranial pressure and cerebral ischemia.

Disturbances of cerebrospinal fluid circulation in patients with hydrocephalus were consequently studied in the reported period. New, improved three-phase infusion test was introduced into clinical practice which allows a separate evaluation of resorption resistance, outflow resistance and values of persisted facilitation in cerebrospinal fluid outflow. Results of treatment of hydrocephalic patients were verified by neuropsychological tests. They showed improvement of cognitive functions especially the most affected ones in the patients treated with valve shunt. Evaluation of cerebrovascular reactivity using transcranial Doppler sonography was proved to be of significant clinical usefulness in evaluation of patients status. Studies of erythrocyte aggregability changes in patients treated with mannitol showed that mannitol administration contributed to the aggregability increase and could negatively influence microcirculation. Evoked somatosensory potentials were studied in electrophysiological research. Clinical usefulness of evoked somatosensory potentials in evaluation of cervical disc herniations was proved. The dynamics of changes in evoked potential records in treatment of foci brain lesions was determined. Studies of evoked somatosensory potential brain mapping have been started in cooperation with the Institute of Experimental Biology (Warsaw).

Evaluation of patients with stereotactic brain lesions revealed memory disturbances in patients with hippocampal lesions. No relation between the type of disturbance and the lesion side was observed.

In experimental research the influence of Ca-canal blocker Dotarizine on cerebral vessels was evaluated. Dilation of cerebral vessels and an increase in cerebral blood flow was proved in animals subjected to hypoxemia and simultaneously treated with the blocker. In another experiment it was shown that Dotarizine reversed the effect of cerebrovascular constriction in the presence of hypocapnia. In studies of cerebral ischemia in gerbils a high correlation between the reduction of CBF and decrease in cerebrovascular reserve was ascertained.

CHARACTERISTICS OF POWER SPECTRUM IN PRIMARY AND SECONDARY EPILEPTIC FOCI OBTAINED USING THE METHOD OF BRAIN MAPPING

Prof. Eugeniusz Mempel

Automatic analysis of frequency and power spectrum and topographic EEG mapping were performed in 20 patients with generalized tonic-clonic seizures and so-called small seizures of "petit mal" or "absence" type. In this kind of seizures the localization of epileptic foci having clinical importance is generally unattainable by means of traditional EEG evaluation.

Among the patients examined localization of single pathological foci was obtained in 6 cases. In 4 cases the coexistence of 2 separate epileptic foci of different localization in both cerebral hemisheres was proved. In 1 case 3 independent epileptogenic foci were identified. The last case, after thorough analysis, was verified by corticography performed upon bilaterally exposed frontal regions of the brain. Corticography confirmed the results of frequency and power spectrum analysis obtained by the method of brain mapping. On this basis we performed 2 frontal topectomies and observed an expected improvement in the operated patient. In 9 patients the exact localization of epileptic focus could not be identified by means of brain mapping.

Our studies showed that in some cases of epilepsy, especially those difficult to diagnose, the method of frequency and power spectrum analysis in brain mapping may be useful for localization of the pathological foci. It was verified by corticography and surgery in the case of a patient with 3 separate pathological foci diagnosed by this mathod. Two of these foci could be removed during one surgery.

The results of brain mapping always have to be confronted with clinical data and results of other modern diagnostic methods like computerized tomography or magnetic resonance imaging of the brain. In our study we did it in every case. The

latter methods are very important diagnostic tools in brain diseases. Nevertheless, our observations showed, that there are epileptic foci without distinct structural changes on CT and MRI scans like in the case of our operated patients.

NEUROMUSCULAR UNIT
Head: Professor Irena Hausmanowa-Petrusewicz
1a Banacha Str., 02-097 Warsaw
Telephone: 658 45 01

STUDIES ON MUSCULAR IMMATURITY IN SOME CONGENITAL NEUROMUSCULAR DISEASES

Prof. Anna Fidziańska-Dolot

Over two years of our study a new syndrome with specific structural defect in skeletal muscle architecture was identified in children. In eight children with progressive congenital myopathy, representing 4 different families, abnormal accumulation of myofibrillar protein was found. Myopathy was characterized by the presence of fine hyaline plaques devoid of oxidative as well as ATP-ase enzyme activity. At the ultrastructutal level plaques were composed of helical filaments which showed strong desmin and ubiquitin immunoreactivity. In addition they were also dystrophin positive.

The exclusive appearance of desmin and dystrophin positive plaques in muscle of 8 children emphasizes uniquess of these plaques.

Regenerative capability of the senile rat muscle in response to injury was studied by means of immunocytochemistry and electron microscopy. It was shown that senile muscle retained its ability for regeneration up to advanced age (33-36 months). However, regeneration process was delayed, non-synchronous and the number of newly regenerated fibres was lowered.

Recently we have started to study the phenomenon of muscle cell apoptosis-programmed cell death which appears to play an important role in muscle cell deletion in fatal congenital neuromuscular diseases.

SPINAL ATROPHY AND NEUROPATHY

Assoc. Prof. Katarzyna Rowińska-Marcińska

The method for quantitative evaluation of the Motor Unit Action Potentials (MUAP) irregularity, developed recently by Zalewska and Hausmanowa-Petrusewicz has been applied to the potential analysis in myopathic lesions (DMD and BMD) and neurogenic lesion (SMA). The aim of this study was to find out

whether there are any differences in the potential irregularity in considered groups of patients. The introduced method evaluates only the potential shape irregularity independently of other features such as amplitude and duration. Thus, it gives supplementary information to the conventional set of potential parameters. It turned out that the mean level of the irregularity coefficient is almost equal in both types of lesion (myo- and neurogenic) but the relation between number of phases and turns and irregularity coefficient is different.

The morphological changes in sural nerve in immunological process have been studied. The authors consider a possibility of variable changes in peripheral nerve, underlying that in some cases the morphological changes and neuropathic symptoms do not appear simultaneously.

The main achievement of this work is the application of the method for quantitative evaluation of complex potentials.

ELECTROPHYSIOLOGICAL AND IMMUNOLOGICAL CORRELATIONS AND MORPHOLOGICAL STUDIES IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

Prof. Irena Niebrój-Dobosz

Electrophysiological studies

112 patients with ALS were examined. In none of them a conduction block was present. Conduction block appeared in three cases suspected of ALS. It was, however, not confirmed either because the diagnosis appeared not to be the right one, or the conduction block in the next electrophysiological examination was not present any more. The conduction block was present in five out of 138 cases with Guillain-Barre syndrome, in 2 patients with hereditary neuropathies and also in one case with pressure neuropathy. In motor neuropathy despite of clinical improvement conduction block persisted in an unchanged form.

The conduction block was examined in a way described previously (Hausmanowa-Petrusewicz I, Rowińska-Marcińska K, Kopeć A "Chronic aquired demyelinating motor neuropathy", *Acta Scand Neurol* 1991, 84, 40-45).

Immunological studies

The titer of anibodies against GM1, AGM1 and Sulfatides in serum were tested in 44 patients with ALS. In 40 of the cases the titer of the anibodies was also examined in the cerebrospinal fluid. The results of the antibody titer and also those of IgG, IgA, IgM and paraprotein content are given in the following table:

	anti-GM1	anti-AGM1	anti-sulfatides	IgG	IgA	IgM	Paraproteins
serum	10/44	20/44	9/44	10/43	21/42	2/42	absent
CSF	14/40	11/40	9/40	1/43	absent		absent

The numerator means the number of patients with abnormal values of the titer, the denominator means the number of tested cases. Elevated titer against the gangliosides was also present in two cases of motor neuropathy.

Morphological studies

Histopathological studies of the brain and spinal cord white matter were performed in six autopsies of ALS cases. It was stated that the white matter deterioration is extended outside the central and peripheral motoneuron. It indicates the necessity to confront it with MRI interpretation, as well as with the results of histological and immunocytochemical methods.

Recently examinations of the grey matter of ALS cases have been started. Histological and immunohistochemical methods are used.

The main research achievements

Our results indicate that the opinion according to which the presence of conduction block permits to distinguish the acquired neuropathies from the congenital ones is wrong. Our results clearly indicate that the conduction block is present also in congenital neuropathies.

Up to now we have no clear opinion whether there is any relationship between the presence of conduction block and the titer of antibodies against gangliosides and sulfatides. In pure motor neuropathies high titer of anti-GM1, anti-sulfatides and anti-MAG are present.

IMMUNOLOGICAL MARKERS: THEIR DIAGNOSTIC SIGNIFICANS IN POLY- AND DERMATOMYOSITIS

Prof. Irena Hausmanowa-Petrusewicz

The studies carried out in collaboration between the Neuromuscular Unit and Laboratory of Molecular Immunology NIH (Bethesda, USA) were continued. Ninty five cases of inflamatory myopathies were examined using immunofluorescence and double diffusion assay. The same cases were examined by dr. Miller for immunogenetic markers. Dr. Kowalska as a fellow of MDA of America, has performed

similar studies in Tuscon (Arizona) on the Latin-American population.

Preliminary results of this study point to the association of 20% cases dermatomyositis (DM) in Poland with immunological marker incidence - Mi 2 was detected mainly in cases of mild DM. This is the first report on detection of Mi 2 in Poland. The other marker Ku, considered to be typical for Aziatic regions, was found in some cases of polymyositis associated with connective tissue diseases.

Fourty seven cases of scleromyositis, confirmed by the presence of PM-Scl antibody were characterized clinically and electrophysiologically. The therapeutic attempt to apply small or moderate doses of steroids was successful.

The detection of above markers allowed their clinical application because of diagnostic and prognostic significance.

MORPHOLOGICAL, ELECTROPHYSIOLOGICAL AND GENETIC STUDIES ON MUSCULAR DYSTROPHIES (OTHER THAN X-LINKED)

Prof. Irena Hausmanowa-Petrusewicz

Seventy five cases diagnosed as limb girdle dystrophy (LGMD) during the period 1980-1993 were re-checked. On the basis of muscle biopsy (detection of dystrophin) and DNA test, 23 cases were separated from this group since they tourned out to be dystrophinopathies (mostly fitting into the group of Becker type). The remaining 52 cases were re-analysed clinically and to study the mode of transmission they are prepared for DNA testing in collaboration with the Institute of Genetics in Newcastle, England. The aim of the study is to check a possible mapping to chromosomes 2, 13 or 15.

On mice mdx the hind limb muscles were compared with diaphragm at different periods of life span. Diaphragm is much more affected by dystrophic process than the hind limb muscles and the occurring changes are similar to those in human muscle dystrophy. To our knowledge, it is the first study comparing dystrophic process in diaphragm and in hind limb muscles of mdx.

ANALYSIS OF DNA IN DUCHENNE AND BECKER DYSTROPHY AND IN CHILDHOOD SPINAL ATROPHY

Prof. Irena Hausmanowa-Petrusewicz
Supported by the State Committee for Scientific Research: grant # 4 0779 91 01

Summary of the results

1. Introduction in Poland DNA tests in x-linked dystrophies and evaluation of the gene product - muscle dystrophin.
2. Analysis of the interrelationship between clinical phenotype, deletion and dystrophin amount.
3. Possibility to determine carriership and to perform prenatal diagnosis in Duchenne and Becker dystrophies, as well as in spinal muscular atrophy.
4. Characterization of atypical cases of Becker dystrophy: their pheno- and genotypes.
5. Possible presence of dystrophic changes in females.
6. Clinical-genetic analysis in spinal muscular atrophy which indicates intrafalimial clinical variability and male preponderance.
7. Participation in the study on linkage disequilibrium in Polish families with spinal atrophy.

IMMUNOCYTOCHEMICAL STUDIES IN NEUROMUSCULAR DISORDERS

Dr. Anna Kamińska
Supported by the State Committee for Scientific Research: grant # 4 0879 91 01

Continuing the previous studies on basement membrane components in neuromuscular disorders it was shown, that chronic denervation of rat skeletal muscle resulted in elevation of fibronectin and - to a lesser extent - laminin in muscle.

The effect of the variety of noxious stimuli such a: bupivacaine, notexin and cold on immature rat skeletal muscle was the subject of our electronmicroscopic studies. It was demonstrated, that despite of the nature of noxious agent, immature muscle cells respond to the injury with apoptosis but not necrosis.

Immunocytochemicals studies of biopsies of patients with spinal muscular atrophy (SMA), and Duchenne muscular dystrophy (DMD) were continued.Increased activity of desmin and vimentin as well as of laminin and fibronentin in small muscle fibres in SMA, suggested their immaturity. In DMA atrophied fibres, showing increased fluorescence of desmin and vimentin as well as high acridine orange activity, result probably from the regenerative processes observed in this disease.

