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CONTINUATION OF THE STUDIES ON THE MECHANISM OF RESPIRATORY REFLEXES MEDIATED *VIA* THE CAROTID BODY CHEMORECEPTORS

Project leader: Mieczysław Pokorski
Contributor: Lidia Faff

The studies continued concerned the carotid body mechanisms in the regulation of respiration. The major technique used was immunohistochemistry for the identification of the putative signal enzymes in the carotid body.

One experimental series was devote to the description of GABA in the cat carotid body. GABA has been found in the chemoreceptor cells, but its content was little compared with that in other species, e.g., the mouse, which underlines the interspecies differences.

Another experimental series was devoted to the identification of protein kinase C isoforms in the cat carotid body. Antisera against 11 known isoforms were used and the reaction product identified under light, confocal, and electron microscope. The classical protein kinase C isoforms: alpha and gamma have been positively identified in the cytoplasm of the chemoreceptor cell. These studies will be continued in the part concerning the possible redistribution of the isoforms in hypoxia.

Yet another series was devoted to the role of the cannabinergic system in respiratory regulation. It was hypothesized that this system may have a tonic inhibitory influence on respiration. In the preliminary studies with the

anesthetized rabbits it was shown that a synthetic cannabinoid agonist, WIN 55212-2 depresses respiration in a dose dependent manner. The depression may be overcome by hypoxia whose stimulatory effect mediated by the carotid body seems enhanced.

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Department of Medical Physiology, The Panum Institute, University of Copenhagen, Denmark (P-E. Paulev)
Laboratory of Cell Ultrastructure, MRC, PASci, Warsaw, Poland (M. Walski)

CONTROL MECHANISMS OF THE NEURAL AND MUSCLE RESPIRATORY ACTIVITY

Project leader: Krystyna Budzińska
Contributor: Beata Sokołowska

In the research on respiratory compensation of the diaphragm failure, we analyzed sets of parameters that are, with high probability, responsible for changes in pulmonary ventilation following the diaphragm paralysis. Partial pressure of oxygen carbon dioxide and pH in the arterial blood were classified with respect to the control levels of arterial carbon dioxide pressure in the intact animal. For the analysis, a static method of pattern recognition with the application of the standard decision rule of k-nearest neighbours and leave one out method was used. We found that the partial pressure of arterial carbon dioxide is the parameter of the strongest correlation with a new state of the respiratory system. The initial arterial CO_2 level predicts the magnitude of the respiratory insufficiency after diaphragm paralysis.

Another approach to study the respiratory compensation consisted of testing of the neural muscle and ventilatory responses to pharmacologically evoked bronchoconstriction after the diaphragm paralysis in the cat.

In intact animals, bronchoconstriction caused a small increase in tidal volume and minute ventilation preceded by a short depression or apnea. When the diaphragm was paralyzed, minute ventilation decreased during bronchoconstriction, presumably due to an unchanged increment of the intercostal muscle activity in comparison to control. This decrease in minute ventilation was absent when bronchoconstriction was evoked during hypercapnia. The results show that during bronchoconstriction the contribution of the diaphragm to minute ventilation increases appreciably. These studies have been continued.

REFLEX CONTRIBUTION OF THE LOWER AND UPPER AIRWAYS TO THE PATTERN OF BREATHING

Project leader: Małgorzata Szereda-Przestaszewska
Contributors: Katarzyna Kaczyńska, Beata Kopczyńska

In research on pulmonary chemoreflex induced by exogenous serotonin administered to the pulmonary circulation in cats, contribution of serotonergic receptors to the ventilatory response was studied. Respiratory sequence of post-serotonin chemoreflex constitutes: expiratory apnoea, followed by resumed breathing of depressed tidal volume and increased respiratory rate.

It was shown, that blockade of 5 HT_2 receptors by their antagonist ketanserin significantly shortened an expiratory apnoea, without affecting the tidal and timing components of the respiratory pattern. This suggest that to the response contribute mainly 5 HT_2 receptors situated in the smooth muscles of the airways.

5 HT_3-receptor antagonist – MDL 72222 (tropanserin) precluded the constellation of pulmonary chemoreflex evoked by serotonin. MDL 72222 was more effective than the supranodose vagotomy, blocking vagal afferent pathways from lung receptors to the sensory endings in the brainstem.

Serotonergic system located on the vagus nerve contribute apparently to the respiratory response to serotonin.

EFFECT OF AMYLOID β UPON THE BIOCHEMICAL MECHANISM OF DNA INJURY AND NEURON(S) APOPTOSIS

Supported by the State Committee for Scientific Research: grant # 4.P05.051.12

Project leader: Robert Strosznajder

The aim of this year activities was to investigate the mechanism of amyloid beta (Aβ) dependent alteration of poly (ADP-ribose) polymerase (PARP; E.C. 2.4.2.30) activity in different parts of the brain. Moreover, PARP activity during aging was investigated in 4, 14 and 24-27 months old rats. The studies indicate that Aβ (25-35), exclusively in aggregated form, significantly enhanced PARP activity in adult (4 months) hippocampus but had no effect in brain cortex or hippocampus of aged (24-27 months) animals. The effects of Aβ was decreased by a noncompetitive inhibitor of NMDA receptor, MK-801 and by a nitric oxide synthase(s) inhibitor, NNLA. Stimulation of glutamate receptor(s) itself enhanced PARP activity in adult hippocampus. However, Aβ (25-35) had no additional stimulatory effect. These results indicate that Aβ through NO and probably other free radicals is involved in the activation of PARP in adult hippocampus. Poly(ADP-ribose) polymerase activation seems to be an early indicator of Aβ evoked DNA damage.

It was also observed that PARP activity increased in hippocampus, cerebellum and cerebral cortex of 14 months old animals comparing to control (4 months old). In aged animals PARP activity was significantly decreased in hippocampus and unchanged in cerebral cortex and cerebellum. There was no direct correlation between PARP activity and free radical evoked lipid peroxidation, determined as TBARS, during brain aging. In addition the experiments concerning the effect of Aβ (1-40) on phosphoinositide-specific phospholipase C were finished. The result suggested that the deposition of aggregated Aβ may alter phosphoinositide signalling in brain.

Publications:

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PHARMACOLOGICAL NEUROPROTECTION IN CHOSEN VASCULARLY-RELATED PATHOLOGIES OF THE CENTRAL NERVOUS SYSTEM

Project leader: Paweł Grieb
Contributors: Mirosław S. Ryba, Stanisław J. Chrapusta

The experiments performed previously, which concerned the assessment of neuroprotective effects of CDP-choline and its hydrolysis products (cytidine and CMP) on CA1 hippocampal neurons in the gerbil following short-term forebrain ischemia have been repeated using animals from the renewed breed. In the present series of experiments it has been conclusively determined that CDP-choline administration provides a significant neuroprotection, while neither cytidine nor CMP is effective. (Collaboration with Department of Neuropathology, MRC).

In the collaborative study with Medical School of Lublin it has been shown that repeated administration of pharmacological doses of 2-deoxyglucose (2-DG) in mice (500 mg/kg x 5 days) leads to a significant impairment of long term memory acquisition and retention. The memory impairment persists for several days after the cessation of the last 2-DG dose, suggestive of that it is consequential to the changes of metabolic pathways of glucose. Prolonged 2-DG administration may thus be considered a simple model of age-independent dementia.

BIODEGRADABLE POLYMERS CONTAINING NUCLEOSIDE ANALOGS FOR TREATMENT OF INTRACRANIAL TUMORS

Supported by the State Committee for Scientific Research: grant # 4.P05F.024.12

Project leader: Paweł Grieb
Contributor: Mirosław S. Ryba

Kinetics of release of a nucleoside from samples of several lactide-glycolide and lactide-caprolactone polymers obtained by various polymerization schemes and loaded with a model nucleoside adenosine have been assessed in the *in vitro* conditions modelling intracerebral environment. The released nucleoside has been assayed by HPLC. Three types of polymers, which displayed particularly long and sustained release of the model nucleoside, sufficient to establish >100 microM concentration for >4 weeks, have been selected for further development. (Polymer samples were produced by dr. M. Bero and collaborators, Institute of Polymer Chemistry, Zabrze, Poland).

MAGNETIC RESONANCE SPECTROSCOPY OF INTRACRANIAL TUMORS

Project leader: Paweł Grieb

A standarized methodology of acquisition and processing of proton magnetic resonance spectra from human brain *in vivo* with the use of Magnetic Resonance Tomograph Elscint Prestige 2 Tesla, which had been developed previously, has been utilized for monitoring metabolic changes in brains of glioma patients who were subjected to radiotherapy following surgical extraction of tumors. It has been found that the increase of the choline signal seems to be a predictor of tumor recurrence, while the increase in the myoinositol/choline signal ratio may be an early marker of tissue radiation damage. (Collaboration with Institute of Oncology, Gliwice).

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Department of Radiology, Institute of Oncology, Gliwice, Poland

Department of Neuropathology, Medical Research Centre, PASci, Warsaw, Poland (R. Gadamski)

Publications:

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LABORATORY OF PHARMACONEUROCHEMISTRY

Head: Professor Jerzy W. Łazarewicz

CALCIUM SIGNALLING IN BRAIN NEURONES: MECHANISMS OF GENERATION AND ROLE IN PLASTICITY AND DEGENERATION

Project leader: Jerzy W. Łazarewicz
Contributors: Mohd Alaraj, Wanda Gordon-Krajcer, Dorota Makarewicz, Halina Nowicka, Elżbieta Salińska, Anna Sobczuk, Aleksandra Stafiej, Apolonia Ziembowicz, Elżbieta Ziemińska

Previous studies concerning the role of excitatory amino acid receptors and calcium in memory formation have shown that training of day-old chicks on the one-trial passive avoidance task results in enhanced increase in NMDA and AMPA-stimulated intracellular calcium concentration in synaptoneurosomes isolated from the forebrain IMHV region. Continuing these studies, the role of calcium mobilisation from the intracellular ryanodine-sensitive pool in memory formation in chicken brain was examined. The results indicated that dantrolene, an inhibitor of intracellular calcium release, abolishes the enhanced training-induced increase in calcium concentration in synaptoneurosomes treated with NMDA, but not with AMPA. Moreover, dantrolene injected intracerebrally 30 min before or after training induced amnesia. These data indicate that calcium release from ryanodine-sensitive intracellular stores may be a necessary stage in the long-term memory storage.

In studies on the role of intracellular calcium in the mechanisms of adaptation to anoxia, attention was focused on the involvement of NMDA receptors

in anoxic preconditioning in the rat cortical slices. It was noted that both long- (10 min), and short-lasting (2 min) anoxia induce sustained post--anoxic increase in the content of intracellular bound calcium, and in the concentration of calcium ions, proportional to the duration of anoxia. These effects were abolished by an NMDA receptor antagonist, 10 μM MK-801. An episode of short preconditioning anoxia preceding of 10 min anoxia prevented the development of anoxia-induced disturbances in calcium homeostasis characteristic for the long-lasting anoxia. This protective effect was completely deleted by the presence of MK-801 during preconditioning. These results demonstrate the role of NMDA receptors in the induction of tolerance to anoxia.

Our further studies on the role of intracellular calcium imbalance in the pathogenesis of ischemic neuronal damage demonstrated, for the first time, a reversibility of postischemic disappearance of immunoreactivity of the calcium binding protein, calbindin D_{28K} in the pyramidal neurones of the rat CA1 and CA2. In agreement with previous findings, calbindin immunoreactivity was lacking between the third day and the end of the first month after the insult. However, it reappeared 6 and 12 months after cardiac arrest, showing the same pattern and percentage of the calbindin-positive neurones, as before ischemia. These results do not support the neuroprotective role of the presence of calbindin in the hippocampal neurones, assuming that the neurones surviving ischemia re-establish their preischemic phenotype.

Studies on the role of calcium induced mitochondrial permeability transition (mPT) in the pathomechanism of NMDA excitotoxicity to the hippocampal neurones, were continued *in vitro* and *in vivo*. It was demonstrated, that cyclosporin A, which *in vitro* partially prevents the calcium-induced swelling of the brain mitochondria, and delays release of potentially proapoptotic cytochrome C, prevented NMDA-induced swelling of mitochondria in the CA1 neurones in the rabbit hippocampus *in vivo* and reduced the NMDA-induced degeneration of these neurones. These results support the hypothesis that inhibition of mPT is a key mechanisms of cyclosporin A-evoked neuroprotection.

In addition, the laboratory participated in several collaborative studies. It was demonstrated that hydroxylamine, a NO donor, applied in the rat model of subarachnoid hemorrhage (SAH), reduces the SAH-induced enhancement of the immunoreactivity of the β-amyloid precursor protein in the cortex and

hippocampus. In other collaborative study it was demonstrated for the first time that glutamine at pathophysiological concentrations induces swelling of brain mitochondria. This effect was partially inhibited by cyclosporin A and completely prevented by the inhibitor of mitochondrial uptake of glutamine, indicating that mPT may be involved in the pathogenesis of hepatic encephalopathy. In another study acute and chronic systemic application of high doses of glucose to healthy adult mice was found to induce swelling of some astrocytic and synaptic mitochondria with intramitochondrial accumulation of the glycogen-like granules. These data point on brain mitochondria as a likely target of glucose neurotoxicity.

Collaborating units:

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- Department of Cell Ultrastructure (B. Gajkowska)
- Laboratory of Experimental Pharmacology (P. Grieb, M.S. Ryba)

NEUROPROTECTIVE EFFECT OF DANTROLENE IN BRAIN HYPOXIA/ISCHEMIA OF NEONATAL RATS

Supported by the State Committee for Scientific Research: grant # 4.P05A.025.16

Project leader: Dorota Makarewicz
Contributor: Anna Sobczuk

Studies are in a preliminary stage. A model of perinatal asphyxia was established and brain hemispheres were collected for further experimentation. A more exhaustive report will follow in the next Annual Report issue.

Publications:

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POSTTRANSLATIONAL PROTEIN MODIFICATIONS IN RESPONSE TO CEREBRAL ISCHEMIA

Project leader: Krystyna Domańska-Janik
Contributors: Agnieszka Bronisz-Kowalczyk, Leonora Bużańska, Małgorzata Nałęcz, Barbara Zabłocka, Teresa Zalewska.

MODULATION OF TYROSINE KINASE, MAPK AND SAPK PATHWAYS BY ISCHEMIA-INDUCED CALCIUM SIGNAL – ROLE OF PROTEIN KINASE C

Supported by the State Committee for Scientific Research: grant # 6.P04.010.14

Project co-ordinator: Krystyna Domańska-Janik

The core pathomechanism of any type of ischemic cellular demise involves membrane depolarisation, calcium and glutamate toxicity and oxidative stress during reperfusion. All of these factors directly impair mitochondria function and stability as well as variety of intracellular signal transduction systems. If stress is severe but not sufficiently potent to cause direct, necrotic cell death, different tissue and pathology-specific repair mechanisms are induced. In parallel in some cells the apoptotic programs, usually under rigorous repressive control, become activated for still unknown reasons. In effect delayed responses to ischemia can be situated in the spectrum from complete functional and morphological recovery up to apoptosis/ necrosis of vulnerable neurones.

Main goal is to characterise factors and processes determining sensitivity of neural cells to delayed death after metabolic/ischemic stress.

Points of interest: a) anomalies in protein phosphorylation/ dephosphorylation balance and kinase /phosphatase activities; b) calpains and their substrates degradation; c) loss of survival and induction of stress signals transduced by

mitogen-activated-kinases (MAPKs) leading to ERK and JNK activation; d) induction of transcriptional factor AP1 and functional changes in its composition; e) immune-competent signalling and NFkB expression/regulation in activated microglia and astrocytes.

Experimental models. ***In Vivo.*** Transient (5 min.) forebrain ischemia in gerbils results in selective neuronal apoptosis becoming evident after 3-5 days of reperfusion in CA1 sector of hippocampus. The cellular reaction on ischemic insult is assessed at various reperfusion time in the two parts of hippocampus: dorsal part containing vulnerable CA1 neurons and abdominal part considered as an ischemia-resistant area. A combination of potentially damaging and compensatory processes are differentially expressed in these two parts of hippocampus.

In vitro. Apoptosis induced by various factors (staurosporine, wortmannin, ammonia) in N2a neuroblastoma and C6 glioblastoma cell lines.

CALPAIN-DEPENDENT PROTEIN KINASE C DOWN-REGULATION IN POSTISCHEMIC NEURONAL APOPTOSIS

Project leader: Teresa Zalewska
Contributors: Teresa Czechmańska, Krystyna Domańska-Janik, Małgorzata Nałęcz

During the last few years we focused our attention on the role of Ca^{2+} - dependent isoforms of protein kinase C (cPKCs) in the development of delayed neuronal damage which occurs selectively in pyramidal neurons of CA1 region following short-term cerebral ischemia. In the course of our previous study we found that ischemia induced biphasic changes of the cPKC activity – early enhancement of phosphorylating activity was followed by its down-regulation. In the present study we have expanded our observations on various isoforms (a classical, a novel and an atypical) of PKCs superfamily in two distinct regions (ischemia resistant abdominal and vulnerable CA1) of hippocampus. The main finding of the present study is that postischemic decrease of PKC levels referred to as down-regulation , is mainly specific for CA1 region. Moreover, the down-regulation of PKC during reperfusion is simultaneously accompanied by the increase of calcium dependent proteolytic activity of calpain, which is expressed by degradation of cytoskeletal

proteins (fodrin and MAP-2) and also restricted to CA1 region. The loss of cPKC protein, as well as enhancement of proteolytic activity during recirculation, corresponds with the occurrence of neuronal apoptosis in pyramidal layer of CA1. We speculate that one of the key events in the signalling cascade of ischemia-induced apoptosis might be not initial translocation/activation of PKC observed regardless of regional susceptibility to ischemia, but rather the subsequent down-regulation and/or degradation of calcium-dependent isoforms specific only for CA1 region.

TRANSLOCATION OF PROTEIN KINASE C ISOFORMS TOWARDS POSTSYNAPTIC DENSITIES AFTER CEREBRAL ISCHEMIA

Project leader: Barbara Zabłocka
Contributor: Krystyna Domańska-Janik

Protein composition of the postsynaptic densities (PSD) undergoes rapid modifications after 15 min postdecapitative as well as 5 min transient global ischemia. In addition to previously described changes (Domańska-Janik et al. 1999) we observe a significant increase in cPKC and nPKC protein content in the postischemic PSD. From calcium-regulated PKC isoforms the α and β subtypes increase in PSD over 10 times above the control values whereas γPKC, an isoform most abundant in the native PSD structure, shows relatively smaller changes under ischemic conditions. For the first time, the PSD membrane translocation of Ca^{2+}-independent isoforms δ and ε is shown. The yield of the PSD protein preparation from the postischemic cortex was 2 times higher compared with control. At the electron microscope level it correlates with an abundant increase in the optical density and changed morphology of this structure. Also the sections from CA1 gerbils hippocampus after transient ischemia show persistent magnification of postsynaptic densities up to 24 hours of reperfusion. It is accompanied by elevation of the PSD/cytoskeleton-connected α, β PKC immunoreactivity and other changes in neuronal and glial cells morphology typical for postischemic degeneration. We conclude, that the sustained molecular changes which are already seen in the PKC composition and organization of postsynaptic membrane during and after ischemia might, due to persisted alternation in synaptic transmission, contribute to delayed neuronal injury.

Collaborating unit:
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INTERRELATIONS BETWEEN NUCLEAR FACTOR KAPPA B ACTIVATION, GLIAL RESPONSE AND NEURONAL APOPTOSIS IN GERBIL HIPPOCAMPUS AFTER ISCHEMIA

Project leader: Krystyna Domańska-Janik
Contributors: Agnieszka Bronisz-Kowalczyk, Barbara Zabłocka.

Nuclear factor kappa B (NFκB) is a transcriptional factor coordinating expression of variety of genes encoding proteins involved in immunity and inflammation. Spatial and temporal relations between NFκB and glia activation in gerbil hippocampus after transient cerebral ischemia has been studied. Activation of protein binding to NFκB consensus oligonucleotide was determined by electrophoretic mobility gel shift assay (EMSA) and by translocation of this factor from cytoplasmatic to nuclear fraction.An early activation of NFκB was observed exclusively in CA1 sector at 3 hr after ischemia and preceded any morphological markers of ischemic tissue injury. This early NFκB activation in CA1 region was followed by microglia activation as visualized by lectin (RCA-120) binding in CA1 at 24 hr reperfusion. Although, at 3 days after the insult when apoptotic DNA fragmentation was clearly seen in TUNEL staining in CA1 pyramidal neurons, a pattern of increased NFκB signalling coincided rather with uniform astrogliosis (GFAP immunoreactivity) in dorsal as well as abdominal hippocampus. From this data can be concluded that NFκB activation precedes both: an early microglia and a later astroglia activation observed at different times of postischemic reperfusion.

MECHANISM OF APOPTOSIS INDUCED BY PKC INHIBITION IN N2A NEUROBLASTOMA

Project leader: Agnieszka Bronisz-Kowalczyk
Contributors: Leonora Bużańska, Krystyna Domańska-Janik

A model for study of the apoptosis *in vitro* was established on the neuroblastoma N2a cell line. By staining of living cells with Hoechst 33258 and Propidium Iodide, or by TUNEL, cJun/AP1 immunoreaction and DNA laddering we show that the N2a cell line responded with apoptosis to PKC inhibition. Two different classes of inhibitors, staurosporine and G06976, at concentrations regarded as PKC-selective (10 and 50 nM respectively) significantly increased the number of apoptotic cells. They started to die 2-3 hr after the treatment then, at 6 and 24 hr approximately 30-40% of cells acquired apoptotic feature. This response was neither dependent upon cell differentiation nor the PKC and bcl2 gene and protein expression, but exclusively on the lack of serum in growth medium. Furthermore, both of experimental manipulations (serum deprivation and PKC inhibition) synergically, but to different extent, suppressed the ERK pathway. However, their pro-apoptotic effects were neither mimicked nor modified by an additional inhibition of MEK/ERK kinase by 50 μM PD 98059 resulting in a 80% inhibition of its enzymatic activity.

The further study showed that apoptosis of the cells was preceded by AP1 upregulation, increased JNK phosphorylation and enhanced phospho-cJun contents in AP1 complex. Moreover apoptotic effect of staurosporine was coupled with decreases of RAF and BAD phosphorylations in N2a cells. The later effect was abrogated by the survival signals induced by serum and transduced by wortmannin sensitive PIP3K-AKT pathway. These data indicate that a targed reaction in staurosporine-induced serum-sensitive N2a apoptosis is a level of BAD phosphorylation and its sequestration out of Bcl2 connected with mitochondria. The extent of BAD phosphorylation is regulated by both, RAS-RAF as well as AKT pathways activity.

RECIPROCAL ROLE OF ERK AND JNK PATHWAYS OF MAP-KINASES CASCADE IN NEURONAL DEATH/SURVIVAL AFTER ISCHEMIA

Project leader: Krystyna Domańska-Janik
Contributors: Barbara Zabłocka, Agnieszka Bronisz-Kowalczyk.

The postischemic activation of transcriptional factor AP1 and two pathways of the MAPK cascade: ERK and JNK were evaluated in gerbil hippocampus in our *in vivo* ischemic model (see above). The neuronal apoptosis

visualized by TUNEL reaction appeared exclusively in CA1 pyramidal cells at 3-4 days after the insult. Concomitantly the enhanced immunoreactivity to anti-cJun/AP1 antibody was observed in this sector only in the cells designed to die. The AP1 activation, estimated by electrophoretic mobility gel shift assay (EMSA), showed biphasic reaction: an early enhancement in ischemia-resistant hippocampal regions and a late one in the vulnerable, apoptogenic CA1 layer. At the time of apoptosis super-shift EMSA with antibodies against cFos and phospho-cJun constituencies of AP1 dimmer revealed an increase of phospho-cJun and a decrease of cFos contents in parallel with activation/ phosphorylation of JNK and inhibition of ERK pathways. In addition successively decreasing contents of phosphorylated BAD in CA1 sector was observed after ischemia during apoptosis induction.

These results indicate 1) an heterogeneity of AP1 complex in various, functionally different phases of cellular postischemic reaction in gerbil hippocampus, 2) the different roles of ERK and JNK pathways: the former, in parallel with BAD phosphorylation being coupled with neuronal survival whereas JNK / cJun phosphorylation with neuronal death.

DELAYED INDUCTION OF APOPTOSIS BY AMMONIA IN C6 GLIOMA AND REACTIVE ADULTS ASTROCYTES IN CULTURE

Project leader: Leonora Bużańska
Contributors: Krystyna Domańska-Janik, Barbara Zabłocka

Ammonia is a neurotoxin implicated in hyperammonemic encephalopathy. Ammonia neurotoxicity is mediated by NMDA receptors and the downstream effects of ammonia include PKC translocation and phosphorylation of microtubule associated proteins 2 (MAP-2). Since the above effects are also involved in the transduction of the apoptotic signalling, we speculated that ammonia might promote apoptosis. In the present study therefore, we tested the potential of a pathogenic (5 mM and 10mM) concentrations of ammonium chloride to induce apoptosis in C6 glioma cells and reactive astrocytes derived from adult rat striatum in culture. Cells grown either in high (10%) or low (2%) serum were incubated with 10 mM ammonium for five days. Each 24 h ammonium treated and non treated cells were screened for the extent of apoptosis by differential staining of living cells with Hoechst

33258 and Propidium Iodide. Morphological observations were additionally verified by DNA laddering. It was shown that 10 mM ammonia causes apoptosis but in low serum conditions only. Significant increase in the number of apoptotic cells is observed after 48 h (9%) and massive response (up to 50%) after 96 h. This dynamics of apoptosis is typically delayed comparing with classical response. However it is consistent with the changes in PKC activation in ammonia toxicity in cerebellar granular cells, where early translocation of PKC is followed by its down regulation. Moreover, the initial activation of NFκB transcriptional factor after ammonia treatment characteristic for glia activation, is followed by its inhibition in the apoptotic phase. We hypothesize that the mechanism of apoptosis induction by ammonia involves its down-stream effects on PKC and NFkB signalling.

Collaborating unit:
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LINEAGEING OF MULTIPOTENT NEURAL HUMAN CELLS TOWARDS NEURONAL OR GLIAL FATE BY TRANSFECTION *IN VITRO*

Project co-ordinator: Leonora Bużańska

Recent studies provided evidence for the existence of neural stem cells in adult vertebrates and the potency of these cells to differentiate into either neurons, astrocytes or oligodendrocytes. However, no data are available as to whether factors capable of differentiating non-human neural cell lines can effectively and in well defined (precisely oriented) manner differentiate human lines into authentic cell types of the human CNS.

In this project using human medulloblastoma DEV cells line we tested the influence of the expression of ten different transcription factor genes on the process of specification of a human neural precursor towards a neuronal or glial fate. It is shown that most of invertebrate or mouse transcription factor genes tested, have conserved their function in the human DEV cell line, qualifying this cell line for the use as a screening tool for experimental verification of the mechanisms underlying lineageing towards neurons and glia.

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Publications:

Domańska-Janik K., Bong P., Bronisz-Kowalczyk A., Zając H., Zabłocka B.: AP1 transcriptional factor and its relation to apoptosis of hippocampal CA1 pyramidal neurons after transient ischemia in gerbils. *J Neurosci Res* 1999, 57, 840-846.

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LABORATORY OF PATHOBIOCHEMISTRY OF THE CNS

Head: Professor Urszula Rafałowska

STUDIES ON DISTURBANCES OF FUNCTION AND METABOLISM OF THE CENTRAL NERVOUS SYSTEM CAUSED BY TOXICITY OF LEAD AND ISCHEMIC PATHOLOGY IN BRAIN

Project leader: Urszula Rafałowska
Contributors: Beata Dąbrowska-Bouta, Aleksandra Lenkiewicz, Lidia Strużyńska, Grzegorz Sulkowski, Jolanta Waśkiewicz

The protective role of astroglia in the early period of experimental lead toxicity in the rat

The purpose of this study was to assess the function of nerve endings and astrocytes after acute lead exposure. Male Wistar rats were used in the experiments. Two cellular fractions: synaptosomes and glia-derived vesicles (GPV) obtained from control and intoxicated animals were used in biochemical

measurements. Previous studies on glial toxicity by lead were mainly performed using cultured glial cells. In contrast to synaptosomes, which are a good model for nerve ending studies, there were no preparations available for the study of glial functions after *in vivo* exposure, until recently. The GPV fraction obtained by the method of discontinuous density-gradient centrifugation by Daniels and Vickroy was verified by the authors for the structural integrity and analyzed for glial markers and functional specificity. The studies provide solid evidence for the highly-enriched glial nature of the GPV fraction. Acute lead exposure lowered the uptake of glutamate to the synaptosomes and increased its KCl-dependent release suggesting an impairment of glutamatergic transmission perhaps leading to the elevation of extracellular amino acid concentration. In contrast, glutamate uptake to the GVP fraction was significantly elevated. The activity of the marker enzyme glutamine synthetase (GS) was also significantly increased. The activation of glial fraction suggest a regulatory role for these cells in the early period of acute lead toxicity.

Alteration of dopamine transport and dopamine D2 receptor binding in the brain of the early and late stage after global ischemia caused by cardiac arrest in the rat

A unique rat model of global cerebral ischemia resulting in cardiac arrest was chosen for our work because it mimics in a well controlled way global cerebral ischemia and postischemia in humans. Our present studies using this model have been designed to focus on the question of whether or not dopamine transport and dopamine D2 receptor binding in CNS observed in long-term post resuscitation differs from the observed in the early phases of this pathological process. The effects of 10 min global ischemia were measured immediately and 1 hour and 7 days post resuscitation. A decrease of dopamine uptake by synaptosomes was noted immediately following global ischemia and 1 hour after resuscitation. No changes in dopamine release were observed at any time period postischemia. There was an increase in the affinity of D2 receptor binding in the early postresuscitation phase, perhaps reflecting adaptation to the loss in D2 binding sites.

Collaborating units:
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- Laboratory of the Cell Ultrastructure (M. Walski)

Publication:
Sulkowski G., Dąbrowska-Bouta B., Waśkiewicz J., Rafałowska U.: Inhibition of dopamine transport and binding of [3H] spiperone to dopamine D2 receptor by acute PB-toxicity *in vivo*. *Folia Neuropathol* 1999, 37, 205-209.