N-CAM AS A MARKER OF MUSCLE CELL IMMATURITY

Prof. Anna Fidziańska-Dolot
Supported by the State Committee for Scientific Research: grant # 4 S405 005 06

Muscle specimen from two neonates with fatal X-linked recessive form of central nuclear myopathy were studied by immunohistochemistry using neural cell adhesive molecules (N-CAM). In both neonate muscles fibres with the myotubes strongly expressed N-CAM. The expression of N-CAM in postnatal human muscle suggests that neonatal X-linked fatal form of centronuclear myopathy is characterized by an arrest of muscle fibre maturation.

MORPHOLOGICAL STUDIES OF THE SENILE RAT SKELETAL MUSCLE

Prof. Irena Hausmanowa-Petrusewicz
Supported by the Deutsche Forschungs Gemeinshaft: grant # 436/Pol 133/63

In our previous study skeletal muscles of senile rats with significant age-dependent functional deficits, examined by light microscopy, showed signs of advanced neurogenic atrophy.

Electron microscopic studies of such muscles showed a variety of changes such as: 1) marked atrophy of muscle fibers; 2) severe muscle fiber fragmentation; 3) aggregation and deformity of nuclei; 4) basement membrane changes with the presence of loops and bridges connecting split muscle fragment; 5) structural changes of muscle fibres with the disorganisation and atrophy of myofibrils and replication of scarcoplasm reticulum membranes.

Such constellation of changes is consisted with chronic denervation.

Experimental studies of induced regeneration in senile skeletal muscle were completed. It seems, that severely atrophied senile muscle retains its ability to regenerate. Regeneration, however, is delayed and non synchronous.

DEPARTMENT OF APPLIED PHYSIOLOGY
Head: Professor Hanna Kaciuba-Uścilko
17 Jazgarzewska Str., 00-730 Warsaw
Telephone: 41 25 05

HEMODYNAMIC, METABOLIC AND HORMONAL RESPONSES TO PHYSIOLOGICAL STIMULI IN RELATION TO AGE AND IN HYPERTENSION

Prof. Krystyna Nazar

Studies concerning a course of changes in the plasma growth hormone (HGH) and blood lactate (LA) concentrations during graded incremental exercise were continued in male athletes different age (from 15 to 56 years). It was found that sensitivity of the hypothalamo-pituitary system to exercise stimulus is markedly enchanced in youngsters in comparison with adult subjects, decreasing with age. A significant correlation was ascertained between the plasma HGH and blood LA levels, whereas no relationships were found between the calculated LA and HGH thresholds. The latter findings suggests lack of causality between LA accumulation in blood and an increase in HGH release.

In order to establish mechanisms underlying the LA threshold effects of prolonged hyperadrenalinemia (up to 60 hours), and experimental hypo- and hyperthyroidism changes in blood LA concentrations were followed in rats during graded treadmill exercise with increasing (to the maximal) intensity. Methodology of the LA threshold estimation in this animal species was previously elaborated in our Department. It was demonstrated that moderate hiperadrenalinemia sustained for 12 hours increases the LA threshold towards higher work loads. Similar effects were noted in the rats with triiodothyronine deficit and exercise. These findings indicate that both adrenaline and triiodothyronine play an important role in determining the anaerobic threshold.

In cooperation with the Institute of Sport (Warsaw), and Department of Sport Medicine of Military School of Medicine (Łódź) studies were continued on development of physical capacity in children and youngsters involved in various sports activities such as swimming, athletics, ski running and untrained. No meaningful effects of training were found on the ability for anaerobic exercise. In boys a significant correlation was ascertained between indices of exercise performance and plasma testosterone concentration, whilst there were no relationships between these indices and resting or post-exercise levels of the plasma growth hormone.

Four-year studies concerning metabolic effects of physical training of girls at the age of 11-15 years have been completed. It was found that training at this age does not affect negatively either somatic development or sex maturation. Besides, it was demonstrated that increased physical activity of the girls results in a slight decrease of their resting metabolic rate and enhancement of the thermogenic effect of glucose. In majority of untrained girls the latter effect did not occur.

A relationship between hemodynamic changes as well as duration of systolic time intervals caused by postural change (from laying to standing position) and age of subjects (20-59 years) has been analized. It was found that ageing causes a decrease in the rate and magnitude of changes in heart rate and cardiac output, and diminishes the ratio of pre-ejection periods to total electromechanical systole and duration of R-R in electrocardiogram.

The effect of prolonged restriction of physical activity on exercise-induced changes in body temperature and skeletal muscle metabolism was investigated in dogs. It was demonstrated that cage confinement accelerates development of exercise hypothermia. This effect can be partly reversed by intravenous glucose infusion during exercise. It was also found that inactivity results in alterations in substrate utilization by skeletal muscles and reduces ATP resynthesis.

Studies on the effects of dehydration on thermoregulation were continued. It was shown that in the rat (opposite to other species) dehydration, induced by 24 hrs water deprivation, does not impair body temperature regulation during exercise. At rest, it increases, however, body temperature during exposure to high ambient temperature. There were no differences in the magnitude of core temperature on heat exposure between young (6 months) and old (18 months) rats.

In cooperation with the National Institute of Cardiology, studies on hemodynamic, hormonal and metabolic responses to oral glucose load in patients with hypertension have been started.

INTERACTIONS BETWEEN LIPID AND CARBOHYDRATE METABOLISM IN SKELETAL MUSCLE

Assoc. Prof. Leszek Budohoski

Continuing studies concerning factors affecting lipid metabolism in skeletal muscle, validity of our model for studying the effects of fatty acid (FA) availability on lipid metabolism in skeletal muscle was further confirmed using immunochemical methods. It was demonstrated that albumin-bound fatty acids (FA), added to the incubation medium, penetrate well to the centre of a muscle sample. Also, using thin layer chromatography, a relationship between incorporation of FA into various

classes of intracellular lipids and FA concentration in the medium was determined. Palmitic acid was incorporated into acylglycerol and phospholipids pools, however, incorporation into phospholipids decreased with an increase in FA concentration in the medium.

Several experiments were performed to study a relationship between FA availability in the incubation medium and the rate of intracellular transport of glucose to the rat soleus muscle. The results obtained so far indicate that only high FA concentrations inhibit the insulin-stimulated glucose transport.

Preliminary experiments were performed in order to establish the effect of the rat hindlimb denervation on lipid and carbohydrate metabolism in the soleus muscle. It was found that sensitivity of lactate production and glycogen synthesis in response to insulin, measured *in vitro*, becomes depressed already after 12 h following denervation. Addition of adenosine antagonist, 8-phenyl-theophylline, to the incubation medium reversed the changes induced by denervation. Besides, in the soleus muscle samples taken after 12 h following denervation, lipoprotein lipase (LPL) activity and triacylglycerol (TG) content were measured. The data obtained so far indicate that denervation causes a decrease in activity of both forms of the enzyme (heparin-releasable and residual), without any significant changes in TG content.

For better explanation of the relationship between lipid and carbohydrate metabolism in skeletal muscle under various experimental conditions, studies will be continued using both rat hindlimb perfusion and *in vitro* techniques. Last year, a unique modern equipment necessary for these experiments has been completed.

In the preliminary studies with hindlimb perfusion it was proved that the rate of FA incorporation into intracellular triacylglycerols in the rat skeletal muscles depend on free fatty acid concentration in the perfusate, time of perfusion and the rate of perfusion fluid flow.

Additionally, in co-operation with the Department of Physiology CNRS, University of Lyon (France), snsitivity of the rat soleus muscle to insulin *in vitro* was studied after hindlimb suspension. It was demonstrated that this type restriction of physical activity results in an increased rate of lactate formation, glycogen synthesis, and glucose transport. This effect was observed already after 24 h of hindlimb suspension, and it was maintained for five weeks. It seems possible that the effect can be attributed to the increased content of glucose transporting protein GLUT-4, and/or to the decreased number of affinity of adenosine receptors.

URINE CONCENTRATION AND DILUTION IN THE EXPLANTED KIDNEY AND IN THE STANDARD ACUTE KIDNEY PREPARATION: ROLE OF ANESTHESIA

Prof. Janusz Sadowski
Supported by the State Committee for Scientific Research: grant # 4 1310 91 01

We have developed recently a new experimental model of rat kidney chronically explanted under the skin, to enable an access to the organ and studies of its function without deep anesthesia and acute surgery. In the present work we designed experimental procedures for the study on the explanted kidney under two opposed functional conditions: high urine concentration and dilution. In euvolemic and moderately volume expanded Wistar rats the ability to concentrate and to the dilute urine was examined under i.v. α-glucochloralose/ethanol (Chl) and under i.v. Inactin/ethanol (In) anesthesia. For comparison, the kidney surgically exposed in an acute experiment under i.p. Inactin anesthesia was also examined. Urine osmolality (Uosm) in excess of 1000 mosm/kg H_2O was found in euvolemic Chl anesthetized rats; distinctly lower and highly variable Uosm was seen in the In group. Volume expansion with 10% ethanol in Ringer solution (2.5% B.W., infused for 1h) lowered Uosm slightly in the Chl and very markedly in the In group. Dilution of urine below isotonicity in response to a water load (2.5% glucose solution infused at 12 ml/h) was seen in all groups. Ethanol addition (10%) to glucose solution did not increase water diuresis. The data indicate that both urine concentration and dilution can be induced in the model of the explanted rat kidney when animals are anesthetized with Chl. Achievement of high Uosm under In was difficult. Both under Chl and In the kidney diluted urine in response to a water load.

RISK FACTORS OF PRIMARY HYPERTENSION

Prof. Krystyna Nazar
Supported by the State Committee for Scientific Research: grant # 4 1310 91 01

The study on hemodynamic, metabolic and hormonal characteristics of normotensive young men with exaggerated blood pressure (BP) response to exercise has been completed. As a criterion of abnormal BP response, systolic BP (SBP) > 200 mmHg at 150 W or lower work load during graded bicycle ergometer test was accepted. Fifteen students with elevated exercise BP (group E) volunteered for examinations. Their resting and ambulatory BP measurements showed high normal values. Eight of them had family history of hypertension. The mean left ventricular

mass index (LVMI) in this group was 127±(SD)15 g·m^{-2}, with 4 subjects meeting the cardiac hypertrophy criteria. Significant correlations were found between exercise SBP and LVMI or average 24 h and day time SBP recordings. In comparison with normal subjects of the same age (group N, n=13) those from group E had similar body mass index, plasma lipid profile, fasting glucose, insulin and catecholamine (CA) concentrations, but showed increased erythrocyte sodium content, slightly elevated resting plasma renin activity, and cortisol level. During exercise E subjects exhibited greater cardiac output (CO) increases than N subjects without differences in heart rate, total peripheral resistance (TPR), and plasma CA. There were no significant differences between groups in hemodynamic and plasma CA responses to posture change from supine to standing. Glucose ingestion (75 g) caused smaller increases in CO and smaller decreases in TPR in E than in N subjects without differences in BP, blood glucose, plsama insulin and CA.

It is concluded that young normotensive men with exaggerated BP response to exercise show some characteristics that may be considered as markers of predisposition to hypertension or factors promoting development of hypertension.

THE EFFECT OF LOW-CARBOHYDRATE DIET ON PHYSIOLOGICAL RESPONSES TO EXERCISE IN MEN

Prof. Hanna Kaciuba-Uścilko

Supported by the State Committee for Scientific Research: grant # 4 S404 028 07

The purpose of the study completed this year was to find out whether three day low carbohydrate (CHO) "ketogenic" diet affects maximal oxygen uptake (VO_2max) and anaerobic threshold (AT) as well as blood pH, metabolite and some hormone concentrations after graded, incremental exercise. Eight untrained volunteers (aged 22±0.9 yrs) participated in this investigation. After overnight fast, they performed bicycle exercise tests until volitional exhaustion (1) after three days of controlled normal diet (134 KJ/kg/day, 60% CHO, 20% fat and 20% protein), (2) after three days of ketogenic diet of the same energy content (50% fat, 45% protein and 5% CHO). In comparison with normal diet, the ketogenic diet resulted in elevation of resting plasma β-hydroxybutyrate (BAOH), free fatty acid (FFA), adrenaline (A), noradrenaline (NA), and cortisol concentrations. Resting levels of insulin and blood lactate (LA) were decreased without significant changes in blood glucose. Respiratory exchange ratio was significantly decreased both at rest and during exercise, while oxygen uptake was enhanced during exercise and VO_2max was elevated without differences in maximal heart rate. Although post-exercise blood pH was lowered after ketogenic diet, LA was reduced and AT occurred at higher work load

(170 vs 138 W, $p<0.05$). During 1h-recovery BAOH decreased after ketogenic diet, whilst at normal diet it rose. Plasma FFA and blood glucose did not change significantly during and after exercise under both conditions. Similarly to initial values the post-exercise plasma catecholamines and cortisol were enhanced, whilst plasma insulin diminished after ketogenic diet.