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MOLECULAR MECHANISMS OF BRAIN AGING AND POSTISCHEMIC ENCEPHALOPATHY AND THE EFFECT OF AMYLOID BETA AND ETHYL ALCOHOL

Project leader: Joanna Strosznajder
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In 1999 the studies were continued on molecular mechanisms of brain aging and on the effects of amyloid beta and ethanol. Moreover, the mechanism of alteration of signal transduction processes during reperfusion after short time of forebrain ischemia was investigated. Our studies have also focused on evaluation of the compounds effective in protecting the brain cells from degeneration. The study was concentrated on the nuclear enzyme, poly(ADP-ribose) polymerase (PARP, EC 2.4.2.30). This enzyme plays an important role in the stability of genome. DNA damage activates PARP. Following limited DNA damage, PARP activation plays a critical role in DNA repair. However, massive DNA damage and the extensive activation of PARP lead to polyADP ribosilation of nuclear protein, NAD and ATP depletion and to cell death. Up till now, the biological role of PARP and in particular, the role of PARP in neurotransmission processes is not fully understood. In our study, the regulation of PARP activity was determined after cholinergic receptor stimulation in different parts of the brain, i.e. in hippocampus, brain cortex and striatum. Concomitantly, the involvement of free radical processes in muscarinic cholinergic receptor regulation of PARP activity was estimated. The activity of PARP was determined by the measurements of nuclear protein ADP-ribosylation using [^{14}C] NAD. Our data indicate that stimulation of muscarinic cholinergic receptor in all investigated parts of the brain activates PARP in about 50% of the experiments whereas in the remaining experiments the signal did not reach this nuclear enzyme. It

has been found that stimulation of PARP in these experimental conditions is not dependent on free radical processes. The results indicate a new role for PARP as the nuclear target for cholinergic receptor mediated signal transduction pathway in the brain. Moreover, we have also observed that stimulation of glutaminergic receptor exclusively in hippocampal slices, evoked an activation of PARP in about 80%. This NMDA receptor dependent PARP stimulation was decreased by about 50% by an inhibitor of nitric oxide synthase (NOS), N-nitro L-arginine (NNLA) and was eliminated by spermine, NMDA receptor modulator and free radical scavenger. However, this effect was not observed in hippocampal slices from aged animals. In nuclear fractions from brain cortex, hippocampus and cerebellum of adult aged (14 months) animals, the enhancement of PARP activity amounted 35%, 100% and 70%, respectively comparing with control value. In aged (24-months) animals, PARP activity was slightly stimulated in the brain cortex. In cerebellum, the activity of enzyme was close to control value. However, in the hippocampus of 24-months animals, enzyme activity was decreased to 50% of the control value. Our data indicate that free radical processes may be involved in the stimulation of PARP activity in adult aged brain. The analysis of Western blot presented no alteration in PARP protein content in aged brain. However, it is impossible to exclude that covalent modification of the enzyme or its interactions with nuclear proteins are responsible for the alteration of its activity. Lower activity of PARP (basal and NMDA receptor regulated) in aged hippocampus may be the consequence of an alteration of neurotransmission processes regulated by NMDA receptor system in aged brain and may suggest the alteration of DNA repair mechanisms in aged hippocampus. In the case of short forebrain ischemia in gerbils, enhancement of PARP activity was observed exclusively in hippocampus, during short time of reperfusion, 15 min after ischemia. Thirty min, 1 h and 2 h after ischemia, PARP activity was decreased and was enhanced again 4 days after ischemic insult. NMDA receptor antagonist, MK-801, administrated intraperitoneally before ischemia protects the brain against enhancement of PARP activity during reperfusion. In brain cortex, ischemic insult has no effect on PARP activity. The preliminary results of Western blot analysis indicated lower immunochemical reaction for PARP in the hippocampus 7 days after ischemia. Studies in this field will continue using different PARP inhibitors to examine their effect on neuronal survival in CA1 layer of the hippocampus.

The efficiency of PARP inhibitors will also be determined in local brain ischemia in the cooperation with the Department of Neuropathology.

Among the agents that may affect NOS and PARP activity, the ethanol was investigated. Basal and NMDA receptor dependent NOS and PARP activity and free radical processes were determined after preincubation of brain slices with ethanol in concentration range of 50-200 mM. It has been found that ethanol in the concentration of 100 mM activates basal NOS activity exclusively in hippocampal slices. However, it has no effect on NMDA receptor regulated NO synthase. It has also been found that in the concentration range of 50-200 mM during 4 h of incubation ethanol has no effect on free radical evoked lipid peroxidation processes. Ethanol directly influences the cytosolic form of NOS dependent of tetrahydrobiopterin (BH4) concentration. In the presence of low BH4 concentration ethanol significantly decreases cytosolic NOS activity. These data indicate that 100 mM ethanol enhances NOS activity in hippocampal slices probably by the effect on membrane and receptor functions. Our studies suggest that ethanol may affect neurotransmission processes regulated by NO and cGMP. Moreover, the data provide evidence that BH4 is an important factor responsible for the effect of ethanol on NOS activity. Complementary to the above studies, the cooperation with other departments was carried out, e.g. with the Laboratory of Experimental Pharmacology and with the Department of Neuropathology on the effect of hydroxylamine on neurological outcome after subarachnoid hemorrhage and on APO E genes in Alzheimer's disease in the central Poland.

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CHANGES OF TRANSPORT OF NEUROACTIVE AMINO ACIDS IN HYPERAMMONEMIC ENCEPHALOPATHY

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The contents of the neuroactive amino acids taurine (Tau), glutamate (Glu) and aspartate (Asp), were measured in the cerebral cortical microdialysates of control rats and rats treated twice with thioacetamide (TAA) at 24h intervals and examined 21 days later, i.e. in the apparent recovery (subclinical hepatic encephalopathy, SHE) phase after hepatocellular damage. When microdialysis was carried out in the awake rats, the dialysate contents of all the three amino acids were found to be higher in SHE rats than in control rats, manifesting both an ongoing neuropathological process (excitotoxic neuronal damage related to increased Glu and Asp), and improved neuroprotection (redistribution of Tau) in the cerebral cortex. Ketamine anesthesia led to an increase of the basal Asp, Glu and Tau content in the microdialysates of control rats, while in SHE rats the Asp and Glu contents in the microdialysates were not different from control. The reduction of the effects of SHE on Glu and Asp content in ketamine-anesthesized rats are likely to reflect interference of ketamine with the NMDA receptor-mediated component of the SHE-evoked efflux and/or reuptake of the two amino acids. The SHE-related increase of Tau content was not affected by ketamine anesthesia, indicating a different mechanism(s) underlying SHE-evoked accumulation of Tau and excitatory amino acids.

The efflux of endogenous amino acids and cell volume (inulin space) was measured in cerebrocortical minislices derived from rats with TAA-induced HE. Since an extra ammonia challenge is often a precipitating factor in he-

patic encephalopathy (HE) patients, we compared the effect of HE or/and *in vitro* treatment of the slices with 5 mM ammonium acetate. It was shown that HE and ammonia stimulate the efflux of endogenous Tau and other neuroactive amino acids independently of producing changes in the cellular amino acid content, confirming the operation of an osmoindependent mechanism of efflux under these conditions. In slices derived from HE rats, cell volume was found unchanged upon superfusion in the absence of ammonia, but was increased in the presence of ammonia. This result is consistent with impaired cell volume regulation being the factor precipitating brain edema and coma in HE patients subjected to extra ammonia challenge.

Collaborating units:
Department of Cell Physiology and Pharmacology, University of Leicester, England (R.O. Law)
Preclinical Research and Development, Merz+Co, GmbH+Co, Frankfurt, Germany (G. Quack, M. Hesselink)

GLUTAMINE AND AMMONIA NEUROTOXICITY

Collaborative project.
Contributors: Jan Albrecht, Monika Dolińska

Considerable evidence indicates that excessive accumulation of glutamine (Gln) in the CNS contributes to pathophysiological symptoms of hyperammonemic encephalopathy, but the underlying mechanism remains unclear. We showed that exposure of rat cerebral mitochondria to Gln at concentrations normally reached in the brain during hyperammonemia induces mitochondrial swelling and induces mitochondrial permeability transition (mPT) induction. Histidine (His), which is a potent inhibitor of high affinity Gln uptake to mitochondria, attenuated Gln-induced decrease of mitochondrial light scattering indicating simultaneous attenuation of mPT. The results thus point to mitochondrial swelling and subsequent activation of mPT, as one of the potential mechanisms by which Gln induces metabolic disturbances in the brain in hyperammonemic conditions.

Collaborating unit:
Laboratory of Pharmaconeurochemistry, Department of Neurochemistry, Medical Research Centre, PASci, Warsaw, Poland (J.W. Łazarewicz, E. Ziemińska)

HEPATIC ENCEPHALOPATHY (HE) AND DOPAMINERGIC SYSTEM

Project leader: Jan Albrecht
Contributors: Hanna D. Borkowska, Wojciech Hilgier.

Investigations on the effect of HE in the clinical stage of the thioacetamide model on the dopaminergic system focused on the role of calcium in the stimulation of dopamine (DA) release under these conditions. HE enhanced the KCl-stimulated [3H]DA release from the striatal and frontal cortical slices and synaptosomes in the presence , but not in the absence of Ca^{2+} in the release medium. We conclude that in both brain regions studied, HE stimulates DA exocytosis triggered by Ca^{2+} influx, without affecting the release through reversed operation of plasma membrane transporter, or exocytosis involving intraterminal Ca^{2+}.

Collaborating units:
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Laboratory of Molecular Neuropathology, Department of Neurochemistry, Medical Research Centre, PASci, Warsaw, Poland (K. Domańska-Janik, L. Bużańska)

GLUTAMINE TRANSPORT IN GLIAL CELLS DURING ACUTE OR CHRONIC EXPOSURE TO AMMONIA

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Project leader: Jan Albrecht
Contributors: Monika Dolińska, Anna Dybel, Wojciech Hilgier, Maria Zielińska

Glutamine transport across the cell membranes of a variety of mammalian tissues is mediated by at least four transport systems: a sodium-inde-

pendent system L, and sodium-dependent systems A, ASC and N, the latter occurring in different tissue-specific variants. We assessed the contribution of these systems to the uptake of [3H] glutamine in C6 rat glioma cells. In the presence of sodium the cells accumulated glutamine with a relatively high affinity (Km~0.12 mM) and moderate capacity (Vmax~2.1 nmoles/min/mg prot.), both parameters similar to those measured by others in cultured rat astrocytes or neurons. The sodium-dependent uptake which accounted for more than 80% of the total uptake, was not inhibited by methylamino-isobutyric acid (MeAiB), indicating that system A was inactive, possibly being depressed by glutamine present in the culture medium. About 80% of the sodium-dependent uptake was mediated by system ASC, which differed from the ASC system common to other CNS- and non-CNS tissues by its pH-dependence and partial lithium tolerance. The residual 20% of sodium-dependent uptake appeared to be mediated by system N, which was identified as a component resistant to inhibition by McAiB + threonine. The N system in C6 cells appeared to be neither fully compatible with the neuronal Nb system, nor with the N system described in astrocytes: it differed from the former in being strongly inhibited by histidine and showing fair tolerance for lithium, and from the latter in its pH-insensitivity and strong inhibition by glutamate. The sodium-independent glutamine uptake differed from the astrocytic or neuronal uptake in its relatively weak inhibition by L-system substrates and a strong inhibition by ASC substrates, indicating a possible contribution of a sodium-independent variant of the ASC system.

The question whether and in what degree Gln uptake to mitochondria is a rate limiting process in Gln metabolism has for many years been subject to hot debate. In this study therefore, we tested the ability of protein and non-protein amino acids and their synthetic structural analogues and derivatives for their ability to inhibit the uptake of L-[14C] Gln and the activity of the major Gln-metabolizing enzyme, phosphate-activated glutaminase (PAG) in nonsynaptic mitochondria isolated from rat cerebral hemispheres. The uptake was inhibited by >50% in the presence of a 10-fold excess of a number of amino acids, most strongly by His, homocysteine (Hcy), Tyr, and Leu, and a newly synthesized alanine analogue, 2'-cyano -(biphenyl) alanine, referred to as MRC01. MRC01, His, Hcy and Leu also inhibited PAG activity by >50% when added at the inhibitor/Gln concentration ratio of 1:2. PAG activity was not affected by Tau, Lys or Pro, compounds which did not inhibit Gln uptake. The results suggest that a number of natural amino acids

function as common endogenous modulators of cerebral mitochondrial Gln uptake and its degradation. MRC01, because of its inhibitory potency towards both mitochondrial Gln uptake and PAG activity, may become a convenient tool in studying the role of Gln transport in its mitochondrial metabolism in intact CNS cell and tissues.

Collaborating unit:
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DELAYED INDUCTION OF APOPTOSIS BY AMMONIA IN C6 GLIOMA CELLS IN CULTURE

Collaborative project.
Contributors: Jan Albrecht, Anna Dybel.

We tested the potential of pathogenic (5 mM and 10mM) concentrations of ammonium chloride to induce apoptosis in C6 glioma cells and reactive astrocytes derived from adult rat striatum in culture. In cells grown in low (2%) serum concentration in the presence of either concentration of ammonium chloride, a significant increase in the number of apoptotic cells was observed after 48 h (9%) and massive response (up to 50%) after 96 h, as revealed by differential staining of living cells with Hoechst 33258 and Propidium Iodide, and additionally verified by DNA laddering. This dynamics of apoptosis was thus delayed comparing with classical response. However it was consistent with the changes in PKC activation previously shown to underlie ammonia toxicity in cerebellar granular cells, where early translocation of PKC is followed by its down regulation. Moreover, the initial activation of NFκB transcriptional factor after ammonia treatment characteristic for glia activation, was followed by its inhibition in the apoptotic phase. We hypothesize that the mechanism of apoptosis induction by ammonia involves its down-stream effects on PKC and NFκB signalling.

Collaborating unit:
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QUALITATIVE AND QUANTITATIVE EVALUATION OF CHANGES IN BRAIN IN THE POSTISCHEMIC ENCEPHALOPATHY IN DIFFERENT EXPERIMENTAL MODELS

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The doctoral thesis entitled: "Effect of Coenzyme Q_{10} on morphological picture and some biochemical processes in rat brain in experimental models of ischemic hypoxia following administration of endothelin 1 and 3" was accomplished. The experimental results on superoxide dismutase activity, morphometric studies in hippocampal neurons and quantitative analysis of GSH, and GSSG to SH ratio are included. The doctoral thesis: "Effect of Coenzyme Q10 and lipoinic acid on histopathological, ultrastructural changes and apoptosis in the experimental streptozotocin-induced diabetes complicated with ischemic hypoxia of the rat brain" is far advanced. The thesis presents, among other, the morphometric studies. The subject of another thesis has been settled and the experiments are in progress. The title is: "Effect of zinc ions on intensity of the early and late injuries of the rat hippocampal neurons in the anoxic model *in vitro*". In collaboration with two German centres, an electrochemical method of quantitative evaluation of the radical scavenging activity of superoxide dismutase under the condition of cerebral ischemia has been elaborated and published. In collaboration with the Laboratory of Pathobiochemistry of the CNS, MRC, changes in dopamine transport and density of D_2 receptors in the rat brain after cardiac arrest

have been described. In collaboration with the Laboratory of Experimental Pharmacology, MRC, the studies on protective effect of CDP-choline on survival of CA_1 hippocampal neurons in the gerbil brain after shortlasting ischemia have been performed. In collaboration with the Laboratory of Neurochemistry of the M. Nencki Institute of Experimental Biology, several series of experimental studies have been performed under the project entitled: "Participation of proinflammatory cytokines and neurotoxic factors in the mechanism of neurodegeneration in hippocampus after ischemia and neurotoxic impairment of brain". In collaboration with the Laboratory of Neuropathology and Molecular Biology of Pediatric Diseases of the New York State Institute for Basic Research in Developmental Disabilities, the characteristics of posttranslative modifications of CLN3 protein, its function and molecular reasons of functional disorders of CLN3 protein having atypical point mutations have been studied. Furthermore, the role of α-synuclein in the neurodegenerative and ischemic processes in the brain has been also studied. The study on the effect of Coenzyme Q_{10} on alterations of glucoconjugates at the cell surface in brain ischemia has been continued.

Investigations have been conducted beyond the statutory program concerning the morphology and immunohistochemical analysis of inflammatory infiltrations and expression of PECAM-1 in lung adenoma (collaboration with the Department of Neurology and the Department of Pathology, Medical School in Warsaw, and astrogliosis with the development of blood-vessels during myelinization of spinal cord. As in the former years, the Department of Neuropathology has conducted histopathological and immunocytochemical diagnostics of brain tumors and case reports. There were 120 such cases.

Collaborating units:

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FENOTYPIC AND GENOTYPIC EXAMINATION OF APOLIPOPROTEIN E ISOFORMS IN ALZHEIMER'S DISEASE AND AGING – CONTINUATION

Project leader: Maria Barcikowska-Litwin
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Apolipoprotein E genotype (APOE) has been identified as a susceptibility factor in the late-onset familial and sporadic Alzheimer's disease (AD). Many studies have shown a strong association between the APO Eε4 allele in both sporadic, early and late onset familial AD in different populations. We examined the association between *APOE* genotypes, and a risk for AD in the Polish patients. Our subjects included 183 individuals divided into 2 groups: controls (n=46) and AD cases (n=137). The AD group met DSM-III R and NINCDS/ADRDA criteria for possible or probable AD and were further divided into three subgroups: pure sporadic AD (p AD) (n=116), mixed dementia (MIX) [n=14] and familial AD (FAD) [n=7]. Our study suggests that the APOEε4 allele frequency in the Polish AD patients is similar to those reported from other Western countries and the APOEε2 allele occurred with significantly lower frequency in AD patients than in agematched controls. Moreover, *APOE* 4/3 and 3/3 had significantly higher frequency in AD than 4/4 genotype and there was neither an association with the age of onset and the number of e4 alleles nor with the specific genotype of the Polish AD patients. However, despite well confirmed evidence for the role of *APOE* in AD, it is not recomended to test for APOEε4 in routine clinical diagnosis and APOEε4 should not be used for predictive testing. The aim of another study was to determine whether *APOE* genotypes can influence the rate of cognitive decline in AD. *APOE* genotypes were identified in 100 persons (64 women and 36 men) with clinically diagnosed AD according to NINCDS/ADRDA criteria, who were followed up longitudinally for 12 months. Statistically significant differences were observed between ε4 carriers and non-carriers regarding measures of cognitive decline. The patients without APOE ε4 alleles progressed faster (3.90 points per year on the MMSE). The rate of decline for patients with other genotypes was slower, at 2.8 points per year on the MMSE ($p<0.5$). We suggest that the APOE ε4 allele is not associated with a more rapid course of AD. Recent studies suggest that α2microglobulin (α2M) may play an important role in the

pathogenesis of AD. The presence of (α2M) G/G genotype is thought to increase the risk of late onset form of AD. We have studied (α2M) polymorphism in a Polish population of AD patients. The (α2M) genotyping was done using a method of restriction isotyping in two groups of people; AD patients (60) and 58 non-demented control group. Among the AD patients the (α2M) genotype G/G, A/G and A/A frequency was 0.06, 0.51 and 0.43 respectively. In the control group the frequencies were: 0.09, 0.58 and 0.33, respectively. We found by now no statistically significant difference between the groups, which may suggest that G/G genotype is not a risk factor for AD.

These results need yet to be confirmed in a progressive study with another known genetic risk factor, such as Cathepsin D, on a larger number of subject. More elaborate statistical analysis may shown further correlations between APO E, α2M and Cathepsin D polymorphisms. Assessing all of them as a possible genetic risk factors my be useful in clinical diagnosis of AD.

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- Department of Neurosurgery (E. Łuczywek)
- Department of Cellular Signalling (M. Łałowski)

NERVE FIBER LESIONS IN THE CENTRAL NERVOUS SYSTEM IN THE COURSE OF AIDS

Project leader: Irmina B. Zelman
Contributor: Mirosław J. Mossakowski

Pathological analysis of 20 cases of the progressive multifocal leukoencephalopathy (PML) appearing in the course of acquired immune deficiency syndrome (AIDS) was done. PML occurred in 10% of all AIDS cases, collected in the period from 1987 to 1999. PML appeared either as the only brain pathology or accompanied HIV-related brain alterations isolated or concomitant with one or several opportunistic infections and/or neoplastic growth (malignant lymphoma). Basing on the pathomorphological picture and clinical symptomatology early, atypical and severe forms of the disease were distinguished. All of them were characterized by typical PML demyelination with oligodendroglial and astrocytic pathology. The group with early

changes revealed widespread, multifocal myelin alterations of a moderate intensity with predominant oligodendroglial abnormalities and less advanced astrocytic changes. Atypical form of the disease was represented by cases with unifocal changes, although containing all key elements of PML pathology. The leading pathological feature of the severe form of the disease consisted in a particular intensity of the demyelination, resulting in tissue destruction often with its cavitation, with typical glial reaction and intense macrophage and lymphocytic infiltration. The other distinguishing feature consisted in strong topographic prevalence of the pathological process either to brain hemispheres or cerebellum.

Differences of PML pathology in the course of AIDS as compared with non-AIDS cases were the subject of special analysis. Due to the relatively high frequency of cases of isolated or strongly predominant involvement of cerebellum, separation of the cerebellar form of the disease has been suggested.

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DEPARTMENT OF DEVELOPMENTAL NEUROPATHOLOGY

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ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDIES OF THE BRAIN DEVELOPMENTAL DISORDERS MAST CELLS IN CNS PATHOLOGY

Project leader: Danuta Maślińska
Contributors: Agnieszka Kaliszek, Radosław Woźniak

The presence of mast cell (MC) heterogeneity is a critical concept in studying the role of these cells in health and disease. The first precise identification of two mast cell phenotypes in humans has been based on differences in the content of the neutral proteases tryptase and chymase, and tryptase (MC_T) and tryptase-chymase (MC_{TC}) phenotypes. The phenotypic characteristics revealed significant differences, between these two mast cell phenotypes, in mediators content, responses to proliferation/survival factors and patterns of sensitivity to agents that induce or pharmacologically modulate degranulation and mediator release. Thus, each mast cell phenotype has different biological properties and may influence the surrounding tissue in a different way. Both types of mast cells are considered to play a pivotal role in a variety of biological responses including local homeostasis, angiogenesis, fibrosis, wound healing, inflammation and tissue remodeling. The association of mast cells with a variety of tumors has long been recognized, but little is known on heterogeneity of these cells. Therefore, the aim of our study was to determine mast cell phenotypes in different brain tumors, by means of specific monoclonal tryptase and chymase antibodies. The preliminary results showed that in benign brain tumors used in the study, mast cells were tryptase-chymase phenotype (MC_{TC}). They were numerous in the tumors and did not infiltrate the sourrounding brain parenchyma. In malignant tumors mast cells were mainly tryptase phenotype (MC_T). They were numerous only in some areas of neoplasm, where they were localized mainly in perivascular

area of some blood vessels. The further study is on the way. Recently, a new important role of mast cells for the pineal gland function has been postulated. According to those findings mast cells as a source of histamine may modulate synthesis of melatonin in the pineal gland. During the last decade much attention has been focused on a functional role for melatonin as the principal hormone of the pineal gland. It has been found that melatonin provides not only photoperiodic information but influences various, immunological and endocrinological functions in human body. Alteration of such a central pineal control may lead to the inability of the body to adapt to the environmental variables, including the capacity for a proper thermoregulation or resistence to bacterial or viral infections. The importance of melatonin and pineal gland function for development of human body was supported by the findings that maternal melatonin crosses the placenta and enters the fetal circulation and that from the 26th week of gestation fetal pineal glands are capable for melatonin synthesis. Data concerning dysfunction of the pineal gland in human fetuses and young children are limited, because in routine brain autopsy pineal gland is not examined. Our study was performed on 117 pineal glands of fetuses and children who died following severe diseases and complicated pregnancies. In the pineal glands of most fetuses delivered over the 25th week of gestation and in all examined children morphological lesions were found. They include hemorrhages, focal necrosis, numerous and different size post-necrotic cavities and cystic changes. Mast cells in these pineal glands were mainly tryptase phenotype. In some fetuses numerous of these cells underwent mineralization and calcium deposits were detected by von Kossa's method. Most numerous mast cells were found in pineal glands of children, who died following leukemia. Clusters of these cells were scattered throughout the pineal parenchyma and in perivascular area of the blood vessels. Pineal lesions in most (95%) cases were concomitant with neuropathological changes in the brain of fetuses and children. The results of our study lead to the conclusion that the developing pineal glands are very often injured by different harmful agents, and that such injuries may altere the control functions of the gland. The role of mast cells in those pineal glands is not clear and requires further study. For last few years much attention has been payed to the expression of cyclooxygenase-2 (COX-2) in the normal and injured brain. In most tissues this enzyme participates in metabolism of arachidonic acid delivered from cell and nuclear membranes

following cell injury. In normal brain small population of neurons contain constitutive COX-2 enzyme. The results of our study provided evidence that COX-2 is constitutively upregulated also in epithelial cells of developing human choroid plexus. In the studies performed on ischemic human brain we found strong COX-2 immunoexpression in numerous non-neuronal cells (mainly macrophages and hypertrophic astrocytes) participating in the late period of post-ischemic inflammatory reaction of the tissue.

DEVELOPMENT OF THE NERVOUS SYSTEM AND ITS DISTURBANCES

Project leader: Maria Dąmbska
Contributors: Izabela Kuchna (postdoctoral fellowship in IBR, USA), Milena Laure-Kamionowska

1. Pathological changes in brains of children died of neoplastic diseases were examined. Cerebellopathy was found as a most frequent paraneoplastic syndrome. Generalized lesions, most important in the cerebral cortex were also observed. They seemed to be the sequela of the prolonged treatment with cytostatic drugs. Our observations were based on examination of 27 brains of children aged from 0 to 11 years of age with neoplastic disease treated and non treated with chemotherapy also with and without neoplastic changes in the CNS.

2. Moderate and small focal anomalies within cerebral cortex were analysed in 12 cases. They correlated topographically with glial-pial barrier lesions. In majority of examined cases the changes within meninges which may be responsible for abnormal vascularisation of cortical ribbon were found. The type of cortical anomalies locked as depending on time of damage leading to their formation.

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LABORATORY OF THE CELL ULTRASTRUCTURE

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STUDIES ON CELL ULTRASTRUCTURE AND CELLULAR SIGNALLING PROCESSES IN APOPTOSIS. CONTINUATION OF ULTRASTRUCTURAL STUDIES ON BRAIN VESSELS AND PERIVASCULAR AREA IN THE CENTRAL NERVOUS SYSTEM IN DIFFERENT PATHOLOGICAL MODELS

Project leader: Barbara Gajkowska
Contributors: Hanna Olszewska-Bądarczuk, Marcin Cholewiński

Studies on proteins from bcl-2 family were conducted on various neoplastic cell lines by means of post-embedding immunocytochemistry for electron microscopy. Bcl-2 proteins play a crucial role in TGF-β1-induced apoptosis. Treatment of cells with TGF-β1 activates transcription and subcellular redistribution of Bax. We showed that Bax is rapidly (15 min) translocated from cytosol to the mitochondria and Golgi apparatus in TGF-β1-treated murine breast cancer cell line HC11. After 60 min, Bax immunoreactivity is detectable in cytosol, mitochondrial membranes, nuclear membrane pores and heterochromatin. Nuclear homing of Bax was confirmed by a new method, embedment-free electron microscopy that revealed Bax immunoreactivity on the core filaments of nuclear matrix. This suggests a nuclear target for Bax but physiological significance of putative Bax signalling in the nucleus remains unknown.

Contributor: Michał Walski

NADPH histochemistry in combination with electron microscopy was employed to detect the activity of NO synthase (NOS) in rat cerebral cortex after trauma. NOS reactivity was found in the elements of the damaged blood-brain barrier, especially in mastocytes and phagocytes. These studies support the role of NOS in the development of traumatic brain damage.

Contributor: Małgorzata Frontczak-Baniewicz

We completed the studies on the reactions of capillary vessels in rat brain in the regions with patent blood-brain barrier and in barrier-free areas. Ischemic cerebral damage was induced by the photochemical platelet aggregation and mechanic brain compression. In both experimental models, ultrastructural analysis revealed similar changes in the endothelium, pericytes and astrocytes with concomitant alterations in the basement membrane and extracellular matrix. Angiogenesis was observed as the reaction to the experimental brain damage.

THE ROLE OF IONIZED CALCIUM AND CALCIUM CHANNELS IN APOPTOSIS IN NORMAL AND CANCER CELLS. EXPERIMENTS USING FLUORESCENT CALCIUM DETERMINATION IN LIVING CELLS AND ELECTRON MICROSCOPY

Supported by the State Committee for Scientific Research: grant # 4 P05A 045 15

Project leader: Barbara Gajkowska
Contributors: Hanna Olszewska-Bądarczuk, Marcin Cholewiński

Biochemical studies of the changes in chromatin and morphological ultrastructural studies of the structure of nuclear matrix during cellular differentiation and death were conducted in 1999. Gene expression and DNA replication depend on the structure of chromatin which determines the accessibility of DNA to transcription factors and other regulatory proteins. In order to follow the changes in chromatin and nuclear matrix structure during differentiation and cell death, we developed an *in vitro* model in which an immortalized keratinocyte cell line HaCaT was differentiated by extracellular calcium or by calcium ionophore the latter inducing programmed cell death. Measurement of binding of selected fluorochromes (propidium iodide, YO-PRO-1, 7-aminoactinomycin) to DNA in intact nuclei revealed an over 10-fold decrease in chromatin accessibility in differentiating cells. Extraction of histones reversed those changes indicating that the observed decrease in chromatophore binding is due to modification in the DNA structure by histones. Moreover, a novel, embedment-free electron microscopy technique has shown that three-dimensional structure of nuclear matrix undergoes marked changes

in differentiating cells. These phenomena may play a crucial role in the regulation of gene transcription in differentiating cells.