It is concluded that low cardohydrate diet does not impair aerobic exercise capacity as indicated by enhanced VO_2max and AT. This may be due to elevated ketoacid and FFA production and utilization. Stimulation of synaptho-adrenal system may be also of some importance for preservation of working capacity.

INFLUENCE OF ORAL CONTRACEPTIVES ON SWEATING AND SHIVERING IN WOMEN EXPOSED TO ENDOGENOUS AND EXOGENOUS HEAT LOADS

Assoc. Prof. Ryszard Grucza

Supported by the State Committee for Scientific Research: grant # 4 0216 91 01

Intake of oral contraceptives reduces menstrual phase related differences in the temperature gain for sweating (Grucza et al. *Eur J Appl Physiol* 67:279-285, 1993). However, it is not clear whether the effect is specific only for sweating response to exercise, or whether it constitutes a constant feature of the thermoregulatory system. The aim of this study was, therefore, to investigate the phase related changes in temperature gain for shivering. Seven young women, taking oral contraceptives over 2 years, participated in this study. The subjects, wearing only T-shirts and shorts, rested in a climatic chamber. They were exposed for 30 min to ambient temperature of 4°C and to speed of air equal to 4 m/s. The experiment was repeated for quasi-follicular (qF) and quasi-luteal (qL) phase of the menstrual cycle. Rectal (Tre), mean skin (Tsk) temperatures, integrated electromyographic activity (IEMG) were measured in the subjects. Cold exposure caused a decrease in Tsk by 13.6 and by 14.6°C, and in Tre by 0.54 and by 0.53°C for both menstrual phases, respectively. The onset for shivering was faster in qF (1 min) than in qL (4 min), $p<0.01$. IEMG showed significantly greater muscle activity for qF (1886 μVs) than for qL (1354 μVs). The Tre gains for shivering were 3.19 mVs/°C and 2.49 mVs/°C, $p<0.05$, respectively. It can be concluded that intake of oral contraceptives reduces menstrual phase related temperature gain for sweating during exercise but it does not influence the temperature gain for shivering in cold exposed women. The results obtained suggest different sensitivity of thermoregulatory mechanisms to heat and cold stress in women under oral contraceptives.

IMPORTANCE OF INTERRELATIONSHIPS BETWEEN AGE, BODY MASS AND PHYSICAL WORKING CAPACITY IN DETERMINING METABOLIC RESPONSES TO GLUCOSE LOAD IN MEN AND WOMEN

Dr. Andrzej Ziemba

Supported by the State Committee for Scientific Research: grant # 4 0219 91 01

Oxygen uptake (VO_2) and CO_2 production (VCO_2), blood glucose (BG), plasma insulin (IRI), adrenaline (A) and noradrenaline (NA) concentrations were measured after ingestion of glucose (75 g) in 48 healthy men (23-67 yrs) and 57 healthy women (21-57 yrs). Body mass index (BMI) in men ranged from 18.8 to 40.8 and in women from 18.9 to 40.1 kg/m^2. Maximal oxygen uptake (VO_2 max), determined during incremental bicycle exercise test, was 2.3±0.15 and 1.7±0.10 l/min in men and women respectively. In addition, in 14 overweight women (BMI 29.2 to 45.1 kg/m^2) aged 31 to 53 yrs, metabolic and hormonal responses to glucose were determined before and after 4 and 8 weeks of energy restricted diet (4.2 MJ daily).

Resting metabolic rate (RMR) and thermal effect of glucose (TEG) were calculated from VO_2 and VCO_2. TEG as well as BG and IRI responses to glucose were calculated as areas under the curves (auc) obtained from 2h determinations following glucose ingestion.

Women showed significantly smaller RMR than men (3.79±0.15 vs 4.60±0.14 KJ/min, $p<0.001$) and their TEG was smaller (38.0±4.0 vs 55.7±5.2 KJ, $p<0.001$), but there were no differences between sexes in relative values of these variables (RMR - 53.0±1.8 vs 56±1.2 J/min/kg, TEG - 8.04±0.92 vs 8.86±0.81 % of total energy expediture during 2h). Responses of BG and catecholamines to glucose load were similar in men and women, whereas those of plasma IRI were greater in women than in men (6947±687 vs 4856±502 μU/ml/min, $p<0.01$).

In men significant correlations were found between TEG (kJ) and BMI ($r=-0.37$), IRIauc ($r=-0.65$), BGauc/IRIauc ratio ($r=-0.55$) and the maximal post-glucose plasma NA level ($r=-0.79$). Similar correlation coefficients were ascertained when TEG was expressed as percentage of total energy expenditure. In women significant relations were found between TEG (kJ) and age ($r=-0.30$, BMI ($r=-0.31$), and VO_2max, IRIauc ($r=-0.38$), BGauc/IRIauc ratio ($r=-0.43$), and the maximal post-glucose plasma NA level ($r=-0.65$).

Eight week energy restricted diet resulting in body mass loss of 7.9±0.4 kg, caused significant decreases in fasting IRI level (20.4±2.0 vs 28.0±3.2 μU/ml, $p<0.05$, BGauc (226±34 vs 273±43 mmol/l, $p<0.05$), initial plasma NA (1.31±0.16 vs 1.80±0.21 mmol/l, $p<0.05$) and maximal post-glucose NA (2.19±0.22 vs 2.83±0.33, $p<0.05$). RMR showed a tendency towards elevation while TEG tended

to decrease 53.4±14.6 vs 62.2±12.6 KJ, ns. and 7.1±1.7 vs 8.88±1.27 , ns.).

The present results demonstrated that the magnitude of thermogenic effect of oral glucose in subjects of both sexes depends to a large extent on insulin response to glucose load and the glucose-induced activation of sympathetic nervous system. The modest inverse relationship between BMI and TEG was found but dietary reduction in body mass did not increase TEG. Significant negative correlation between TEG and age and positive between TEG and physical capacity were found only in women. The data suggest that overweight and aging, leading to decreased insulin sensitivity, may reduce thermogenic effect of food, which in turn promotes further body mass gain.

THE CORTICO-MEDULLARY ELECTROLYTE GRADIENT IN RAT RENAL INTERSTITIUM: EFFECTS OF HORMONES CONTROLLING SALT TRANSPORT IN HENLE'S LOOP

Prof. Janusz Sadowski

Supported by the State Committee for Scientific Research: grant # 4 1310 91 01

In anesthetized rats tissue electrical admittance of the inner medulla (a measure of total ion concentration in the interstitium), outer medullary blood flow (laser-Doppler technique) and renal clearances were measured simultaneously before and during i.v. infusion of glucagon (110 and 330 ng/min kg body wt). Addmitance increased modestly, 5.4% after the large glucagon dose ($p<0.01$), while medullary blood flow was stable. The glomerular filtration rate increased transiently, and then fell during the large-dose glucagon infusion. The increase in tissue electrolyte (mostly NaCl) concentration in the medulla observed with stable medullary blood flow and decreasing glomerular filtration rate indicates that stimulation by glucagon of NaCl reabsorption in the medullary ascending limb of Henle's loop was the mechanism underlying augmentation of medullary ionic hypertonicity. This suggests that glucagon can contribute to urine concentration process.

CONSTRUCTION AND SOFTWARE DESIGN OF A DEVICE ENABLING 24-h MONITORING OF HEMODYNAMIC HEART ACTIVITY USING IMPEDANCE-CARDIOGRAPHY - RHEOMONITOR

Dr. Gerard Cybulski
Supported by the State Committee for Scientific Research: grant # 8 S506 013 05

In cooperation with Warsaw Politechnics a model of device consisting of the microrheograph and a part recording signals on PCMCIA chart was constructed basing on 85562 processor. Using this model the first version of rheomonitor was made, and tested under laboratory conditions. The data obtained served for corrections of the prototype. Electrodes for a long-term transmission of the rheographic signals are being elaborated.

The main part of the graphic programme for Windows, presenting the data collected during registration was also prepared. Simultaneously, programs analyzing rheographic signals (detection of ECG segments, calculations of systolic time intervals, values and trends of changes in stroke volume, cardiac output etc) have been worked out. Elaboration of the methods of data compression for this application is in progress.

TRIGLYCERIDE METABOLISM IN SKELETAL MUSCLE

Prof. Hanna Kaciuba-Uścilko
Supported by Polish/USA Maria Skłodowska-Curie Joint Fund II,
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In the second year of the project, studies on the relationship between fatty acid (FA) utilization by skeletal muscle and FA availability were continued applying *in vitro* and *in vivo* models. In both models fate of FA was followed using palmitate labelled with ^{3}H or ^{14}C.

In the *in vitro* experiments performed at the Department of Applied Physiology, MRC in Warsaw a series of investigations was completed on the effect of FA concentration (0.5-2.0 mmol/l) in the incubation medium on FA uptake and then their oxidation to CO_2 and triacylglycerol (TG) synthesis in the rat soleus muscle. High correlations were ascertained between medium concentration of palmitate and its incorporation into muscle TG ($r=0.90$) and FA oxidation ($r=0.60$). Thin layer chromatography (TCL) was used for separation and identification of particular lipid classes in the chloroform-methanol (2:1) muscle extract. At all medium FA concen-

trations approx. 30% of radioactivity was detected in TG. Percentage of radioactivity in phospholipid fraction (PL) decreased with FA concentration from approx. 18% at 0.5 mmol/l to 10% at 2.0 mmol/l, whilst that in mono- and diacylglycerol fraction (MG/DG) from 10 to 5%. The relative content of free FA rose with the concentration of FA in the medium from approx. 30 to 60%. In order to find out whether the free FA in the muscle extract are of intra- or extracellular origin, muscle extracellular water content was determined with ^{3}H-inulin. It was found that extracellular fluid volume in the rat soleus amounts to 260±14 µl/g w.w. It appears, therefore, that most of FA in the muscle extract may derive from the extracellular space. Thus, it seems that muscle cells (at least *in vitro*) do not accumulate free FA, but rapidly esterify or oxidize them. The total uptake of FA (calculated as the sum of palmitate molecules incorporated to TG, MG/DG, PL and oxidized to CO2 was found to correlate with medium FA concentration (r=0.85).

In the Department of Physiology, SUNY Health Science Center in Syracuse, N.Y. experiments were performed using the technique of rat hindquarter perfusion. TG synthesis was determined in three muscle types at various FA concentrations in perfusate and at different rates of perfusion. Muscle blood flow was measured using ^{85}Sr - 15 µ spheres. It was found that TG synthesis in all three muscle fiber types is dependent on plasma FA concentration but not on FA delivery *per se*.

Using the *in vitro* model (in Warsaw) investigations on the effect of experimental hyperthyroidism induced by 3-day injections of triiodothyronine (T_3) on FA utilization by rat soleus have been completed. The data indicate that T_3 excess results in increased FA uptake into muscle lipid pool (TG+PL), and only slightly enhanced FA oxidation.

OUTPATIENT CARDIAC UNIT FOR DIAGNOSIS AND THERAPY
Head: Dr. Ewa Wójcik-Ziółkowska
5 Pawińskiego Str., 02-106 Warsaw
Telephone: 658 46 43

CARDIAC FAILURE IN THE COURSE OF LONG-TERM CORONARY HEART DISEASE IN PATIENTS WITHOUT AND AFTER MYOCARDIAL INFARCTION: CLINICAL AND PROGNOSTIC ASPECTS

Dr. Ewa Wójcik-Ziółkowska

Fifty three patients with coronary heart disease (CHD) were examined. This group included 11 patients without myocardial infarction (MI) - subgroup 1, and 42 patients who had uncomplicated MI, 16±3 years before the study (subgroup 2). The results showed greater variability of ischemic changes in resting ECG ($p<0.01$), and more frequent ventricular arrhythmias above the III class of Lown ($p<0.05$) in the patients after MI than in those without MI. Only in the former group segmental contractility disturbances were demonstrated by echocardiography. The mean left ventricle end-diastolic diameter was increased in both subgroups, but it was significantly greater in the patients after MI than in those without MI ($p<0.01$). No significant differences were ascertained between subgroups in the shortening fraction (FS). Symptoms of increased end-diastolic pressure were more frequent in patients without MI than in those after MI ($p<0.01$), while the mitral valve insufficiency was more frequent in the latter subgroup ($p<0.01$). Heart volume, calculated from rtg was similarly increased in both subgroups.

ASSESSMENT OF CORONARY BLOOD FLOW RESERVE IN SILENT MYOCARDIAL ISCHEMIA BY NONINVASIVE METHODS

Dr. Ewa Wójcik-Ziółkowska
Supported by the State Committee for Scientific Research: grant # 4 S402 022 04

Comparison of the results of noninvasive assessment of coronary blood flow reserve between patients after myocardial infarction (MI) with and without angina did not reveal significant differences. This indicates necessity of special care of the patients with silent myocardial ischemia and their frequent examination using a

broad range of noninvasive tests in order to follow up the time-course of the disease. Usefulness of coronary perfusion assessment with radioisotope examination (SPECT Tc99 MIBI) and post-exercise echocardiography in patients without angina was documented. Presence of transient, and in particular of transient and multifocal perfusion deficits during exercise in patients with coronary heart disease risk factors makes diagnosis of the disease feasible despite the lack of clinical symptoms. Standard diagnostic methods, such as e.g. exercise tests, Holter ECG recordings, appeared to be inadequate for diagnosis of silent myocardial ischemia. Since motivation of coronary patients without angina for coronarography is rather low, qualification for this examination should be based on the results of several noninvasive tests.

CARDIOVASCULAR LABORATORY
Head: Professor Krystyna Cedro-Ceremużyńska
3 Dworkowa Str., 00-784 Warsaw
Telephone: 49 73 55

THE ROLE OF FREE RADICAL-MEDIATED PROCESSES IN ISCHEMIC MYOCARDIAL INJURY

CLINICAL INVESTIGATION UPON SIGNAL-AVERAGED ELECTROCARDIOGRAM IN THE COURSE OF MYOCARDIAL INFARCTION

Prof. Krystyna Cedro-Ceremużyńska

Ventricular late potentials, detected on a signal-averaged electrocardiogram, are known to be a risk factor for ventricular tachycardia and a predictor of sudden cardiac death in the postinfarction patients. Late potential activity is related to slowed and inhomogenous conduction within damaged cardiac tissue and represents an "arrhythmogenic substrate" required for ventricular tachyarrhythmias. There is an ample experimental evidence that oxygen free radicals known to be produced during myocardial ischemia alter the function of myocardial membranes in the manner that might contribute to arrhythmias, whereas antioxidants increase electric stability and resistance to ischemia and protect myocardial cells from free radical-induced damage.

Considering the above, we have hypothesized that an association exists between oxygen free radical-induced component of myocardial ischemic injury and altered electric function that underlies the genesis of ventricular late potentials in the course of myocardial infarction. If so, antioxidants (vitamins C and E) may prevent alterations of signal averaged electrocardiogram.

Sixty one consecutive patients with acute myocardial infarction were randomized to receive conventional treatment and vitamins C and E aa 600 mg/day orally for 14 days (supplemented group, n=33) or conventional treatment only (control group, n=28). Signal averaged electrocardiogram was performed on 1-2 and 9-13 (mean 10) day. Serum ascorbic acid, tocopherol and oxygen free radical production by isolated leukocytes were measured on 1-2 and 12-14 day. Signal averaged electrocardiogram was recorded with orthogonal bipolar lead configuration (XYZ) and three conventional indices were calculated. Mean value of each variable and number of patients with late potentials was evaluated according to standard criteria.

In the control group, signal averaged electrocardiography showed an increase in mean QRS ($p<0.001$) and low amplitude (below 40 μV) signal ($p<0.001$) durations

and a decrease in the root mean square voltage of the last 40 ms of QRS ($p<0.002$). In vitamin-supplemented patients, all these indices remained unchanged. There was a tendency towards fewer late potentials, and oxygen free radical production by leukocytes was decreased ($p<0.02$). Supplementation was confirmed by elevation of serum ascorbic acid ($p<0.001$) and tocopherol ($p<0.001$) levels.

The results obtained support the hypothesis that in acute myocardial infarction,oxygen free radical-induced cellular damage contributes to alterations in electric function of the heart reflected by signal-averaged electrocardiogram. In view of prognostic significance of an abnormal signal-averaged electrocardiogram in identifying patients prone to life-threatening arrhythmias and sudden cardiac death, an intervention able to prevent its abnormalities is warranted. Investigation on a larger number of patients is required to confirm preliminary results obtained in this study.

DEPARTMENT OF SURGICAL RESEARCH AND TRANSPLANTATION
Head: Professor Waldemar L. Olszewski
5 Chałubińskiego Str., 02-004 Warsaw
Telephone: 621 49 23

The experimental and clinical studies of the Department concentrate around the problem of regulation of *in vivo* immune cell traffic in the physiological conditions (in skin, liver, and peritoneal cavity) and pathophysiological, surgically - oriented states as trauma, lymph stasis, transplants and tumors. The purpose of studies is to evaluate the antigenic signals and immune and neurohormonal mediators participating in the process of mobilization and recruitment of immune cells at the site of antigen deposition, in the animal and clinical models.

THE EFFECT OF CYTOKINES ON EXTRAVASATION OF LYMPHOCYTES TO SKIN AND LYMPH NODES

Prof. Waldemar L. Olszewski

The effect of IL1 and IL2 on skin leukocyte extravasation

Extravasation of leukocytes into inflammatory lesions is regulated by interleukin 1 (IL1), 2 (IL2), 6 (IL6), 8 (IL8), tumor necrosis factor (TNFα). Besides of the effects on expression of adhesion molecules on leukocytes and ligands on endothelial cells, the cytokines participate in the process of excessive accumulation of nonspecifically activated leukocytes in inflammatory lesions. In the present study, the effects of intradermally injected cytokines IL1 and IL2 on extravasation of leukocytes and their traffic to afferent peripheral lymph was investigated in a dog model. Interestingly, IL2 had no effect on the extravasation of leukocytes when injected into the skin. IL1 caused an increase in cell extravasation, most probably both through activation of migrating cells and a direct effect on endothelial cells. It seems that local cytokine administration could be a method facilitating the traffic of immune cells to the skin. Our previous study has shown that normal human skin lymph contains significant levels of IL1 and IL6, but not of IL2. These concentrations of IL1 and IL6 may be sufficient to promote the physiological extravasation of immune cells to the skin.

Lymphatic transport of cells from hematoma

The mechanism of resorption of blood extravasated to tissue remains unknown. Thus, the lymphatic transport component of erythrocytes and leukocytes from extravasated blood was studied. Fresh autologous venous blood, without heparine, was injected subcutaneously in three locations between toes in mongrel dogs and, subsequently, peripheral lymph was collected for 6-12 hours. The concentration of erythrocytes and white blood cells was measured in fresh lymph and differential analysis of cells in MGG and monoclonal antibody-stained cytospines was performed. Electrophoretic analysis of lymph proteins was carried out. Subcutaneous injection of antologous venous blood brings about a significant increase in the lymph of white blood cells count with preponderance of neutrophils. An increase in the β-globulin fraction in lymph fluid was observed.

ORTHOTOPIC TRANSPLANTATION OF LIMB-SEARCH FOR OPTIMUM DOSAGE OF IMMUNOSUPPRESSIVE DRUGS

Prof. Waldemar L. Olszewski

Transplantation of a large mass of alloantigen in the grafted limb as well as transplantation of tissues with different immunogenicity require appropriate immunosuppressive drug dosage. Orthotopic vascularized hind limb transplantation from Brown Norway to Lewis rats was performed and recipients were treated with cyclosporine A (5 mg/kg b.w.). In the control non-treated rats, skin was totally destroyed on day 7. Desquamation of endothalial cells in the femoral artery, and mononuclear cell infiltration around vessels supplying muscles and nerves were observed. There was an increase in number of class II positive cells in the lymphoid organs of the recipients. In the CsA treated group, on day 7 skin revealed accumulation of class II positive cells under the epidermis. There were scanty infiltrates around femoral vessels. Spleen, lymph nodes and bone marrow of the recipients demonstrated few class II positive cells. In a 100-days allogeneic hind limb graft, skin underwent chronic rejection, despite CsA therapy. Muscles and nerves were degenerated, bone marrow was replaced by recipient cells, with few remaining hemopoietic cells. Lymphoid organs of recipients were depleted of cells, most likely due to CsA treatment and/or chronic response to an excess of donor antigen. The results suggest that the dosage of CsA necessary for a long-term survival of the transplanted limb should be based on the immunohistopathological picture of rejecting skin.

REGULATION OF CELLULAR REJECTION OF TRANSPLANTED TISSUES

Prof. Waldemar L. Olszewski

Tolerogenic properties of liver lymphocytes

Data accumulating during the recent years suggest that liver sinusoidal passenger cells may display tolerogenic properties upon transplantation to an allogeneic recipient. In the present study, the in vivo immuno- and tolerogenic properties of rat liver sinusoidal washout cells (LSWC) and their capacity to prolong heart allograft survival time were investigated. It was found that LSWC were less immunogenic than mononuclear cells from peripheral blood (PBMC). The degree of elimination of subsequently injected allogeneic PBMC was lower in recipient immunized with LSWC than with PBMC. The level of immunization was strain-dependent. LSWC were more immunogenic in the BN to LEW than in LEW to DA strain combinations. Lower immunogenicity after LSWC immunization correlated with the survival time to heart allografts. It was permanent after LSWC injection in the LEW to DA, and slightly prolonged (by 6 days) in BN to LEW strain combinations.

Cyclosporin A impairs the *in vivo* lymphocyte migration

It is generally acknowledged that a limited recruitment of host lymphocytes into the graft and lower rate of local proliferation of invading lymphocytes may be responsible for a diminished amount of host cellular infiltrates in allografts in recipients treated with cyclosporin A (CsA). It is also possible that less infiltrates is caused by impairment of lymphocyte migration. We have investigated the migratory properties of lymphocytes treated with CsA. The changes in migration and homing of thoracic ductus lymphocytes (TDL): 1/ naive; 2/ pretreated *in vivo* with CsA; 3/ immunized *in vivo* with alloantigen (splenocytes) and pretreated with CsA, were studied in recipients of syngeneic and allogeneic intraperitoneally transplanted skin. It was found that accumulated of naive TDL in lymphoid organs of syngeneic graft recipients was higher compared to that in recipients of allogeneic grafts. The accumulation in lymphoid organs of CsA-pretreated TDL and TDL immunized *in vivo* with allogeneic splenocytes was lower compared with naive TDL, both in allo- and syngeneic graft recipients. Conversely, TDL obianted from rats immunized *in vivo* with alloantigen and treated with CsA accumulated at a higher level in lymphoid organs of allograft recipients compared with recipients of syngeneic grafts. We conclude that CsA treatment affects the distribution and homing of syngeneic TDL to lymphoid organs of graft recipients.

MECHANISM OF BONE MARROW ACTIVATION FOLLOWING BLOOD TRANSFUSION

Prof. Waldemar L. Olszewski

Blood transfusions have a clinically important immunomodulatory effects on the immune system of recipients. The present study was designed to examine immune changes occuring in various lymphoid compartments following transfusion of allogeneic blood in a rat model. Transfusion of allogeneic blood caused an increase in the bone marrow myeloid and lymphoid cell compartments, as well as a rise in the percentage of $OX7^+$ stem cells in bone marrow. The changes in hemopoiesis were accompanied by diminished responsiveness of blood, spleen and bone marrow lymphocytes to mitogens. In our previous study, in which rats were transfused with syngeneic blood, similar effects to the present ones were observed. We conclude that transfusions of both allogeneic and syngeneic blood affect hemopoiesis and decrease immune responsiveness in the *in vitro* testing.