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CONTINUATION OF STUDIES CONCERNING EVALUATION OF THE DISTURBANCES OF INTRACRANIAL HOMEOSTASIS

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Clinical research is a continuation of studies concerning disturbances of cerebrospinal fluid (CSF) circulation. Further 68 cases of the dilation of the ventricular system were examined. High correlation was found between the values of CSF outflow resistance in three-phase infusion test and hydrocephalus index calculated on the basis of digital analysis of the CT images. This finding will contribute to the effective and less invasive diagnosis of hydrocephalus in the future.

Studies concerning the diagnosis of tightness of CSF spaces based on the results of lumbar infusion test with physiological loadings were continued. Clinical usefulness of the developed method has been confirmed.

Studies of the cerebral blood flow (CBF) evaluated by Transcranial Doppler Sonography (TCD) in a group of 127 patients in cooperation with the Neurosurgical Department in Wrocław, have shown that localization of the ruptured aneurysm and distribution of the extravasated blood correlates with an increase in blood flow velocity in the vessels from which the aneurysm originates. Multifactorial analysis of CBF enabled the determination of patients at risk of developing delayed ischemic deficits following vasospasm

after subarachnoid hemorrhage (SAH): age over 50 years, group III according to Hunt-Hess scale, grade III according to Fisher scale and high CBF values detected on the 3rd day after SAH. Other CBF studies have shown that the patients operated on the 2nd day after SAH are at the highest risk of developing vasospasm.

In the cooperation with the Neurosurgical Centre in Freiburg, an analysis of 6850 biopsies in the patients suspected of brain tumor was performed and showed that an ischemic lesion imitating brain tumor on the imaging studies (CT or MRI) was found in 43 cases.

In the neuropsychological research, the dynamics of blood flow in the middle cerebral arteries (MCA) in the left-handed patients was investigated. Twenty three individuals were examined using TCD. It has been found that the values of flow in MCA are bilaterally similar in this group as opposed to the right-handed patients in whom a significantly higher flow values in the left MCA were observed. In cervical discopathies, the evoked potentials with the potential mapping were investigated. Twenty four patients were examined. The disturbances of stimuli transmission appears to be prognostic in the evaluation of the severity of spinal cord injury. This observation is helpful in the qualification for surgical treatment and has a prognostic value.

EEG examinations, evoked potentials with EEG and potential mapping were performed in 22 patients with epilepsy and in 15 patients with Parkinson syndrome. These examinations in the epileptic patients are helpful in the optimalization of pharmacological treatment and the qualification of patients for surgery. Based on EEG examination and cortical potential mapping, the results of pharmacological treatment and the results of stereotactic procedures were evaluated. In experimental research, an influence of orally administered dotarizine on the cerebrovascular reactivity in rabbits was investigated. During hyperventilation and following anoxia, stabilizing effect of dotarizine upon the basilar artery was more pronounced than that in the MCA.

The method of application of standardized physiological loadings (body position changes, Valsalva manoeuvre, anoxia) in evaluation of the reserve of an intracranial volume in cats was developed. The results will be useful in a clinical practice.

IN VITRO MR SPECTROSCOPY OF HIGH RESOLUTION IN AN INTRACEREBRAL TUMOR TISSUE AND CEREBROSPINAL FLUID – APPLICATION IN CLINICAL DIAGNOSTICS

Supported by the State Committee for Scientific Research: grant # 4 P05B 054 14

Project leader: Zbigniew Czernicki
Contributors: Dariusz Horsztyński, Jerzy Walecki

Spectroscopic evaluation of 54 tumor samples obtained intraoperatively was performed. Spectra of 12 malignant gliomas (grade III and IV according to WHO classification) and 22 meningiomas (grade I and II) were analyzed. MR signals of inozitol, glicerol-phosphocholine, phosphocholine and choline, creatine and phosphocreatine, glutamine, acetate, alanine and lactate were evaluated using two methods. The first enabled quantitative analysis of the above compounds by measuring the height of spectra. The second method allowed for comparison of spectra by normalization of the spectra heights to the signal for creatine and phosphocreatine. Statistically significant differences between the spectra of meningiomas and gliomas were found.

EXPRESSION OF NUCLEAR T3 AND RETINOID RECEPTORS IN GLIOMAS

Supported by the State Committee for Scientific Research: grant # 4 P05C 005 15

Project leader: Zbigniew Czernicki
Contributor: Paweł Nauman

Obtained results have shown that in all gliomas examined – except for 2 cases of astrocytoma and 1 case of glioblastoma – the expression of TRα gene was significantly lower than in the non-pathological brain tissue. Expression of TRβ gene was generally very low in the non-pathological brain tissue. In the majority of glioma samples (except for 1 case of oligodendroglioma), it was impossible to detect TRβ gene expression using Northern blot method. An increase in the expression of the receptor protein TRβ correlated with the grade of malignancy. Expression of TRα was also altered in comparison with that of the non-pathological brain tissue.

NON-INVASIVE METHOD TO EVALUATE HEMODYNAMIC CHANGES AND AUTOREGULATION IN SEVERE BRAIN TRAUMA AND CEREBROVASCULAR DISEASES

Supported by PHARE-SCI-TECH: grant # PL9611-03-02

Project leader: Jerzy Jurkiewicz
Contributors: Zbigniew Czernicki, Katarzyna Jarus-Dziedzic, Wojciech Sapieja, Elżbieta Łuczywek

A new TCD device for bilateral monitoring of the cerebral circulation (two probes) with the possibility of simultaneous monitoring of intracranial pressure, arterial pressure and central venous pressure was constructed.

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Publications:

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STRUCTURE AND FUNCTION OF MUSCLE AND NERVE IN THE DEVELOPMENT AND AGING AND IN GENETIC AND ACQUIRED DISEASES

Project leader: Irena Hausmanowa-Petrusewicz
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New findings in x-linked muscle dystrophy

a) Motoneuron involvement in Duchenne dystrophy. The rate of firing of motor units was examined in 26 Duchenne patients and in a group of age matched controls. Only in one subgroup the firing rate was higher than in controls. In all patients the relationship between the standard deviation of interspike intervals and their mean values was shifted towards shorter intervals and lower standard duration. Quantitative analysis of the data disclosed a correlation between shift's value and severity of the disease. Experimental computer simulation data indicate that the break-point of many interspikes intervals values are correlated with motoneuron properties i.a. with the duration of afterhyperpolarization. In dystrophy, this point corresponds to the shorter interspike values. This study raises a very fundamental question of motoneuron alteration in such a "pure" primary muscular process, as has been regarded dystrophy.

b) Investigations on dystrophinopathy in 24 females showed a high activity of CK, varying degree of myopathic EMC changes and mosaic pattern of dystrophin in muscle biopsy. Quantitative evaluation of dystrophin in carrier's by Western blot showed its reduced abundance, with normal or abnormal molecular weight. These changes are more expressed in isolated carriers.

c) Autosomal dominant and autosomal recessive forms of Emery-Dreifuss dystrophy were analyzed with respect to clinico-genetical correlations. In the X-linked variant, the defect linked with the absence of emerin is manifested by nuclear membrane abnormalities with the extrusion of chromatin.

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Clinical-genetic correlations in neuropathies

Project leader: Hanna Jędrzejowska
Contributors: Irena Hausmanowa-Petrusewicz, Hanna Drac

A duplication in chromosome 17p11.2 (HMSN type IA was disclosed in 11 out of 19 families with dominant demyelinating form of HMSN. The course of the disease was mild, there was a significant slowing of conduction velocity and prolongation of F wave latency. Onion bulb formations with concentric Schwann cell proliferation in sural nerve were seen. Three members of one family exhibited signs designated in the past as Roussy-Levy syndrome. In a patient with hereditary neuropathy with a liability to pressure palsies and 17p11.2 deletion clinical and electrophysiological features as well as sural nerve histology were typical for this disease. However, in EM some nerve fibers were seen to contain uncompacted myelin which is rarely noted in abnormal peripheral nerves. In two families in whom clinical, electrophysiological and histological data suggested axonal forms of HMSN, linkage to AR demyelinating CMT chromosomal loci (8q 13-21.1 and 11q23) was documented.

Collaborating units:
Department of Molecular Genetics, Medical Academy, Poznań, Poland (A. Kochański)
Department of Neurology, Medical Academy, Warsaw, Poland (B. Ryniewicz)

Improvement of sensitivity and reliability of motor unit potentials analysis

Project leader: Irena Hausmanowa-Petrusewicz
Contributor: Katarzyna Rowińska-Marcińska

The aim of this study was to compare the effectiveness of parameters such as amplitude, duration, area, size index, number of peaks and irregularity coefficient applied to the classification of motor unit action potentials in neurogenic and myogenic muscle disorders. The percentages of potentials unclassified by amplitude, duration, area/amplitude, area and size index were 31%, 34%, 28%, 15% and 15%, respectively. Neither the number of phases and turns nor the irregularity coefficient are of importance in the differentiation between neuro- and myogenic groups. There was also a set of potentials that were not properly classified by any parameter. Among the unclassified potentials there were more potentials from myogenic cases. These unclassified myogenic potentials were predominantly irregular, whereas most of unclassified neurogenic potentials were simple. In neurogenic cases, parameters describing the size of the potential (amplitude, duration, area) were not significantly correlated with those describing the shape (number of phases and turns), whereas in myogenic cases some correlations between these parameters were significant.

Motor unit quantities have different sensitivity. Indexes were more sensitive than simple parameters and were also much more effective in the evaluation of atypical potentials.

Motor unit action potential (MUAP) may be characterized by two complementary features: global and detailed. The global feature represents the magnitude of the signal and is related to the number of fibers contributing to the MUAP. The detailed feature indicates the single fiber potential's synchronization. The global feature is characterized by amplitude and duration and may be quantified either by area/amplitude index or size index, which is interpreted as of the potential "thickness". The detailed feature is characterized by the number of phases and turns. The irregularity coefficient, which is related to the "length" of the curve, combines the number of phases and turns with their amplitudes. The aim of this study was to analyze the relationship between the global and detailed features of MUAPs in various neuromuscu-

lar disorders. In neurogenic cases, with the exception of the correlation between the duration and number of turns (r=0.58), no correlation between any other parameter related to global and detailed features were significant (r<0.4). In myogenic cases, a significant correlation between the irregularity coefficient and duration (r=0.71), irregularity coefficient and area/amplitude (r=0.54) rather than between the irregularity coefficient and area (r=0.32), has occurred. The correlations between parameters were more significant in more chronic processes.

The study on repetitive discharges (RD's) indicates that in lower motor lesion, of the voluntary activated motor unit reflect the hyperexcitability of the MU and are a sign of membrane changes leading to MU degeneration. The RD's can originate from different sites throughout the MU and may be one of the first signs of the MU dysfunction.

Collaborating unit:
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New data on myelin maturation and some defects in muscle development

Project leader: Anna Fidziańska
Contributors: Janina Rafałowska, Anna Kamińska

Our comparative study on human fetal muscles and on biopsies taken from patients with neuromuscular disorders indicate that a failure in muscle maturation plays an important role in the pathogenesis of some congenital myopathies. A block of fusion at the stage at which the primary myotube changes into the mature one is the cause of the myotubular myopathy.

Cap disease seems to be another example of the disturbance of fusion during myogenesis. An abnormal sarcomere pattern seen in the core area in central core disease seems to be a consequence of an error in the synthesis of muscle proteins.

Immaturity of blood vessels in myelinated rat spinal cord was investigated. Two types of blood vessels were observed. In one type of small vessels endothelial cells were hypertrophied, rich in organelles and the lumen was very narrow. The luminal surface showed invaginations and numerous

microvilli. This feature resembles immature blood vessels observed during fetal life. The second type of blood vessels with a very narrow endothelial cells and wide lumen are identical with mature blood vessels. These findings indicate that intensive spinal cord vascularization closely correlates with the myelination process.

Two adult familial cases with hereditary inclusion body myopathy associated with desmin storage were investigated. Morphological findings, identical in both cases, were characterized by tubulofilamentous nuclear and cytoplasmic inclusions 16-21 nm in diameter. In addition, large deposits of desmin were observed in numerous muscle fibers. These channels may either represent two coexisting disease processes or merely reflect an abnormal form of muscle fiber degeneration.

Collaborating unit:
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CLINICAL AND GENETIC STUDIES ON INFANTILE AND JUVENILE SPINAL MUSCULAR ATROPHY. PHENOTYPE–GENOTYPE CORRELATIONS

Supported by the State Committee for Scientific Research: grant # 4 P05E 001 12

Project leader: Irena Hausmanowa-Petrusewicz
Contributors: Anna Fidziańska, Maria Jędrzejowska, Małgorzata Dorobek

Introduction in 2 hospital centers of the technique of identification of deletion of SMN and NAIP genes, permitted an analysis of phenotype/genotype correlation in the Polish population, derived from the analysis of 40 families (120 members). Preliminary results indicate a correlation between the size of deletion and severity of the disease. Deletion of NAIP was associated with deletion of SMN only in severe infantile cases. In no family deletion was found in non-affected members.

Clinical intrafamilial variability raises the need of determining nonhereditary factors.

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CHEMOKINES MIP-1α AND MCP-1 IN MULTIPLE SCLEROSIS (MS) PATIENTS TREATED WITH INTERFERON BETA 1α

Project leader: Jacek Losy
Contributors: Grażyna Michałowska-Wender, Mieczysław Wender

Chemokines MCP-1 and MIP-1α may play an important role in the pathogenesis of multiple sclerosis, influencing migration of leukocytes to the CNS. One of possible mechanisms of the action of interferon beta is an effect on chemokines. We have measured MCP-1 and MIP-1α chemokines in sera of 24 patients with MS treated with interferon beta 1a after 3 months of therapy and in 15 control patients. There was a significant increase of MIP-1α concentration in sera of MS patients. After the interferon beta 1a treatment MIP-1α and MCP-1 levels in sera did not differ from values before therapy. Investigations will be continued after longer time of treatment with interferon beta.

CLADRIBINE TREATMENT INFLUENCES β-2 MICROGLOBULIN AND SOLUBLE INTERCELLULAR ADHESION MOLECULE 1 (ICAM-1) CONCENTRATIONS IN CEREBROSPINAL FLUID AND SERA OF PATIENTS WITH MULTIPLE SCLEROSIS

Project co-ordinator: Jacek Losy

β-2 Microglobulin (β2M) – is low molecular weight protein located extracellularly and associated with class 1 antigens of the major histocompatibility complex and is considered as a marker for disease activity in immune disorders. Cladribine (2-chloro-2-deoxyadenosine, 2-CDA) is a potent lymphocytotoxic agent under investigation in the treatment in MS patients.

Suspecting that β2M levels might indicate inflammatory events in CNS we determined CSF-β2M and serum β2M level in patients with relapsing-remitting MS before and after cladribine treatment as well as in a control group. There was a significant decrease in sera but not in CSF of β2M concentration in MS patients after the cladribine treatment, associated with a slight but significant clinical improvement measured by Kurtzke's Expanded Disability Status Scale. Similar observations were made for sICAM-1 concentrations. We conclude that β2M serum as well as sICAM-1 CSF concentration may be used as a marker in therapy monitoring in patients with MS during cladribine treatment.

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Publications:

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HEMODYNAMIC, METABOLIC AND NEUROHORMONAL RESPONSES TO VARIOUS PHYSIOLOGICAL STIMULI IN HUMAN SUBJECTS OF DIFFERENT AGE AND PHYSICAL ACTIVITY

Project leader: Krystyna Nazar

Influence of a 3 day-bed rest on the activity of the sympatho-adrenal system and its reactivity to various physiological stimuli in young men

Contributors: Hanna Kaciuba-Uściłko, Krystyna Nazar, Barbara Bicz

The aims of the present study were: (1) to examine the effects of three days of bed rest on plasma adrenaline (A) and noradrenaline (NA) basal concentrations and the plasma catecholamine responses to physiological stimuli, such as glucose ingestion, graded bicycle exercise, changing of body position (lying to standing), cold pressure test (hand cooling), and (2) to find out whether the level and kind of physical activity preceding bed rest modifies its effects. Twenty three young sedentary subjects, 18 endurance trained and 20 strength trained athletes participated in the investigations. The study showed that 3 days of bed rest (1) decreases plasma NA level under basal conditions (in supine subjects after overnight fast), (2) diminishes the plasma catecholamine responses to an oral glucose load and to 8 min standing, (3) causes an earlier activation of the symphathetic nervous system during graded exercise test (only in the endurance trained subjects), (4) does not influence catecholamine response to the hand cooling (2 min). It is concluded that remaining recumbent for only three days inhibits the basal activity of the sympathetic nervous system and reduces its responses to stimuli mediated by arterial baroreceptors (posture change and postglucose vasodilation in splanchnic region and skeletal muscles). The effects were most pronounced in the endurance trained subjects.

Collaborating units:
NASA Ames Research Center, Moffett Field, California, USA
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Hemodynamic responses to posture changes in trained (swimmers) and untrained men

Contributors: Alicja Kodrzycka, Wiktor Niewiadomski, Gerard Cybulski

The changes in heart rate (HR) and stroke volume (SV by impedance cardiography) were estimated in 11 young, male swimmers, and 18 healthy, male sedentary students in the supine and standing positions. The supine mean R-R interval was significantly longer while SV was greater in swimmers than in controls. In the standing, mean R-R intervals were similar in both groups. After standing up SV decreased by 22% in swimmers, and by 20% in controls. Thus, the greater standing-induced decrease of R-R intervals in swimmers than in untrained subjects can not be attributed to a greater SV decrease.

Variability of hemodynamic parameters in patients with atrial fibrillation

Contributors: Gerard Cybulski, Wiktor Niewiadomski, Alicja Kodrzycka

Our prototype impedance cardiography ambulatory device, which enables simultaneous monitoring of ECG and central hemodynamic signals, was applied to evaluate the beat-to-beat variability of hemodynamic parameters in 5 male patients with atrial fibrillation and 5 healthy males (control) during day and night. The coefficients of variation (CV) of stroke volume (SV), cardiac output and the amplitude of impedance signal (AMP) were higher in patients than in controls, whereas there were no differences in the ejection time (ET), preejection period (PEP) and PEP/ET ratio. It is suggested that the increased SV variability in patients is possibly caused by the end diastolic volume variability but not by variations of cardiac contractility.

Collaborating units:
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Outpatient Cardiac Unit for Diagnosis and therapy, MRC, PAsci, Warsaw, Poland (E. Ziółkowska)

Menstrual cycle abnormalities, hormonal profile, physiological and psychological characteristics of girls from the ballet high school

Contributors: Renata Zabielska, Andrzej W. Ziemba

Complex investigation was started with the aim to evaluate health risk in girls of very high physical activity (ballet) in the circumpubertal period. In comparison with the regular high school girls (controls), pupils of the ballet school had delayed menarche, more frequent secondary amenorrhea or oligomenorrhea, lower plasma estradiol and growth hormone concentrations, lower body mass and fat percentage, higher aerobic capacity with similar resting metabolic rate adjusted for body mass. Analysis of physiological questionnaires revealed lower self esteem, lower indices of happiness and vigor with greater inclination to depression in the ballet girls than in controls.

Exercise training in obesity

Contributor: Barbara Kruk

The effects of moderate aerobic training (walking) and the respiratory muscle training (Yoga system or breathing by resistance tube) on metabolic responses to standard exercise and on glucose tolerance were studied in overweight men. The preliminary results suggest that the training regime increases contribution of free fatty acids to resting and exercise metabolism without changing glucose tolerance.

Collaborating unit:
Department of Physiology, University in Kuopio, Finland

Physiological effects of the medical rehabilitation program in lower-limb disabled persons

Contributor: Krzysztof Krzemiński

Thirty patients with lower-limb dysfunction, aged 20-45 years participated in the study. They were classified according to functional and/or muscular deficit and level of habitual physical activity. An arm cranking ergometer test was used to examine cardiovascular responses to exercise and peak oxygen intake. Physical Working Capacity test was used to determine work loads corresponding to heart rate 130, 150, and 170 beats per minute. Similar examinations will be repeated after one year of medical rehabilitation.

Collaborating unit:
Central Institute of Labour Protection, Warsaw, Poland

An influence of oral creatine supplementation on exercise tolerance of elite athletes

Contributor: Jolanta Chwalbińska-Moneta

In continuation of the previous studies the effect of 5 day creatine treatment (20 g of creatine monohydrate daily) on circulatory and metabolic responses to prolonged submaximal exercise followed by supramaximal exercise was evaluated in 14 elite rowers during their intensive training. No significant influence of creatine supplementation on the athletes' ability to perform long-lasting submaximal exercise, or on tolerance of subsequent supramaximal exercise was found.

Pre-Olympic study on adaptation to the rapid change of 8 time zones in athletes

Contributors: Jolanta Chwalbińska-Moneta, Hubert Krysztofiak

Twenty one athletes participated in this study during 16-18 days after the flight from Warsaw to Sydney. Total and lean body mass, body water, the

maximal muscle force and the cardiovasculatory responses to static exercise (hand grip) and orthostatic test as well as the anaerobic threshold, tolerance of submaximal exercise loads and anaerobic capacity were determined. The results indicate that the time course of adaptation to the time zone change in athletes has a phasic pattern. An improvement of the most of evaluated indices was noted 5 to 7 days after arrival to Sydney, but in many sportsmen some disadvantageous alteration occurred again on the 10th-13th day.

Collaborating unit:
National Center of Sport Medicine, Warsaw, Poland

EFFECT OF DIETARY CREATINE SUPPLEMENTATION ON HIGH-ENERGY PHOSPHATES AND MITOCHONDRIAL ENZYMES IN THE MYOCARDIUM AND SKELETAL MUSCLE OF TRAINED RATS

Project leader: Hanna Kaciuba-Uściłko
Contributors: Krystyna Nazar, Zofia Brzezińska, Ilona Falęcka-Wieczorek

The investigation was performed on 32 adult, male Wistar rats; 20 of them were submitted to a moderate treadmill training (group T) and 12 served as sedentary controls (group S). The animals assigned to exercise-training group run for 1 hour per day, 5 days per week, for 3 weeks. Ten exercising and 6 sedentary rats received creatine (Cr) supplementation (500 mg daily for 7 days and 200 mg daily for the next 2 weeks), whilst 10 exercised and 6 sedentary rats were fed the rat chow without Cr. Powdered chow diet was made to 2.5% Cr, mixed into a paste with water, formed into pellets, and dried. At the end of experiments, all rats were anesthetized with pentobarbital sodium. The soleus muscle and the apex part of heart ventricles were excised, deep frozed within 15 s in liquid nitrogen, and then stored at -80^{0}C until assayed. In the muscle specimens total creatine (TCr), phosphocreatine (PCr), Cr, adenylate nucleotide (ATP, ADP and AMP) contents as well as citrate synthase (CS) and β-hydroxy-acyl-CoA-dehydrogenase (HAD) activities were determined.

Cr supplementation for 21 days resulted in an increase TCr (by approx. 25%) with a significant increase in PCr in the myocardium and soleus of the rats from both groups. ATP content was enhanced in the myocardium of the

rats from group S and in the soleus of rats from group T. Activity of HAD was significantly increased in both muscles in Cr supplemented rats from group S whilst in trained rats activity of this enzyme was enhanced only in the soleus. Activity of CS was diminished by Cr supplementation in the myocardium of rats from both groups and it was unchanged in the soleus.

It is concluded that Cr supplementation increases cardiac and skeletal muscle TCr and high energy phosphate reserves independently of animals' physical activity. The influence of dietary Cr treatment on mitochondrial enzymes is, however, ambiguous and needs further exploration.

FACTORS INFLUENCING LIPID METABOLISM IN SKELETAL MUSCLES

Project leader: Leszek Budohoski

Effect of fatty acid availability on glucose utilization in the rat soleus muscle *in vitro*

Contributors: Monika Górecka, Anna Dubaniewicz, Ewa Żernicka

The aim of this investigation was to compare the *in vitro* effect of various concentrations (0-2.0 mM) of palmitic acid in the incubation medium on the rates of glucose transport and lactate production in the soleus muscle from euthyroid and hypothyroid rats. Sixty male Wistar rats were divided into two groups: 1. control, euthyroid (C); 2. hypothyroid (THY+PTU, surgically thyroidectomized, and then treated for 30 days with 0.04% propylthiouracil in drinking water). Glucose transport and lactate (LA) production were estimated in the presence of insulin (100 μU/ml) in the incubation medium. These was no effect of palmitic acid concentration on LA production in the isolated soleus muscle of C rats, and a slight decrease in the rate of 2-deoxy-glucose transport to this muscle occurred only when palmitic acid concentration in the incubation medium was very high (2 mM). In THY+PTU rats a significant decrease in the rate of LA formation in the soleus muscle at 1.5 and 2.0 mM of palmitic acid concentrations was found and the rate of 2-deoxy-glucose transport to the muscle was diminished at 1.0, 1.5 and 2.0 mM of palmitic acid concentrations. In this group of animals significant negative correlations ($p<0.01$) were ascertained between palmitic acid con-

centration and the rates of 2-deoxy-glucose transport or LA formation. The present data confirmed the inhibiting effect of fatty acids on glucose utilization by rat skeletal muscles (Randle cycle) only in hypothyroid rats.

Effect of 24-hour cold exposure on lipid metabolism in the rat

Contributors: Zofia Brzezińska, Ewa Żernicka, Hanna Kaciuba-Uściłko, Krystyna Nazar

The aim of this study was to find out whether 24-hour cold exposure influences indices of lipid utilization by skeletal and cardiac muscles in female rats. For this purpose, plasma free fatty acid (FFA) and triacylglycerol (TG) concentrations, muscle TG content, two forms of muscle lipoprotein lipase (LPL) activities (intracellular and extracellular), mRNA of this enzyme, total, free, and short and long chain acyl carnitine contents, muscle cytochrome c oxidase (COX) activity were measured in control Wistar female rats kept at 28^0C (n=8), and rats exposed to 6^0C for 24 hours (n=8). In comparison with controls, in the cold exposed rats, plasma FFA concentration was increased while that of TG was decreased, soleus muscle TG content showed a tendency to decrease and increased significantly in myocardium. A marked enhancement of both forms of LPL (especially of extracellular, active form) was noted in myocardium and soleus. It was accompanied by an increase in LPL mRNA content only in the soleus. There were no significant changes in total and acyl carnitine in the soleus whilst in cardiac muscle total carnitine content decreased and long-chain acyl carnitine significantly increased during cold exposure. Activity of COX was enhanced only in the myocardium. These findings suggest an enhanced potential of skeletal and cardiac muscles for blood lipid utilization in rats exposed to cold.

Collaborating unit:
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Stimulation of hormone sensitive lipase (HSL) activity by contractions in rat skeletal muscle

Contributor: Józef Langfort

Expression of immunoreactive HSL in the rat skeletal muscles was previously evidenced using the Western-blotting technique of isolated muscle fibres. The present study showed that HSL activity in the rat soleus increases when this muscle is stimulated electrically for 1-5 min. Longer stimulation (10 and 60 min) did cause further changes in the enzyme activity. The contraction-induced enhancement in HSL activity was not abolished by propranolol treatment. Electrical stimulation of epididymal fat did not affect HSL in adipose tissue which proved that stimulation of muscle causes activation of HSL in myocytes but not in interfiber fat.

Collaborating unit:
Copenhagen Muscle Research Centre, Panum Institute, University of Copenhagen, Denmark

EFFECT OF OCCUPATIONAL WORK ON CARDIOVASCULAR, HORMONAL AND METABOLIC INDICES IN PATIENTS WITH CHRONIC CIRCULATORY AND METABOLIC DISEASES

Supported by the Strategic Governmental Program
"Labor safety and health protection in work environment", grant no 04.10.6

Project leader: Krystyna Nazar
Contributors: Hanna Kaciuba-Uściłko, Krzysztof Krzemiński, Barbara Kruk, Gerard Cybulski, Andrzej W. Ziemba, Jolanta Chwalbińska-Moneta, Wiktor Niewiadomski, Hubert Krysztofiak

The aims of this project are (1) to assess the effects of occupational stress on cardiovascular, hormonal and metabolic indices in patients with coronary heart disease, hypertension, diabetes and obesity, (2) to adapt a model of laboratory tests for prediction of the patients' responses to the real life stress occurring in occupational work, (3) to elaborate a system of evaluation of health risk connected with occupational work in patients with chronic circulatory and metabolic diseases. In 1999, 90 patients with hypertension and/or

CHD, aged from 35 to 65 years and 15 healthy men were examined. They are full time employed as business managers, school or university teachers, lawyers, medical doctors, research workers etc. The type of behavior of the patients (type A and B) and conditions of their work were assessed by using respective questionnaires. During 4 separate week days ambulatory blood pressure and electrocardiogram were recorded for 24 hours using Holter methods. The patients were asked to choose two more and two less stressful days, and to make notes of their activities and events during each of these days. The patients' mood status during work time was evaluated on the basis of *ad hoc* mood questionnaire. The pharmacological treatment was not discontinued. Among the subjects examined 38% represented type A of behavior and only 5% type B. The subjects assessed their job as intellectually demanding, with a high level of responsibility. Most of them had a control over the work situation and approx. half positively estimated the social support, psychological and physical well-being. During occupational work moderate to high anxiety scores were found in 30% of subjects. Other mood characteristics were within the normal range. The systolic blood pressure was significantly higher in patients and healthy subjects during their occupational work than during a leisure time. Variability of blood pressure measurements was greater during a leisure time than during work. In patients a strong tendency towards a greater frequency of supraventricular but not ventricular ectopy was noted during the leisure time and night sleep. For assessment of the patients' stress reactivity the standardized laboratory testing program was elaborated. It includes cardiovascular and hormonal responses to mental stress (arithmetic Kreapelin test), exposure to noise, static hand-grip, and orthostatic manoeuvre.

Collaborating unit:

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Publications:

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EFFECT OF INHIBITION OF SODIUM TRANSPORT IN THE LOOP OF HENLE ON INTRARENAL DISTRIBUTION OF BLOOD FLOW: STUDIES OF THE UNDERLYING MECHANISM

Project leader: Janusz Sadowski
Contributors: Leszek Dobrowolski, Bożena Bądzyńska, Monika Grzelec-Mojzesowicz

In addition to the known effect of furosemide (F) as an inhibitor od sodium transport in the ascending loop of Henle's loop (a "loop diuretic"), the drug has been reported to alter renal and systemic circulation. These vascular effects may be involved in furosemide's immediate beneficial action observed in some patients with congestive heart failure and possibly also in the not infrequent resistance to diuretic therapy.