MONONUCLEAR CELLS ADHERE TO LIVER CELLS BUT NOT TO LIVER TUMOR METASTASES

Prof. Waldemar L. Olszewski

It has been well documented that under normal conditions certain populations of blood mononuclear cells are transiently halted in liver sinusoids. It seems that these cells play a principal role in a local destruction of tumor cells that form metastases in the liver. However, the mechanisms which regulate the molecular recognition of tumor cells, adherence to and cytotoxic activity of liver associated lymphocytes, remain largely unknown. Recently, we have worked out an assay which enables a semiquantitative evaluation of the topography of adherence and a type of the cells adhering to the liver tissue sections. Ten patients with metastatic liver tumors, all undergoing partial hepatectomy, were examined. Liver-associated lymphocytes were isolated from the sinusoids by vascular perfusion. Frozen liver sections, comprising both tumor and adjacent normal tissue, were overlaid with liver-associated lymphocytes. The number and the phenotype of cells adhering to the tissue were examined. There were evident differences between the number of cells adhering to the tumor and non-tumoral liver tissue. The number of liver-associated lymphocytes sticking to the metastatic tissue was about ten times lower in comparison to the normal liver tissue. The distribution of phenotypes of adhering lymphocytes to the tumor was similar to their distribution to the non-tumoral liver

tissue. Lack of adherence of the selected populations of liver-associated lymphocytes to adenocarcinoma tissue points to their limited recognition of tumor cells.

IMMUNE CELLS OF HUMAN PERITONEAL CAVITY IN GASTROINTESTINAL TRACT TUMORS

Dr. Urszula Kubicka
Supported by the State Committee for Scientific Research: grant # 4 S402 128 07

Little is known about the involvement of peritoneal cell subpopulations in local immune response in the human peritoneum in the digestive tract cancer. We have analyzed the composition of peritoneal cells (PCs) of patients with gastric nd colon cancer and compared them with PCs from patients with chronic cholecystits (C). PCs were obtained by intraoperative peritoneal lavage in patients with adenocarcinoma of stomach stage II (group I) and colon stage II (group II) with no evident involvement of serosa. A decrease in the percentage of $CD68^+$ macrophages and an increase in the percentage of $CD14^+$ monocytes in both groups was observed compared with C. There were evidently more $CD19^+$ B cells in peritoneal fluid of tumor patients, and an elevation of CD11b, CD11c and CD54 adhesion molecule expression on all PC. A drop in the percentage of memory/effector cells ($CD45R0^+$) in the CD4 and CD8 lymphocyte populations in gr I as compared to groups II and C was observed. Changes in the proportions of various subsets of free cells and a higher prevalance of $CD14^+$ monocytes, $CD19^+$ B cells, and $CD54^+$ (ICAM-1^+) cells suggest that the peritoneum of patients with tumor remains in a state of an immune alert, even in the absence of penetration of the neoplasm through the serosa.

SKIN INFLAMMATORY CHANGES IN LYMPHEDEMA

Prof. Waldemar L. Olszewski
Supported by the World Health Organization (WHO)

Forty patients in different stages of lymphedema were examined. Bacteriological analysis of the skin surface, tissue fluid lymph, inguinal lymph nodes and immunohistochemical investigations of the toeweb and calf skin were performed. It was found that flora of the skin was dominated by different subtypes of Bacillus species. Corynebacteria, Gram-positive cocci and Gram-negative cocci were also isolated, with a respectively decreasing frequency. In tissue fluid, lymph and lymph nodes there was a domination of Gram-positive cocci, particularly of Staphylococcus

epidermidis. Gram-positive cocci were sensitive to gentamycin, erytromycin and less so to penicillin. Infiltrates observed in the skin of patients were localized mainly around the skin venules and epidermo-dermal junctions, are there was high concentration of macrophages and dendritic cells in infiltrates. A protocol for prophylactic antibiotic treatment of patients was established: systemic treatment - an administration of penicillin with a prolonged activity and local treatment - administration of gentamycin, neomycin, erytromycin and clotrimazole. This protocol was approved by the WHO experts.

EXPERIMENTAL TRANSPLANTATION OF VASCULARIZED BONE MARROW

Assoc. Prof. Barbara Lukomska
Supported by the State Committee for Scientific Research: grant # 4 4622 92 03

We have reported previously that bone marrow transplantation in an orthotopic hind-limb graft to lethally irradiated syngeneic rat recipients brings about a complete repopulation of bone marrow cavities within 10 days. The present studies revealed that vascularized bone marrow transplantation promotes also rapid replenishment of lymphoid organs in irradiated rats. It was observed that 10 days after hind-limb transplantation the responsiveness of repopulated mesenteric lymph node lymphocytes to mitogens was restored to normal values. Furthermore, sera (10% v/v) of hind-limb graft recipients revealed an additive stimulatory effect on the third party mesenteric lymph node lymphocytes stimulated with PHA. The serum IL-1α level, which is important in the repopulation of lymphohematopoietic stem cells, their growth and differentiation, was significantly higher in irradiated recipients of vascularized bone marrow graft than in control rats. In conclusion, vascular bone marrow transplantation caused rapid replenishment of lymphoid organs of lethally irradiated syngeneic recipients. The repopulating subsets were fully responsive to mitogens. Sera from reconstituted bone marrow contained a high level of growth promoting factors (e.g. IL-1α), present in low concentrations in normal rats. Intravenous infusion of an equivalent number, as in the grafted bone, of bone marrow cell suspension was much less efective, and the repopulation of lymphoid organs was delayed.

SKIN GRAFTING - CELLS INITIATING ALLOGRAFT REJECTION

Dr. Hanna Gałkowska
Supported by the State Committee for Scientific Research: grant # 4 1129 91 01

Cluster formation is the first phase of cooperation between dendritic cells and lymphocytes. In a 4h assay, an effect of endo- and exogenous TNFα and IL6 on lymph cells from afferent lymphatic vessels of dog hind limb was estimated. Neutralization of endogenous TNFα with anti-TNFα monoclonal antibody resulted in a reduction of the percentage of clusters in lymph. Furthermore, cluster formation was significantly enhanced in the presence of exogenous TNFα. Pentoxyfilin, an inhibitor of TNFα production, caused a dose-dependent reduction of cluster formation. The IL6 and anti-IL6 antibody had no effect on lymph cell clustering. It seems that proinflammatory cytokines, TNFα and IL1β, which have an effect on dendritic cell-lymphocyte clustering, may influence the lymph cell clustering, most probably through their effect on the expression of CD54 and CD58, the molecules playing a key role in the process of lymph cell clustering.

TRANSPLANTATION OF CELLS - EXPERIMENTAL ASPECTS

Prof. Waldemar L. Olszewski
Supported by the State Committee for Scientific Research: grant # 6 P207 011 05

Successful transplantation of hepatocytes is hampered by the lack of the proper cellular (stromal) and humoral environment at the site of implantation and rapid destruction of these cells after transplantation by host phagocytes. In the present study, a mechanism of elimination of the intravenously injected hepatocytes to syngeneic (LEW-LEW) and allogeneic (BN-LEW) rat recipients was examined. It was found that a high number of intravenously infused hepatocytes was eliminated within 6 hours. No evident differences in the elimination rates were observed between syngeneic and allogeneic hepatocytes. Leukopenic rats had a lower rate of elimination of transplanted hepatocytes. Moreover, granulocytes showed *in vitro* cytotoxicity towards syngeneic and allogeneic hepatocytes. Blocking of CD11/18 and CD54 antigens did not abrogate granulocyte-mediated cytotoxicity. Monoclonal antibodies against some membrane antigens of hepatocytes effectively blocked granulocyte cytotoxicity. Both syngeneic and allogeneic rat sera were *in vitro* cytotoxic towards hepatocytes. The results indicate that the molecular structure of isolated hepatocytes may be recognized by native phagocytes as "nonself" and ac-

tivate immune proteins in serum (e.g. complement). The initiated process may lead to the destruction of transplanted hepatocytes.

IMMUNE RESPONSIVENESS TO TRAUMA IN MEN

Dr. Irena Grzelak
Supported by the State Committee for Scientific Research: grant # 4 0369 91 01

Operative trauma substantially affects immune responsiveness of a patient. This may put a patient at an increased risk of infection, cancer reccurrence and prolonged wound healing. The phenotypic characterization of peripheral blood mononuclear cells in patients undergoind elective cholecystectomy was carried out. Particular attention was addressed to the $CD34^+$ cells, released from bone marrow to the blood circulation after endotoxin challenge, chemotherapy and cytokine treatment. It was found that the percentage of $CD34^+$ cells increased significantly on days 3 and 7 after the operation. The appearance of $CD34^+$ cells in blood after the operation may be a result of an expansion of the total bone marrow cell number following surgery.

DEPARTMENT OF ENDOCRINOLOGY
Head: Professor Janusz Nauman
1a Banacha Str., 01-201 Warsaw
Telephones: 659 75 62, 633 86 33

INTERACTION BETWEEN NEUROPEPTIDES IN PAIN TRANSMISSION PROCESSES

Assoc. Prof. Andrzej W. Lipkowski

The endogenous opioid system plays a major role in suppresion of pain signal transmission in both central and peripheral nervous system. Nevertheless, there is a growing evidence that other neuropeptides also play a role in the transmission and suppression of pain signals. In particular, multidisciplinary experimental approaches have demonstrated that spinal peptidergic systems may participate in the integration of nociceptive information. Different neuropeptides, in addition to pain modulation, may play a role in mediation of a variety of somatomotor, autonomic and other neural functions, including modulation of side effects of opioids. Therefore, we believe that using selected neuropeptides or their analogues or antagonists as additives to opioids could be one of the effective approaches of increasing effectiveness of pain treatment with possible reduction of side effects. The available data show that substance P and neurotensin are present in the spinal cord. Substance P antagonists or neurotensin itself produce a very weak and short-lasting antinociceptive effect, after intrathecal (i.t.) application. Nevertheless, we found that the co-injection of substance P antagonists as well as native neurotensin with opioids results in an elevation of anticociception produced by low doses of opioids without any visible neurological disfunction. The program is accomplished under scientific cooperation with the Department of Anesthesia, Massachusetts General Hospital, Boston, USA.

STRUCTURE-ACTIVITY RELATIONSHIPS OF OPIOID LIGANDS

Assoc. Prof. Andrzej W. Lipkowski
Supported by the State Committee for Scientific Research: grant # 6 6337 92 03

In order to overcome the undesirable and toxic side effects of opioids, currently used as drugs, we have targeted our structure-activity relationship studies of endog-

enous opioid peptide analogues and their peptide mimetics on a design of the new generation of antinociceptive drugs with various receptor selectivity, pharmacokinetic and pharmacodynamic profiles. Using a combination of computer assisted design, conformational and topographical considerations, synthetic chemistry, and multiple pharmacological assay methods we have elaborated a series of compounds with new, unusual biological and pharmacological profiles. The most promissing avenues for new drug discovery are new series of opioid peptide analogues, biphalin (with wide opioid receptor selectivity) and deltorphin (selective for delta opioid receptors) families. The program is accomplished under scientific cooperation with the Department of Anesthesia, Massachusetts General Hospital, Boston, and Department of Chemistry, University of Arizona, Tucson, USA.

THE LIBRARY

Head: Krystyna Marczakowska
5 Pawińskiego Str., 02-106 Warsaw
Telephone: 658 46 77

The library constitutes one Department of the Medical Research Centre and acts as an information source for scientists.

Scope and the subject profile:
physiology, neurosciences and experimental surgery, including transplantology.
Present holdings:
books - monographic and serial volumes (Polish and foreign) - 17263
periodicals, newspapers (number of titles) - 163.
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- subject: books
main card-files
- bibliographical list of papers published by scientists of the Medical Research Centre, Polish Academy of Sciences from 1967.

Users:
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Bibliography of library: a list of new books and current periodicals is prepared weekly. On the basis of the Scientific Citation Index a report of citations of papers published by MRC scientists in 1994 was prepared. It contains 550 citations.

MEDIPAN
Head: Andrzej Lasek
1/13 Flory Str., 02-586 Warsaw
Telephone: 48 22 62
Tel./fax: 49 30 79

MEDIPAN is a manufacturer of a special equipment needed by various medical service units. In the past few years it has concentrated on construction and production of microprocessor infusion pumps and microprocessor infusion syringe pumps. In 1994 pumps constructed and manufactured in the previous years i.e. Model 604 microprocessor infusion drip pump and Model 611 and 612 microprocessor infusion syringe pumps were considerably improved in technical aspects as well as modified in their appearance so as to comply with modern worldwide requirements.

Due to technical modifications all these models are comparable to those produced by western companies s far as their reliability, programming system, range of technical possibilities, simplicity of operation and safety requirements are concerned.

A new design of the pumps' casing not only made them more attractive in their appearance but also considerably improved their ergonomy. Both the shape of a casing, colouring and graphic arrangement of the front panel have been completely changed.

In 1994 two new models of infusion pumps were worked out and constructed.

Model 610-2 double-syringe microprocessor infusion pump has been constructed for intravenous injections from two simultaneously but independently working syringes. Four volumes of syringes i.e. 10, 20, 50 and 60 ml can be used in this model. Model 610-2 combines two independent infusion systems under one cover.