This study in the anesthetised rat examined the influence of F (0.25 or 0.5 mg min^{-1} kg^{-1} infused i.v. during 1 h) on renal cortical and medullary blood flow (CBF, MBF) measured by laser-Doppler flowmetry. The responses were compared with simultaneously measured changes of renal excretion and of the tissue admittance (Y), an index of medullary ionic hypertonicity of the interstitium.

Renal vascular responses to furosemide were significant but not dose dependent. During low-rate F infusion CBF decreased 11.5±0.9% and MBF decreased 32.3±3.5% (difference CBF vs. MBF significant at $p<0.001$). During high rate infusion the decreases were by 13.5±1.4% and 29.3±3.8%, respectively (difference significant at $p<0.001$).

Sodium excretion increased 15-fold (by 3.7±0.4 µmol min^{-1}) and 30-fold (by 5.9±1.1 µmol min^{-1}) during low- and high-rate infusion of F, respectively. By contrast, medullary tissue Y decreased similarly with the two doses: maximally by 13.4±1.4% and 10.9±0.9%, respectively.

The exaggerated post-furosemide decrease in blood flow within the medulla coinciding with decreasing tissue Y in this zone, and the observation that neither MBF nor Y changes were related to the dose, suggest a causal relationship between interstitial ionic hypertonicity and vascular resistance. The evidence from this part of the study suggests that post-furosemide decrease in medullary tissue NaCl depressed medullary circulation by inhibiting local generation of vasodilatory prostaglandins.

In further studies we attempted to explore the relation of F effects on the renal transport and on the renal vasculature by introducing alterations of tubular transport processes and of the status of the renin-angiotensin system. The animals were preloaded i.v. with hypertonic saline to increase the delivery of NaCl to the tubular system, or pretreated with captopril, to inhibit generation of endogenous angiotensin II, or pre-treated with losartan, to selectively inhibit angiotensin AT1 receptors.

None of the above experimental manoeuvers significantly modified the cortical vascular response to furosemide. On the other hand, we found that pre-loading animals with hypertonic saline clearly abolished the usual post-furosemide difference between the decrease in blood flow between cortex and medulla, due to a major reduction of medullary vasoconstriction. Captopril pre-treatment modestly reduced the post-furosemide decrease in the medullary blood flow and losartan virtually abolished this response.

The abolishment of the exaggerated post-furosemide decrease of blood flow within the medulla by salt loading and by losartan suggests that the medullary vasosonstriction was related to renal tubular transport changes and the status of the renin-angiotensin system. On the other hand, the observation that furosemide dependent depression of circulation within the cortex was not modified by our experimental manoeuvers suggests that the underlying mechanism was some direct effect of the drug on the diameter of cortical arterioles.

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SILENT ISCHEMIA AND ANGINA PECTORIS – THE MECHANISM OF CORONARY PAIN PERCEPTION

Project leader: Ewa Wójcik-Ziółkowska
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Coronary chest pain is still considered to be a characteristic symptom of coronary heart disease (CHD). The pain intensity, duration and association with effort are the main criterions of diagnosis and severity of CHD. However, in 1974 Stern and Tsivoni reported the existence of silent myocardial ischemia detected in ECG Holter monitoring.

It is well known that approx. 5% of general population and 40-60% of patients with CHD may present with episodes of silent myocardial ischemia. It is still controversial: which is worse – painful or painless CHD.

In our previous investigations (in 1977) we found the same severity of CHD and left ventricular damage among symptomatic and angina patients – both groups after myocardial infarction. Patients without the pain signal are more exposed for crossing threshold of ischemia. So, they should be under the same medical care as those with angina.

In 1999 we tried to answer the question if the pain perception among symptomatic and asymptomatic patients is the same? (regarding the influence of anti-angina drugs). The lack of pain may depend on central or peripheral sensivity disturbances. It has been investigated by Roskamm and Droste – they suggest a lower pain sensivity and perception among asymptomatic patients with CHD. Falcone et al. reported a higher endorphin serum concentration in these patients, it which may decrease the pain perception.

Aims of our study were following:

1. Coronarography analysis – comparison between painful and silent patients with CHD (diagnosed previously by non-invasive methods).

2. The pain threshold investigation – studied by "cold test" or/and Marstock test.
3. Treadmill test – stopped after 1 mm ST depression in angina patients and after 2 mm depression in silent patients. The exercise test enables the measurement of silent ischemia duration time.
4. Beta-endorphin serum concentration level in both groups.
5. The influence of Naloxon (1-2 mg i.v.) on perception of coronary pain in silent and symptomatic patients.

This is an ongoing study that is performed on a group of 30 patients. The group is subdivided into those with and without overt angina. A full report will be submitted after completion of the study.

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HUMORAL AND METABOLIC REACTION TO ACUTE MYOCARDIAL INFARCTION

Project co-ordinator: Krystyna Cedro-Ceremużyńska

Decreased plasma thyroid hormone levels in relation to clinical severity

Alterations in serum levels of thyroid hormones in acute non-thyroidal illnesses including myocardial infarction (MI) have long been recognised. Studies determining changes in serum triiodothyronine (T3), reverse triiodothyronine (rT3), thyroxine (T4) and thyroid stimulatory hormone (TSH) in this disease report a fall of serum T3, the most active thyroid hormone, combined with elevation of rT3 and relatively unchanged T4 and TSH levels. However, there is little information as to the relation of these hormonal changes with the severity of the disease and their prognostic value. An early pilot study performed by our group in late seventies indicates that a fall of T3 is particularly pronounced in patients with severe clinical course of MI. This observation found some support in later reports of other authors. A relatively small number of patients included in these studies inclined us to reinvestigate this intriguing problem and to evaluate thyroid status in acute MI in relation to the severity of clinical course. In a series of 50 consecutive patients admitted to the Coronary Care Unit, Grochowski Hospital, Warsaw within 24h from onset of symptoms of acute MI, determination of plasma T3, T4, TSH by immunoenzymatic methods was performed on days 1, 4 and 7 of hospital stay. Results have shown that the patients with complicated clinical course of MI (n=16) show a profound sustained fall of T3 as compared to the patients with no complications (n=34). Plasma T3 levels on days 1, 4 and 7 were below lower normal limit (1.64 pg/ml), significantly ($p<0.001$) less than respective values in uncomplicated group. There were no significant changes in T4 and TSH and no significant differences in levels of these hormones be-

tween the two groups. After having confirmed an original observation that the patients with extremely low plasma T3 are those more seriously ill, it is tempting to speculate on the mechanism of this phenomenon. The hypothesis we propose states that low blood T3 results predominantly from enhanced T3 uptake into tissues induced by high circulating catecholamines. This reasoning is based on the following: 1) the patients with MI of complicated course are predominantly those with enhanced humoral response, including high blood catecholamines; 2) our early studies have shown that in experimental myocardial infarction accompanied by high circulating catecholamines, T3 binding to specific nuclear receptors in tissues (myocardium, liver) is enhanced; adrenaline infusion imitating quantitatively release in experimental MI increases T3 binding to tissues. Measurement of blood catecholamines and their relation to blood T3 levels in patients with MI of both mild and severe course is required to provide support for our hypothesis. This study is actually in progress.

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IMMUNE CELLS AND CYTOKINES IN INFLAMED HUMAN SKIN, JOINTS AND TISSUE FLUID

Project leader: Waldemar L.Olszewski
Contributors: Hanna Gałkowska, Marzanna Zaleska, Dorota Zolich, Bożenna Interewicz

Lymph draining skin and joints of the lower extremity represents the tissue fluid. Immune events developing in the tissue take place in the intercellular space and change the composition of tissue fluid. Thus, analysis of lymph draining a fragment of tissue gives insight into the immune events in this tissue. There are major differences in the intensity of the immune response depending on the evoking etiological factor.

Lymph obtained from the sites of local immune reaction contains infiltrating cells and their products. The type of cells, their activation level and production of humoral factors can be measured. The intensity of local inflammatory reaction as well as the effect of drugs on this reaction can be quantitatively evaluated. In a group of patients with rheumatoid arthritis we have found an increased level of pro- and antiinflammatory cytokines and chemokines in lymph draining foot joints. The concentration was several fold higher than in normal lymph and serum. This suggests local production of humoral factors. Administration of steroids evidently decreased the cytokine level and cellular influx into the tissue. Simultaneous measuring of serum cytokines did not reveal any evident effect of steroids.

Local inflammatory changes of bacterial origin developing in the skin may be followed by systemic septic reaction. We found that bacteria colonizing tissues with lymph stasis may proliferate in tissue fluid and lymph and

be translocated to blood circulation. In around 12% of patients, bacteremia can be detected. Forty percent of lymph isolates are of the same phenotype as blood isolates. This observation explains the systemic symptoms in patients suffering from acute attacks of local dermatolymphangioadenitis.

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TRANSPLANTATION TOLERANCE INDUCED BY ADMINISTRATION OF DONOR BONE MARROW CELLS

Project leader: Waldemar L.Olszewski
Contributors: Michał Maksymowicz, Bożenna Interewicz, Ewa Cybulska, Marek Durlik, Maria Mościcka-Wesołowska, Joanna Mijal, Edyta Szyper

Microchimerism is considered to be responsible for prolongation of allograft survival time and decreased responsiveness to donor transplantation antigens in the *in vitro* tests. Donor lymphocytes are detected in lymphoid organs and blood in the recipients of surviving organ allografts. We studied the presence of donor cellular and free DNA in recipient tissues and fluids during allograft maintenance and rejection. In the stage of maintenance, the donor cells were found in the recipient tissues up to 100 days after organ transplantation. At the time of rejection, donor cells disappeared and donor free DNA could be detected in all recipient tissues. This study suggests lack of influence of donor microchimerism on the allograft survival.

Immunological "enhancement" in organ grafting is a phenomenon of prolongation of allograft survival after administration to the recipient of donor lymphocytes and recipient-anti-donor antiserum on day 11 and 10 before organ transplantation, respectively. We found that in heart or skin allograft recipients, the complexes of lymphocyte-antiserum locate mostly in the spleen and less in other lymphoid organs. Interestingly, there was no deposition of complexes in the grafts. We propose a concept of " continuous antigen elimination". Donor antigen-recipient antidonor antiserum complexes are continuously eliminated in spleen and liver. Primarily, they are formed from

the administered donor lymphocytes and antiserum, and after organ grafting from shed donor antigens and antiserum produced by the recipient against shed donor antigens.

Allogenic skin transplants are acutely rejected despite of intensive immunosuppressive therapy. In order to study the mechanism of this phenomenon, canine skin was grafted to scid mice, followed by i.v. administration of canine lymph Langerhans cells or lymphocytes and blood mononuclear cells. Langerhans cells revealed the highest ability to initiate rejection of grafted skin. Resistance of Langerhans cells to cyclosporin A may explain lack of effectiveness of this drug in the prevention of rejection of skin allografts.

CELLULAR AND HUMORAL DEFICIT IN PROTRACTED WOUND HEALING

Project leader: Waldemar L.Olszewski,
Contributors: Hanna Gałkowska, Urszula Wojewódzka, Bożenna Interewicz, Ewa Stelmach

The mechanism of apoptosis was studied in granulation tissue of human leg venous ulcers. We found an increase of proapoptotic bax and caspase 3 and less antiapoptotic bcl2 in keratinocytes encroaching the granulation tissue. In keratinocytes located at distance from the ulcer, high expression of bcl2 was noted.

An inflammatory focus is a site of an active apoptotic process. DNA released from the disintegrated cells is drained away with tissue fluid and lymph to the regional lymph nodes. We studied the presence of free DNA in lymph derived from the inflammed foot joints in patients with rheumatoid arthritis. The concentration of apoptotic DNA (fragments of 400 bp) amounted to 80-100% of total lymph DNA, compared with 20% in normal subjects. Unexplained remains the effect of high concentration of afferent lymph DNA on lymph node cells.

TRANSPLANTATION OF ISOLATED CELLS

Project leader: Waldemar L.Olszewski
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Hepatocytes transplanted into subcutaneous tissue, spleen, peritoneal cavity or liver are immediately eliminated by immigrating granulocytes and mononuclear cells. We studied the mechanism of granulocyte/monocyte cytotoxicity against hepatocytes in an *in vitro* rosette formation model. Granulocytes revealed higher cytotoxic activity than monocytes during 2 and 4 h observation period. Mononuclear cells with cytotoxic activity expressed the ED1 surface molecule.

Rejection of allografts is mediated by recipient lymphocytes. The migration of lymphocytes to the graft can be controlled by immunosuppressive drugs. We found that allografting itself produces major changes in the kinetics of lymphocyte migration before cyclosporin A is administered. After heart allotransplantation, recipient anti-donor lymphocytes accumulated in lymphoid organs. Moreover, even non-commited recipient lymphocytes had a tendency to accumulate in lymphoid tissue.

SECONDARY LYMPHEDEMA - IN VENOUS STASIS, AFTER TRAUMA AND SKIN INFECTIONS

Supported by the State Committee for Scientific Research: grant # 4.P05C.079.13

Project leader: Waldemar L.Olszewski
Contributor: Hanna Gałkowska

Lymphoscintigraphic, bacteriological and immunohistochemical investigations were continued. Isotopic studies revealed presence of enlarged and newly formed lymph nodes in areas remote from the inflammatory focus. Bacterial isolates were found in 20% of nodes from areas with lymph stasis. Inguinal lymph nodes were found largely depleted of lymphocytes. The newly formed lymph nodes were populated mostly by CD4 and CD8 lymphocytes and produced cytokines IL1beta, IL6, TNF alpha and GM CSF. Local infective processes bring about major degenerative changes in inguinal lymph

nodes and simultaneously stimulate lymphatic follicles located along lymphatic vessels to proliferate.

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ACCUMULATION OF LYMPHOCYTES IN LIVER SINUSOIDS AND THEIR ROLE IN LOCAL REACTION AGAINST TUMOR CELLS

Supported by the State Committee for Scientific Research: grant # 4.P05A.047.12

Project leader: Waldemar L.Olszewski
Contributors: Sergiusz Durowicz, Joanna Dłużniewska, Dorota Laszuk, Barbara Łukomska,

Studies were continued on the phenotypes of host cells infiltrating metastatic adenocarcinoma foci in the liver. Accumulation of CD3, CD4, CD5, CD8, MHC class II, ED1 and sporadically NK cells was found around the neoplastic tissue. In the tumor tissue, the CD3, CD4, CD8, class II and CD5 cells predominated. Interestingly, no NK cells could be identified. Remote liver areas revealed presence of single CD3, CD4 and class II cells in the sinusoids. ICAM1 molecule was expressed on the cells around the tumor tissue, and in the tumor itself on single infiltrating cells and endothelial cells. The CD18 molecule revealed similar distribution. The CD11a and CD11b molecules were found weakly expressed on the cells around the neoplastic tissue. The results indicate a relatively weak reaction of the host to proliferating tumor cells.