The new 606 Model of microprocessor infusion drip pump has ben enriched with several new technical possibilities in comparison with Model 604. Due to these changes the range of pump's applications may be considerably widened. It can be used for parenteral feeding and infusion of blood substitutes. Its programming system allows to program the occlusion pressure levels. Due to RS 232 output installed in the pump there is a possibility of connecting to computer systems.

INTERNATIONAL COOPERATION

VISITING SCIENTISTS

Department of Neurophysiology

Mamoru Aoki	Department of Physiology, Sapporo School of Medicine, Sapporo, Japan
P.-E. Paulev	Department of Medical Physiology, PANUM Institute, University of Cooenhagen, Denmark

Department of Neuropathology

C. Gajdusek	National Institutes of Health, Bethesda, USA
A. Gurvich	Laboratory of CNS Postresuscitation Pathology, Institute for General Reanimathology, Russian Academy of Medical Sciences, Moscow, Russia
A.S. Lossinsky	New York State Institute for Basic Research in Developmental Disabilities, New York, USA
L. Nikolaishvili	I.S. Beritashvili Institute of Physiology, Georgian Academy of Sciences, Tbilisi, Georgia
I. Victorov	Institute of Brain Research, Russian Academy of Sciences, Moscow, Russia
K. Wiśniewski H. Wiśniewski	New York State Institute for Basic Research in Developmental Disabilities, New York, USA

Laboratory of Developmental Neuropathology

L. Gerhard	Laboratory of Neuropathology, University of Essen, Germany

Department of Neurosurgery

A. Baethmann	Institut für Chirurgische Forschung, Munich, Germany
A. Hartmann	Neurologische Klinik, Bonn, Germany
A.N. Konovalov	Burdenko Neurosurgical Institute, Moscow, Russia
H. Kostron	Universität-Klinik für Neurochirurgie, Innsbruck, Austria
A. Kravtchuk	Burdenko Neurosurgical Institute, Moscow, Russia
N. Kuridze	I. Beritashvili Institute of Physiology, Georgian Academy of Sciences, Tbilisi, Georgia
T. Kuroiwa	Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan
B. Lichterman	Burdenko Neurosurgical Institute, Moscow, Russia
A. Marmarou	Division of Neurosurgery, Medical College of Virginia, Richmond, USA
G. Okladnikov	Department of Neurosurgery, Medicinskij Institut, Novosybirsk, Russia
J.D. Pickard	Neurosurgery Unit, University of Cambridge, Cambridge, UK
H.J. Reulen	Ludwig-Maximilians-Universität, Klinikum Grosshadern, Neurochirurgische Klinik Munich, Germany
H. Wassmann	Neurochirurgische Klinik, Munich, Germany
H. Whitehouse	Department of Neurosurgery, University of Cambridge, UK
O. Zaitsev	Burdenko Neurosurgical Institute, Moscow, Russia

Neuromuscular Unit

J. Jabre	Neuromuscular Center, University School of Medicine, Boston, USA

Department of Surgical Research and Transplantation

C. Hammer	Institute for Surgical Research, University of Munich, Germany
G. Manokaran	University of Madras, Indian National Science Academy, India
A. Ostrowski	Pediatric Clinic, University of Buenos Aires, Argentina
M. Winnock	Laboratory of Cellular Interaction, University of Bordeaux, France

Department of Endocrinology

G. van Binst	Department of Organic Chemistry, Vrije University, Brussels, Belgium
D.B. Carr S. Szyfelbein	Massachusetts General Hospital, Harvard Medical School, Boston, USA
W. Kazimierski	Borroughs WELLCOME, Research Triangle Park, N.C., USA

VISITS ABROAD

Department of Neurphysiology

J. Romaniuk	Metro Health Medical Centre, Case Western Reserve University, Cleveland, Ohio, USA (long term visit)
M. Szereda-Przestaszewska	Department of Pathophysiology, Medical Faculty Safarik University, Kosice, Slovakia

Department of Neurochemistry

M. Łałowski	Department of Pathology, New York University Medical Center, New York, USA (long term visit)
A. Stafiej	Department of Anatomy and Cell Biology, University of Goeteborg, Sweden
J. Strosznajder	Institute of Biochemistry, University of Perugia, Italy

Department of Neuropathology

H. Borkowska	Department of Comparative Physiology, Hungarian Academy of Sciences, Budapest, Hungary
	Department of Biomedical Sciences, and Tampere Brain Research Center, University of Tampere, Finland (long term visit)
L. Faff-Michalak	Department of Cell Neurophysiology, Institute of Physiology, Leipzig, Germany

	Max-Delbruck-Centrum für Molekulare Medizin, Berlin-Buch, Germany
W. Hilgier	Department of Emergency Medicine, Wright State University School of Medicine, Dayton, Ohio, USA
E. Kida	Institute for Basic Research in Developmental Disabilities, New York, USA (long term visit)
S. Krajewski	La Jolla Cancer Research Foundation, La Jolla, USA (long term visit)
M.J. Mossakowski	Institute for Basic Research in Developmental Disabilities, New York, USA
R. Pluta	Institute for Basic Research in Developmental Disabilities, New York, USA

Laboratory of Developmental Neuropathology

M. Dąmbska	Institute for Basic Research in Developmental Disabilities, New York, USA

Laboratory of the Ultrastructure of the Nervous System

B. Gajkowska	Laboratoire de Biologie et Ultrastructure du Noyau de l'Institut de Recherches Scientifiques sur le Cancer, Villejuif, France

Department of Neurosurgery

Z Czernicki	Institute für Neuropathologie, Berlin, Germany
	Neurochirurgische Klinik, Munich, Germany
	Neurochirurgische Klinik, Bonn, Germany

Neuromuscular Unit

J. Borkowska	Institute of Human Genetic, University of Bonn, Germany
A. Kamińska	Institute of Neuropharmacology, Free University of Berlin, Germany
E. Kowalska-Olędzka	Health Sciences Centre, University of Arozina, Tucson, Arizona, USA (long term visit)

Department of Applied Physiology

L. Budohoski	Department of Biochemistry, University of Oxford, United Kingdom
	Department of Physiology, Health Science Center, State University of New York, Syracuse, USA (long term visit)
Z. Brzezińska	Department of Rehabilitation Medicine, University of Goeteborg, Sweden (long term visit)
L. Dobowolski	Physiologisches Institut der Ruprecht-Karls--Universität, Heidelberg, Germany (long term visit)
R. Grucza	Department of Physiology, University of Kuopio, Finland
	Laboratory of Work Physiology, Medical Faculty, Pitie-Salpetriere, Pierre and Marie Curie University, Paris, France
H. Kaciuba-Uściłko	Institute if General and Experimental Pathology, University of Graz, Austria
	Department of Environmental and Occupational Medicine, University Medical School, Aberdeeen, Scotland, UK

	Institute of Physiology, Justus-Liebig University, Giessen, Germany
B. Kruk	Department of Physiology, University of Kuopio, Finland
K. Krzemiński	Department of Health Sciences, Sargent College of Allied Health Professions, Boston University, USA (long term visit)
K. Nazar	Institute if General and Experimental Pathology, University of Graz, Austria
	Department of Environmental and Occupational Medicine, University Medical School, Aberdeeen, Scotland, UK
	Institute of Physiology, Justus-Liebig University, Giessen, Germany

Department of Surgical Research and Transplantation

I. Grzelak	Norwegian Radiumhospital, Oslo, Norway
	University of Genova, Ospedale S. Martino, Italy
U. Kubicka	Indian National Science Academy, Madras, Pondicherry, India
B. Łukomska	Centre National de Recherche Scientific, Bordeaux
W.L. Olszewski	Indian National Science Academy, Madras, Pondicherry, India
	Norwegian Radiumhospital, Oslo, Norway
	University of Genova, Ospedale S. Martino, Italy

	Institute of Surgical Sciences, University of Munich, Germany
	Department of Humanbiology, University of Kassel, Germany
M. Zaleska	Indian National Science Academy, Madras, Pondicherry, India

Department of Endocrinology

R. Gniadecki	Department of Dermatological Research, Leo Pharmaceutical Products, Copenhagen, Denmark
J. Kiljański	Thyroid Eye Disease Center, Allegheny-Singer Research Institute, Medical College Pennsylvania, Pittsburgh, USA (long term visit)
A. Lipkowski	Department of Chemistry, University of Arizona, Tucson, USA
	Massachusetts General Hospital, Harvard Medical School, Boston, USA
A. Minich	Department of Organic Chemistry, Vrije University, Brussels, Belgium
A. Misicka-Kęsik	Department of Organic Chemistry, Vrije University, Brussels, Belgium

PARTICIPATION IN INTERNATIONAL MEETINGS

MDA Symposium on the Molecular Mechanisms of Neuromuscular Disease, Tucson, USA, January 23-25, 1994: *I. Hausmanowa-Petrusewicz*

International Symposium in Cirrhosis, Hyperammonemia and Hepatic Encephalopathy, Valencia, Spain, January 24-27, 1994: *J. Albrecht*

International Conference on Aging, Depression and Dementia, Graz, Austria, February 21-25, 1994: *J. Strosznajder*

3rd International Congress of the Immune Consequences of Trauma, Shock and Sepsis. Mechanisms and Therapeutic Approaches, Munich, Germany, March 2-5, 1994: *M. Grzelak, W.L. Olszewski*

Biophysical Sciences Annual Meeting, New Orleans, USA, March 6-10, 1994: *A. Misicka*

3rd International Symposium "Intracranial Hypertension and Cerebral Ischemia in Clinical Practice", Warsaw, Poland, March 17-18, 1994: *Z. Czernicki, J. Dziduszko, W. Grochowski, D. Horsztyński, J. Jurkiewicz, P. Marszałek, E. Mempel, M.J. Mossakowski, S. Piechnik, W. Sapieja, G. Stępińska, J. Szumska, G. Uchman, B. Witkiewicz, W. Zabolotny*

Third International Symposium of Lymphology, Brussels, Belgium, March 17-19, 1994: *W.L. Olszewski*

Conference in Honor of Prof. A. Stuppler, Munich, Germany, March 19, 1994: *I. Hausmanowa-Petrusewicz*

Conference of Association of European Academies of Sciences, Paris, France, March 20-21, 1994: *M.J. Mossakowski*

International Symposium: Dementia in Parkinson's Disease, Jerusalem, Israel, March 20-25, 1994: *M. Barcikowska*

First European Meeting on Glial Cell Function in Helath and Disease, Heidelberg, Germany, March 24-27, 1994: *L. Faff-Michalak, P. Grieb*

First Czech-Polish International Symposium "Human adaptability to work and environment", Zakopane, Poland, March 28-30, 1994: *J. Chwalbińska-Moneta, H. Kaciuba-Uścilko, B. Kruk, H. Krysztofiak, J. Langfort, K. Nazar, A.W. Ziemba*

First International Congress of the Polish Sleep Research Society, Warsaw, Poland, April 15-16, 1994: *L. Czerwosz, U. Jernajczyk, A. Kukwa*

First European Kidney Research Forum, Erlangen, Germany, April 23-27, 1994: *L. Dobrowolski, E. Kompanowska-Jezierska, J. Sadowski*

IVth European Meeting on Myasthenia Gravis "Euromyasthenia IV", Versailles, France, May 3-6, 1994: *M. Strugalska-Cynowska*

Second International Congress of the Cell Transplant Society, Minneapolis, USA, May 1-4, 1994: *M. Jaskłowska-Englisz, W.L. Olszewski*

XVIIIth Meeting of European Group of Lymphology, Brussels, Belgium, May 6-7, 1994: *W.L. Olszewski*

6th Cyprus Conference on New Methods in Drug Research, Limassol, Cyprus, May 8-14, 1994: *A.W. Lipkowski, A. Misicka*

Symposium to Honor Leon Wolfe "Lipids in the Nervous System: From Structure to Signal Transduction", New Orleans, USA, May 10-12, 1994: *J. Strosznajder*

29th Congress of the European Society for Surgical Research, Montpellier, France, May 16-19, 1994: *M. Durlik, I. Grzelak, W.L. Olszewski*

The Second Symposium of Jagiellonian Medical Research Centre "Nitric Oxide in Cardiovascular System from Basic Research to Clinic", Kraków, Poland, May 22-25, 1994: *K. Białynicka-Birula, M. Chalimoniuk, K. Domańska-Janik, B. Kwiatkowska-Patzer, E. Speina, E. Wojtal, B. Zabłocka*

Symposium Bio'94 "Biotechnology in Environmental Protection", Świnoujście, Poland, May 25-27, 1994: *A.W. Lipkowski*

Second Meeting of the Society of Natural Immunity, Taormina, Italy, May 25-28, 1994: *B. Łukomska*

2nd North Sea Meeting on Venous Diseases, Amsterdam, The Netherlands, May 27-28, 1994: *W.L. Olszewski*

Annual Conference of American College of Sports Medicine, Indianapolis, USA, June 1-4, 1994: *L. Budohoski*

Workshop on 2-chlorodeoxyadenosine, Brussels, Belgium, June 2, 1994: *P. Grieb*

Polish-German Joint Symposium "Cerebro-vascular disease", Poznań/Kraków, Poland, June 2-4, 1994: *Z. Czernicki, W. Gordon-Krajcer, A. Kapuściński, J. Łazarewicz, M.J. Mossakowski, R. Pluta, M. Samochocki, W. Sapieja, J. Strosznajder*

2nd Meeting of Polish Society of Research on Arteriosclerosis, and International Conference New Therapeutic Approaches of Coronary Risk Factors, Międzyzdroje, Poland, June 4-7, 1994: *H. Kaciuba-Uścilko, K. Nazar, A.W. Ziemba*

International Conference "Contribution of Biomedical Engineering to Biology and Medicine", Bethesda, June 13-16, 1994: *I. Hausmanowa-Petrusewicz*

12th European Immunology Meeting, Barcelona, Spain, June 14-17, 1994: *I. Grzelak, M. Jasklowska-Englisz, U. Kubicka, B. Łukomska*

CPDD 56th Annual Meeting the College on Problems of Drug Dependence, Pałm Beach, Florida, USA, June 18-23, 1994: *I. Maszczyńska*

XXX Congress of Italian Association of Neuropathology, St. Vincent, Italy, June 20-22, 1994: *H. Kroh*

3rd International Symposium on Dendritic Cells in Fundamental and Clinical Immunology, Annecy, France, June 19-23, 1994: *H. Gałkowska*

2nd Joint Polish-German Neurosurgical Symposium, Olsztyn, Poland, June 23-25, 1994: *Z. Czernicki, D. Horsztyński, J. Jurkiewicz, H. Kroh*

IV Meeting of the European Neurological Society, Barcelona, Spain, June 25-29, 1994: *P. Grieb*

FEBS Special Meeting "Biological Membranes", Helsinki, Finland, June 2 - July 1, 1994: *R. Strosznajder*

VIIIth European Congress of Clinical Neurophysiology, Budapest, Hungary, July 3-7, 1994: *I. Hausmanowa-Petrusewicz*

The VIII International Congress on Neuromuscular Diseases, Kyoto, Japan, July 10-15, 1994: *I. Hausmanowa-Petrusewicz*

Ninth International Conference "Biochemistry of Exercise" , Aberdeen, Scotland, July 21-26, 1994: *M. Górecka, H. Kaciuba-Uścilko, J. Langfort, K. Nazar, E. Żernicka*

The Fourth International Conference on Alzheimer Disease and Related Disorders, Minneapolis, USA, July 29 - August 3, 1994: *M. Barcikowska*

Ninth International Symposium "Pharmacology of Thermoregulation", Giessen, Germany, August 7-11, 1994: *R. Grucza, H. Kaciuba-Uścilko*

XVII International IAUTA Congress "Preparation for Ageing", Jyväskylä, Finland, August 12-14, 1994: *M. Pokorski*

10th Meeting of the European Society for Neurochemistry, Jerusalem, Israel, August 14-19, 1994: *M. Chalimoniuk, J. Łazarewicz, M. Pokorski, U. Rafałowska, E. Salińska, M. Samochocki, J. Strosznajder, L. Strużyńska, J. Sypecka, B. Zabłocka*

XVth World Congress of the Transplantation Society, Kyoto, Japan, August 28 - September 2, 1994: *M. Durlik, M. Jasklowska-Englisz, B. Łukomska, M. Maksymowicz, A. Namysłowski, W.L. Olszewski*

17th Annual Meeting of the European Neurosciences Association, Vienna, Austria, September 4-8, 1994: *M. Barcikowska, H. Borkowska, B. Dąbrowska-Bouta, K. Domańska-Janik, M. Pokorski, J. Sypecka, B. Zabłocka*

7th International Symposium on Cells of the Hepatic Sinusoid, Kyoto, Japan, September 4-8, 1994: *B. Łukomska, W.L. Olszewski*

23rs European Peptide Symposium, Braga, Portugal, September 4-10, 1994: *A.W. Lipkowski, A. Misicka*

XIVth Martin's Days of Respiration, Martin, Slovakia, September 6-7, 1994: *B. Szereda-Przestaszewska*

Japan-Poland Surgical Research Conference in 1994, Tokyo, Japan, September 9, 1994: *B. Łukomska, W.L. Olszewski*

XIII World Congress of Cardiology, Berlin, Germany, September 10-14, 1994: *K. Cedro-Ceremużyńska*

XXV FIMS World Congress of Sports Medicine, Athens, Greece, September 10-16, 1994: *J. Chwalbińska-Moneta, R. Grucza*

16th International Congress of Biochemistry and Molecular Biology, New Delhi, India, September 16-19, 1994: *J. Strosznajder*

Multiple Sclerosis Medical Satellite Symposium, Budapest, Hungary, September 17-19, 1994: *P. Grieb*

XII International Congress of Neuropathology, Toronto, Canada, September 18-23, 1994: *M. Dąmbska, E. Kida, M.J. Mossakowski*

London Conference on Modelling and Control of Ventilation, London, Great Britain, September 17-20, 1994: *M. Pokorski, B. Szereda-Przestaszewska*

27th Danube Symposium for Neurological Sciences, Krems, Austria, September 22-24, 1994: *A. Kapuściński, P. Marszałek, M.Śmiałek*

68th International Meeting of American Thyroid Association, Chicago, USA, September, 1994: *J. Kiljański*

IV Simposio Internacional Sobre Linfedema, Buenos Aires, Argentina, September 30 - October 10, 1994: *W.L. Olszewski*

ERS Annual Congress, Nice, France, October 1-5, 1994: *W. Janczewski, M. Pokorski*

IX European Congress of Anaesthesiology, Jerusalem, Israel, October 2-7, 1994: *R. Pluta*

36emes Journees de l'Association pour l'Etude du Foie, Lyon, France, October 20-21, 1994: *B. Łukomska*

First National Lymphedema Network Conference, San Francisco, USA October 21-23, 1994: *W.L. Olszewski*

Neurosurgical Symposium "Intracranieller Druck, Hirnödem und Hirndurchblutung", Düsseldorf, Germany, October 28-29, 1994: *Z. Czernicki*

XIX Congreso del Grupo Europeo de Linfologia (GEL), San Sebastian, Spain, October 28-29, 1994: *W.L. Olszewski*

1st European Federation of Neurological Societies Congress on Muscle Disorders, Athens, Greece, October 31 - November 1, 1994: *H. Drac, I. Hausmanowa-Petrusewicz*

Seminar "The Floppy Infant", University of Mainz, Germany, November 2, 1994: *A. Fidziańska*

24th Annual Meeting of Society for Neuroscience, Miami Beach, Florida, USA, November 13-18,1994: *W. Hilgier*

Conference on Medical Faculty of Safarik University, Kosice, Slovakia, November 15, 1995: *B. Szereda-Przestaszewska*

Meeting of the European Federation of Neurological Societies, Poznań, Poland, November 24-26, 1994: *M. Chalimoniuk, Z. Czernicki, P. Grieb, I. Hausmanowa-Petrusewicz, J. Jurkiewicz, A. Kamińska, I. Koładkiewicz, D. Maślińska, M.J. Mossakowski, M. Samochocki, J. Strosznajder*

British Society for Immunology Second Annual Congress, Harrogate, Great Britain, December 5-7, 1994: *H. Gałkowska*

V° Simposio Nazionale Della Societa Italiana di Linfangiologia, Genova, Italy, December 10, 1994: *W.L. Olszewski*

Topical Workshop "Quality Assessment and Quantification MRS", Copenhagen, Denmark, December 11-13, 1994: *P. Grieb*

SCIENTIFIC DEGREES

DOCTOR'S DEGREES

Robert Gniadecki

Cutaneous Effects of KH 1060: A Potent Analogue of 1,25-$(OH)_2$ D3; Studies in Hairless Mice

(Department of Endocrinology)

Urszula Jernajczyk

Disproportions in the activities of the respiratory nerves - phrenic and hypoglossal - in the pathogenesis of the sleep apnea syndrome

(Department of Neurophysiology)

Albert Stephen Lossinsky

Mechanisms of trans-endothelial transport in some pathological conditions of the blood-brain barrier

(New York State Institute for Basic Research in Developmental Disabilities)

Wiktor Niewiadomski

Automatized calculation and characterization of chosen heart rate variability (HRV) indices

(Department of Applied Physiology)

HABILITATIONS

Jolanta Chwalbińska-Moneta

Anaerobic threshold - physiological and biochemical basis

(Department of Applied Physiology)

Jacek Dziduszko

Evaluation of clinical application of spinal sensory potentials in patients with herniated lumbar discs

(Department of Neurosurgery)

SCIENTIFIC MEETINGS

ORGANIZED BY THE MEDICAL RESEARCH CENTRE

Scientifc Symposium "Wound Healing", January 11, 1994, Warsaw

Practical Course of Staining with Monoclonal Antibodies, February 15-16, 1994, Warsaw; sponsored by DAKOPATTS, Denmark

3rd International Symposium on Intracranial Hypertension and Cerebral Ischemia in Clinical Practice, March 17-18, 1994, Warsaw

First Czech-Polish International Symposium "Human Adaptability to Work and Therma Environment", March 28-30, 1994, Zakopane

XXVII Course of Basic Microsurgical Techniques, March 28-30, 1994, Warsaw; sponsored by AMERICAN CYANAMID COMPANY

"SMA update" - Satellite Meeting of The VIII International Congress on Neuromuscular Diseases, July 10-15, 1994, Kyoto

Practical Course of Staining with Monoclonal Antibodies, October 11-12, 1994, Warsaw; sponsored by DAKOPATTS, Denmark

Symposium "Up-date and future of tissue and organ transplantation in an animal model, November 9, 1994, Warsaw

XXVIII Course of Basic Microsurgical Techniques, December 5-7, 1994, Warsaw; sponsored by AMERICAN CYANAMID COMPANY

II Confrence of Neurochemistry "Biochemistry, pathophysiology, and molecular biology of neurotransmission and signal transduction in the nervous system", December 16, 1994, Warsaw

PUBLICATIONS

1. Albrecht J, Bender AS, Norenberg MD: Ammonia stimulates the release of taurine from cultured astrocytes. *Brain Res* 1994, 660, 288-292.

2. Albrecht J, Faff L: Astrocyte-neuron interactions in hyperammonemia and hepatic encephalopathy. *Adv Exp Biol Med* 1994, 368, 45-54.

3. Albrecht J, Hilgier W: Similarities of the *in vivo* and *in vitro* effects of mercuric chloride on [^{3}H] ouabain binding and potassium activation of Na^+/K^+-ATPase in isolated rat cerebral microvessels. *Toxicol Lett* 1994, 70, 331-336.

4. Albrecht J, Hilgier W, Januszewski S, Kapuściński A, Quack G: Increase of the brain uptake index for L-ornithine in rats with hepatic encephalopathy. *Neuroreport* 1994, 5, 671-673.

5. Albrecht J, Hilgier W, Walski M: Ammonia added in vitro, but not moderate hyperammonemia *in vivo*, stimulates glutamate uptake and H^+-ATP-ase activity in synaptic vesicles of the rat brain. *Metabol Brain Dis* 1994, 9, 3, 257-266.

6. Albrecht J, Szumańska G, Gadamski R, Gajkowska B: Changes of activity and ultrastructural localization of alkaline phosphatase in cerebral cortical microvessels of rat after single intraperitoneal administration of mercuric chloride. *Neurotoxicology* 1994, 15, 897-902.

7. Amaning E, Olszewski WL: Kinetics of distribution of recirculating lymphocytes during whole body hyperthermia. *Archivum Immunologiae et Therapiae Experimentalis* 1994, 42, 107-113.

8. Baranowska B, Lipkowski A, Marczak E, Makulec I: Keratin of the waste pig bristle as a source of cosmetic and pharmaceutical raw materials. In: *Biotechnology in Environmental Protection. Proc. of Symposium Bio'94, Ekochem*, Szczecin 1994, pp. 191-196.

9. Barcikowska M, Friedman A: Clinicopathological study of 10 cases with dementia and parkinsonian syndrome. In: *Proceedings of the International Symposium "Dementia in Parkinson's disease", Jerusalem, Israel, 20-25 March, 1994*. Ed.: A Korczyn, Monduzzi Editore, Bologna, Italy, 1994, pp. 203-205.

10. Barcikowska M, Papierz W, Klimek A, Liberski PP: Glial reaction in the cerebral cortex and spinal cord in cases of "non-respiratory". Amyotrophic Lateral Sclerosis (ALS). *Neurobiol Aging* 1994, 1, 119.

11. Bauminger ER, Barcikowska M, Friedman A, Galązka-Friedman J, Hechel D, Nowik J: Does iron play a role in Parkinson disease? *Hyperfine Interactions* 1994, 91, 853-857.

12. Bednarczuk T, Nauman A: Type II iodothyronine 5'-deiodinase activity in brain regions of euthyroid and hypothyroid adult rats. *Endokrynologia Polska* 1994, 45, 419-425.

13. Bednarczuk T, Nauman A: Effects of thyroid hormones on the central nervous system. *Endokrynologia Polska* 1994, 45, 97-198.

14. Berdyga J, Czernicki Z, Czosnyka M: Evaluation of intracranial volume compensation analyzing changes of harmonic components of intracranial pressure pulse wave. *Neurol Neurochir Pol* 1994, 28, 195-199 (in Polish).

15. Berdyga J, Czernicki Z, Jurkiewicz J: Intracranial volume pressure reserve assessment based on ICP pulse wave analysis. *Neurol Neurochir Pol* 1994, 28, 49-55 (in Polish).

16. Bryła P, Olszewski WL: Sympathectomy decrease extravasation of immune cells in lymphoid and non-lymphoid tissues. *Lymphology* 1994, 27 (Suppl - *Progress in Lymphology XIV*), 234-236.

17. Chwalbińska-Moneta J: *Anaerobic threshold - physiological and biochemical basis*. MRC Polish Acad Sci, Warsaw, 1994 (monography in Polish).

18. Chmura J, Nazar K, Kaciuba-Uściłko H: Choice reaction time during graded exercise in relation to blood lactate and plasma catecholamine thresholds. *Int J Sports Med* 1994, 15, 172-176.

19. Czarnowski D, Langfort J, Pilis W, Górski J: Effect of a low-carbohydrate diet on plasma and sweat ammonia concentrations during prolonged nonexhausting exercise. *Eur J Appl Physiol* 1994, 69, 70-74.

20. Czernicki Z, Berdyga J, Stępińska G, Pawłowski W, Grochowski W, Jurkiewicz J: Application of physiological loadings in evaluation of intracranial volume-pressure relations. II. Clinical studies. *Neur Neurochir Pol* 1994, 28, 43-47 (in Polish).

21. Czernicki Z, Kuroiwa T, Ohno K, Endo S, Ito U: Effect of acetazolamide on early ischemic cerebral edema in gerbils. *Acta Neurochirurgica* 1994, Suppl. 60, 329-331.

22. Czernicki Z, Luczywek E, Fersten E: Cerebral blood changes during neuropsychologic tests assessed by transcranial Doppler sonography. Preliminary report. *Neur Neurochir Pol* 1994, 28, 861-868 (in Polish).

23. Czernicki Z, Stępińska G: Transcranial Doppler's ultrasonography for determination of brain death. *Anestezjologia Intensywna Terapia* 1994, 26, 49-53 (in Polish).

24. Czernicki Z, Tomita H, Ito U, Jurkiewicz J, Stępińska G, Walecki J: Application of transcranial Doppler ultrasonography in the assesment of cerebrovascular reactivity studies and cerebral vascular reserve estimation in patients with focal brain injury. *Neur Neurochir Pol* 1994, 28, 351-361 (in Polish).

25. Dąmbska M: Morphological aspects of neuronal maturation. In: *The normal and pathologic development of the nervous system (Rozwój układu nerwowego - norma i patologia)*. The First Spring Neurobiological School, Warszawa, 15 kwietnia 1994, pp. 1-8 (in Polish).

26. Dąmbska M, Kuchna I, Nowicki K: Neuropathological variants of cystic encephalopathy in infants. *Folia Neuropathol* 1994, 32, 1, 31-35.

27. Dąmbska M, Kozłowski PB: Central nervous system changes in infants with HIV infection. I. Epidemiology and neurology. *Neur Neurochir Pol* 1994, 28, 379-384 (in Polish).

28. Dobrzyński B, Lewicki R, Kaczorowski K, Nazar K: Relationship between physical fitness indices obtained in laboratory and field tests in adolescent athletes. In: *Proceedings of the Third International Congress Sport Kinetics 1993*. Eds: W Osiński, W Starosta. AWF Poznań, 1994, pp. 99-106.

29. Durlik M, Łukomska B, Cybulska E, Olszewski WL: Tissue injury promots bone marrow proliferation in rats. *Intensive Care Medicine* 1994, 20, S.1, 84.

30. Faff-Michalak L, Reichenbach A, Dettmer D, Kellner K, Albrecht J: K^+-, hyperosmolarity, and NH_4^+-induced taurine release from cultured rabbit Müller cells: The role of Na^+ and Cl^- ions and relation to cell volume changes. *Glia* 1994, 10, 114-120.

31. Fersten E, Szatkowska I, Łuczywek E, Herman A, Grabowska A: The effect of the damage to the medial temporal lobe structures on the storage of sensory inflamation. *Studia Psychologiczne* 1994, 32, 79-94 (in Polish).

32. Fidziańska A, Goebel HH: Aberrant arested in maturation neuromuscular junctions in centronuclear myopathy. *J Neurol Sci* 1994, 124, 83-88.

33. Fidziańska A, Warlo I, Goebel HH: Neonatal centronuclear myopathy with N-Cam decorated myotubes. *Neuropediatrics* 1994, 25, 158-161.

34. Friedman A, Barcikowska M: Dementia in Parkinson's disease. *Dementia* 1994, 5, 12-16.

35. Fredman A, Bauminger E, Gałązka-Friedman J, Barcikowska A, Suwalski J, Hechel D, Dymecki J, Nowik I: Mossbauer spectroscopy of iron in substantia nigra in Parkinson's disease and controls. *Neurol Neurochir Pol* 1994, 28, 145-155 (in Polish).

36. Gadamski R, Kroh H: Immunoreactivity of astroglia after brief ischemia resulting in asymmetrical damage to the hippocampal CA1 sector in the Mongolian gerbil. *Folia Neuropathol* 1994, 32, 2, 95-99.

37. Gajkowska B, Domańska-Janik K, Viron A: Protein kinase C-like immunoreactivity in gerbil hippocampus after a transient cerebral ischemia. *Folia Histochem Cytobiol* 1994, 32, 2, 71-77.

38. Gajkowska B, Gadamski R, Frontczak-Baniewicz M: Effect of staphylococcal and toxin on the ultrastructure of the rat hypothalamo-neurohypophyseal system. *Acta Neurobiol Exp* 1994, 54, 219-225.

39. Gajkowska B, Gniadecki R: Immunolocalization of serotonin in the hypothalamo-neurohypophysial system in rat. *Endokrynologia Polska* 1994, 45, 303-309.

40. Gajkowska B, Kochman H, Kochman K: A rapid reaction of the neurons of arcuate nucleus in the hypothalamus of the female rat after single injection of progesterone. *Neuroendocrinology Lett* 1994, 16, 5-6, 285-290.

41. Gajkowska B, Mossakowski MJ: Ischemia inhibits GABA-ergic neurons of the rat thalamic reticular nucleus. An immunocytochemical study. *Folia Neuropathol* 1994, 32, 139-149.

42. Gajkowska B, Viron A: GABA and glutamate in microvesicles of neurohypophysis. Immunocytochemical study. *Neuroendocrinology Lett* 1994, 16, 209-214.

43. Gałkowska H, Olszewski WL: Modulation of skin dendritic cell-lymphocyte interactions *in vivo*. *Lymphology* 1994, (Suppl - *Progress in Lymphology XIV*), 27, 868-871.

44. Gałkowska H, Wojewódzka U, Olszewski WL: Spontaneous dendritic cell-lymphocyte interaction in skin afferent lymph. *Immunology* 1994, 83, Suppl 1, 71 (abstract).

45. Gołębiowski M, Barcikowska M, Pfeffer-Baczuk A, Łuczywek E: Monitoring CT in clinical practice of patients with Alzheimer's disease. *Polski Przegląd Radiologiczny* 1994, 58, 1-2, 16-19 (in Polish).

46. Góra-Tybor J, Robak T, Warzocha K, Grieb P: Influence of 2-chloro-2'--deoxyadenosine alone and in combination with cyclophosphamide or methotrexate on murine leukemia L1210. *Arch Immunol Ther Exp* 1994, 42, 39-42.

47. Górski A, Castronovo V, Stępień-Sopniewska B, Grieb P, Ryba M, Mrowiec T, Korczak-Kowalska B, Wierzbicki P, Matysiak W, Dybowska B, Piekarczyk J, Gawatkiewicz J, Kryast P, Borkowski A, Tołłoczko T: Depressed immune surveillance against cancer: role of deficient T cell: extracellular matrix interactions. *Cell Adh Commun* 1994, 2, 1-9.

48. Górski A, Grieb P: Cladribine (2-chloro-2'-deoxyadenosine): new perspectives in clinical immunosuppression. *Arch Immunol Ther Exp* 1994, 42, 11-12.

49. Grabowska A, Łuczywek E, Fersten E, Herman A, Szatkowska I: Memory impairment in patients with stereotaxic lesions to the hippocampus and amygdala. *Acta Neurobiol Exp* 1994, 54, 393-403.

50. Grieb P: Discovery, mechanism and expected clinical significance of selective cytotoxicity of 2-chloro-2'-deoxyadenosine (2 CdA). *Arch Immunol Ther Exp* 1994, 42, 1-5.

51. Grieb P, Ryba M, Janczewski W, Sawicki J, Jagielski J, Andrychowski J: 2-chloro-2'-deoxyadenosine (2 CdA) combined with cyclosporine A succesfully prevents rejection of fetal brain stem allograft in rabbits. *Arch Immunol Ther Exp* 1994, 42, 43-46.

52. Grieb P, Ryba M, Stelmasiak Z, Nowicki J, Solski J, Jakubowska B: Clodribine treatment of multiple sclerosis (Letter to the Editor). *Lancet* 1994, 344, 538.

53. Grochowski W, Czernicki Z: Repeatedly recurrent meningitis after removal of anterior fossa meningioma. *Neurol Neurochir Pol* 1994, 28, 775-777 (in Polish).

54. Grucza R, Myamoto Y, Nakazono Y: Effect of work rate on cardiorespiratory response to rhythmic-static exercise. *J Physiol Pharmacol* 1994, 45, 573-583.

55. Grucza R, Smorawiński J: Cardiovascular responses to static exercise (handgrip) in men and women. *Medycyna Sportowa* 1994, 38, 2-3 (in Polish).

56. Grzelak I, Olszewski WL, Engeset A, Oftebro R: The effect of human skin lymph on growth of tumor cells. *Lymphology* 1994, 27 (Suppl - *Progress in Lymphology XIV*), 864-867.

57. Grzelak I, Zaleska M, Olszewski WL: Transfusions of blood cells cause rise in bone marrow stem cell population followed by their release into circulation. *Cell Transplantation* 1994, 3, 241.

58. Grzelak I, Zaleska M, Olszewski WL: Blood transfusions influence hemopoiesis and immune responsiveness. *European Surgical Research* 1994, 26, S.1, 92-93.

59. Grzelak I, Zaleska M, Olszewski WL, Oftebro R: Blood transfusions cause a rise in the OX7^{+} stem cell population in rat bone marrow followed by its release to blood circulation. *Intensive Care Medicine* 1994, 20, S.1, 132.

60. Grzelak I, Zaleska M, Olszewski WL, Oftebro R: Transfusions of blood cells cause a rise in the bone marrow stem cell population followed by their release to blood circulation. *Transplantation Proceedings* 1994, 26, 6, 4060.

61. Grzelak I, Zaleska M, Ziółkowska A, Durlik M, Łagiewska B, Muszyński M, Rowiński W, Olszewski WL.: Increased frequency of hematopoietic progenitor cells in blood circulation following operative trauma. *Intensive Care Medicine* 1994, 20, S.1, 80.

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