PATHOMECHANISM OF POSTTRAUMATIC EDEMA OF LOWER EXTREMITIES

Supported by the State Committee for Scientific Research: grant # 4.P05C.037.10

Project leader: Waldemar L.Olszewski
Contributor: Grzegorz Szczęsny

The study was completed on the pathomechanism of development of posttraumatic edema. Clinical investigations revealed major reaction in the

lymph nodes of the affected extremity and only in few cases, thrombotic changes in large veins. No interruption of lymphatic vessels was found. It was inferred from the experimental studies that hematoma had no effect on lymphatic drainage of the traumatized extremity, whereas bone marrow cells spilled from the fractured bone and bacteria populating the skin cause evident changes in the lymph nodes and may be responsible for the inflammation seen at the site of injury and in the regional lymph nodes.

Collaborating unit:
Surgical Department, Clinical Hospital of Ministry of Internal Affairs, Warsaw, Poland

DIABETIC FOOT ULCER IMMUNOHISTOCHEMICAL ANALYSIS OF INFLAMMATION, THE ROLE OF BACTERIAL INFECTION, THERAPY WITH ANTIBIOTICS COMBINED WITH CYTOKINES REGULATING WOUND HEALING

Supported by the State Committee for Scientific Research: grant # 4.P05B.023.16

Project leader: Hanna Gałkowska
Contributors: Waldemar L. Olszewski, Joanna Mijal, Urszula Wojewódzka

Diabetic foot is one of the major complications of diabetes leading to the loss of a part or whole lower limb in about 20% of all hospitalized patients with diabetes type II. An insight into the pathomechanisms of foot ulceration, ischemia and bacterial infection of deep tissues would allow to correct or prevent ulceration, resulting in lowering of the percentage of leg amputations. Our studies were carried out in the patients with stage 1-4 according to Wagner diabetic ulcer classification and adjusted glycemia. Inflammatory process in deep tissues was evaluated by scanning with radiolabelled granulocytes. Bacterial infections were studied using ulcer swabs. Genomic DNA similarity of bacteria species cultured from the ulcer surface, perineum area and toe-web swab of the same patient was analyzed using PCR reaction and RAPD analysis. We adapted the cytofluorometric method for evaluation of oxygen burst and NO activity in granulocytes from peripheral blood of diabetic foot patients. Ulcer biopsies were characterized immunohistochemically for the presence of infiltrating cells (granulocytes, monocytes/ macropha-

ges, lymphocytes), growth factors and their receptors responsible for angiogenesis and leukocyte recruitment into the wound. Markers of cell apoptosis in ulcer biopsies were also studied.

Scintigraphic studies showed accumulation of granulocytes and reconstruction of foot bones at the sites clinically apparently normal, besides those revealing ulcers and bone sequesters. Initial immunohistochemical evaluation revealed lack of extravasation of granulocytes, lymphocytes and precursors of Langerhans cells in the foot skin.

Collaborating unit:
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GROWTH FACTORS IN LIVER REGENERATION AFTER PARTIAL HEPATECTOMY IN PATIENTS WITH LIVER TUMORS

Supported by the State Committee for Scientific Research: grant # 4.P05B.023.16

Project leader: Barbara Łukomska
Contributors: Joanna Dłużniewska, Bożena Mecner

The aim of the study was to investigate the relationship between the expression of local growth factors in liver cells and the rate of liver tissue regeneration after partial hepatectomy in patients with benign and malignant liver tumors. Twenty patients undergoing partial hepatectomy for benign (10 cases) and metastatic colon adenocarcinoma (ACC) (10 cases) liver tumors were studied. There was evidently higher regeneration rate of liver tissue measured in CT 30 days after liver resection in the malignant (53.0±33.2%) than benign (11.1±12.6%) tumor groups. High expression of PCNA was demonstrated 7 days after partial hepatectomy. Interestingly, PCNA was expressed in nontumoral liver tissue of ACC patients before resection but not in the normal liver with benign tumors. HGF, TGFa, VEGF, TGFß1 and cMET/HGF-R, EGF-R/TGFa-R, flk-1/VEGF-R, TGFß-RI, TGFß-RII were detected in normal liver tissue, however the expression of these molecules was higher in the patients with malignant than benign liver tumors. An increase of growth factors and their receptors expression was observed in the regenerating liver in both group of patients 7 days after surgery. Additional-

ly HGF but not cMET were detected in adenocarcinoma cells. Overexpression of growth factors and their receptors in regenerating liver tissue biopsies taken from metastatic ACC patients maybe responsible for high rate of liver tissue regeneration. HGF derived from microscopic residual tumors remaining in nonresected liver fragments may influence the liver tissue growth in ACC patients.

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THE MECHANISM OF FAST HEMATOPOIETIC RECONSTITUTION IN RATS AFTER VASCULARIZED BONE MARROW TRANSPLANTATION

Supported by the State Committee for Scientific Research : grant # 4.P05A.049.15

Project leader: Barbara Łukomska
Contributors: Sława Janczewska, Anna Ziółkowska, Bożenna Interewicz, Tomasz Majewski, Waldemar L. Olszewski

The study was continued on rapid repopulation of bone marrow cavities and lymphoid tissue in lethally irradiated rats after vascularized bone marrow transplantation. The population of cell retrieved from blood and lymph nodes was evaluated (VBMTx). Ten days after VBMTx rapid replenishment of cells in BM, PB and MLN in comparison with BMCTx were observed. Cytometry analysis of PBL in VBMTx rats revealed no differences from the normal values in the percentage of CD90, CD43, CD5 and CD8 positive cells. The percentage of CD4+ cells in PB was lower in VBMTx rats than in normal rats. Moreover, two subsets expressing different level of CD4 molecule were found. There was more T lymphocytes (CD5+) in PB of VBMTx rats than in bone marrow cells in suspension (BMCTx) recipients. The percentage of CD4+ cells in VBMTx and BMCTx was found similar, but CD4+ cells in PB of BMCTx rats presented low expression of CD4 molecule. The CD4+ cells in PB expressed also CD5+ molecules which suggests they were immature T cells. These types of cells were not found in MLN of VBMTx

and BMCTx recipients, all MLN CD4+ cells revealed high expression of these molecules. The proportions of various phenotypic subsets of MLNL isolated from VBMTx and BMCTx rats followed the pattern found in peripheral blood of these animals. The results of our study indicate transplanted hemopoietic tissue is more efficient than isolated BMC in fast lymphopoietic repopulation of irradiated recipients. The presence of immature lymphocytes in PB but not in MLN suggests that in adult recipients of BMTx the differentiation processes of T cell progenitors occur in the lymph nodes.

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STRUCTURE-ACTIVITY STUDIES OF OPIOID PEPTIDES

A tetraptide dimer, biphalin, originally synthesized by this team, possesses unique analgesic properties comparing to many other opioid peptide analogues studied since. Biphalin is reported to have high affinity for all three opioid receptor types (μ, δ and κ). Last years structure-activity studies performed in collaboration with Professor Hruby group (University of Arizona, Tucson) resulted in establishing minimal structural requirements responsible for the high analgesic properties of biphalin. According to our studies, other groups, with similar physico-chemical properties could replace the fragment not necessary for high analgesic properties. This prompted attempts to synthesize new types of peptide analogues in which opioid fragments were hybridized with pharmacophores of other neuroreceptors. First such a compound of this series, AA501, hybridizes biphalin opioid pharmacophore with a fragment expressing substance P antagonistic properties. Biological tests done in collaboration with Professor Carr showed that AA501 expresses high analgesic properties in both acute and chronic pain in animal models.

PEPTIDES WITH AMPHIPHILIC AMINO ACIDS

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Well topographically defined hydrophobic and aromatic interactions are most critical for membrane peptide receptor-ligand complex formation and

stability. We have hypothesized that proper location of stable hydrophilic counterparts to lipophylic and/or aromatic residues may stabilize the complex of peptide ligands with receptor pocket. Last year, we studied the consequences of introducting α-hydroxymethyl group into the α-position of phenylalanine (3) and/or valine (5 or 6) of deltorphin II. We observed that the significance of such modification was strongly dependent on the position of the primary amino acid in the peptide chain. One of the obtained analogues expressed the highest affinity and selectivity to δ opioid receptors, among other analogues synthesized since. Close collaboration with chemical group headed by Dr. Aleksandra Olma was critical for success of this project.

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Publication:

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IODINE UPTAKE AND THYROID HORMONE METABOLISM AFTER IMPLEMENTATION OF THE OBLIGATORY IODINE PROPHYLAXIS IN POLAND

Project co-ordinator: Janusz Nauman

To study the results of the recently implanted system of obligatory iodine prophylaxis in Poland we compared the parameters of iodine delivery and metabolism in several groups of subjects before and after start of prophylaxis.

We found that in the group of patients with thyroid disorders and in healthy control subjects, mean urinary iodine excretion increased significantly after the onset of iodine prophylaxis. The subjects who eliminated dietary salt continue to have low iodine excretion.

To study the influence of iodine prophylaxis on 5'monodeiodination we studied serum TSH, free T3 and free T4 levels and urinary iodine concentrations in the patients with Graves' hyperthyroidism. Mean urinary iodine after onset of iodine prophylaxis was significantly higher than before; mean serum TSH level had not changed and the fT3/fT4 ratio decreased. We conclude that in both normal subjects and in patients with rapid thyroid hormone metabolism and increased iodine turnover, implemented iodine prophylaxis resulted in normal iodine delivery. Elimination of dietary salt results in iodine deficiency. Moreover, iodine supplementation resulted in normalisation of monodeiodination of T4 to T3, rendering hyperthyroidism treatment effective.

Collaborating unit:
Department of Internal Medicine and Endocrinology, University Medical School, Warsaw, Poland (W. Grzesiuk, A. Kondracka, M. Słoń).

EXPRESSION AND FUNCTION OF THYROID HORMONE RECEPTORS (TR) AND 9-cis RETINOIC ACID RECEPTORS (RXR) IN THYROID CANCERS (DEPENDENT ON RADIATION?)

Supported by the State Committee for Scientific Research: grant # 4 P05B 041 15

Project leader: Monika Puzianowska-Kuźnicka
Contributor: Janusz Nauman

To study whether triiodothyronine and its receptors take part in tumorigenesis we measured the expression of TRα and TRβ genes in thyroid cancers and control thyroid tissue. Analysis of Northern blots (probed with full coding regions of TRα1 or TRβ1) showed that in most analyzed cancers and expression of TRα and TRβ genes was markedly (1.77x and 3.5x, respectively) decreased as compared to normal thyroid tissue. In contrast, it was found that TRα1 protein level was 1.36x higher and TRβ1 – 1.82x higher in cancers than in healthy tissue. In addition, coding regions of TRα1 or TRβ1 genes were cloned by RT-PCR method and screened for mutations that might alter receptor function. Coding regions of RXRα and RXRγ were also cloned by RT-PCR and will be used as probes in Northern analysis of the same RNA as mentioned above.

Collaborating units:
Centre for Medical Postgraduate Education, Warsaw, Poland
– Department of Biochemistry (M. Ambroziak)
– Department of Gastroenterology (L. Trzeciak)
Department of Endocrinology, University Medical School, Warsaw, Poland (A. Madej)

THYROID HORMONE RECEPTORS (TR) AND 9-cis RETINOIC ACID (RXR) IN HUMAN RENAL CLEAR CELL CARCINOMA

Project leader: Monika Puzianowska-Kuźnicka
Contributor: Janusz Nauman

Transcriptional activity of TRα and TRβ genes was estimated on the basis of the amount of specific mRNA in human kidney cancer and in nor-

mal renal tissues. It was shown that in 90% of analysed cancers, the activity of TRα gene was decreased in comparison to healthy surrounding tissue, and that this decrease was most pronounced in well differentiated tumors. Examination of TRβ gene activation showed that in 70% of analysed cases the activity of this gene was decreased approximately 3 times, while in remaining 30% cases this activity was increased (5-40%) in comparison to normal surrounding tissue. On the protein level, Western blot experiments showed that the amount of TRα1 protein was 1.6x higher, while the amount of TRβ1 protein was approximately 10x lower in cancer tissue as compared to the healthy tissue. In addition, coding regions of TRα1 and TRβ1 were cloned by RT-PCR from 20 cancers and 7 control, normal kidneys. Sequencing of these genes showed that T3 binding domain of TRβ1 was mutated in 7 cancers, while T3 binding domain of TRα1 was mutated in 3 cancers. It seems that the percentage of mutated receptors is even higher, since mutations have been found in other domains, too.

Collaborating units:
Department of Biochemistry, Centre for Medical Postgraduate Education, Warsaw, Poland (A. Nauman)
Department of Endocrinology, University Medical School, Warsaw, Poland (A. Madej)

VITAMIN D RECEPTORS (VDR) IN HUMAN RENAL CLEAR CELL CARCINOMA

Project leader: Janusz Nauman
Contributor: Monika Puzianowska-Kuźnicka

Western blot experiments showed that in 80% of well-differentiated, 56% of poorly differentiated kidney cancers and 50% of cancers on intermediate level of differentiation the amount of VDR protein was increased in comparison to normal renal tissue.

Cooperating units:
Department of Endocrinology, University Medical School, Warsaw, Poland (A. Madej)
Department of Biochemistry, Centre for Medical Postgraduate Education, Warsaw, Poland (A. Nauman)

AUTOANTIBODIES REACTIVE WITH EXTRACELLULAR MATRIX (ENM) PROTEINS IN PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY (TAO)

Supported by the State Committee for Scientific Research: grant # 4.P05A.138.14

Project leader: Tomasz Bednarczuk
Contributors: Zbigniew Bartoszewicz, Janusz Nauman

To study the role of autoantibodies reacting with extracellular matrix (ECM) proteins in the pathogenesis of the thyroid-associated ophthalmopathy (TAO) we examined the humoral immune response against specific ECM proteins, namely: collagen types I, III, IV, V (CI, CIII, CIV, CV), fibronectin (FN) and laminin (LM) using ELISA. Overall, sera from 50% of patients with TAO contained antibodies reactive against one or more ECM proteins, compared to 27% with Graves' disease (GD) without evident eye involvement, 28% with Hashimoto's thyroiditis (HT) and 9% of normal subjects. Serum anti-CI, -CIII, -CV and LM levels were significantly ($p<0.05$) higher in patients with TAO than in normals. To determine the structural epitopes of these proteins, we performed immunoblotting studies on CNBr-derived peptides of CI and CV. Results of our study suggest that a variety of ECM proteins (CI, CV, LM) may be secondary autoantigens, that are recognised by antibodies in TAO. These antibodies react with epitopes expressed on both native and denatured proteins, and may therefore have the potential to bind to ECM *in vivo*.

Collaborating units:

Thyroid Centre, Allegheny University of the Health Sciences, Pittsburgh, USA (C. Stolarski, S. Kubota, J.R. Wall)

Department of Endocrinology, University School of Medicine, Warsaw, Poland (M. Rowiński, E. Pawlik, M. Słoń)

Department of Endocrinology, University Medical School, Kurume, Japan (Y. Hiromatsu)

GLYCOSYLATED ISOFORMS OF PROLACTIN HORMONE – CHARACTERIZATION AND INFLUENCE OF SUGAR MOIETY ON HORMONE BIOLOGICAL ACTIVITY

Project leader: Zbigniew Bartoszewicz
Contributor: Katarzyna Sikorska

There is substantial evidence that glycosylated prolactin (GPRL) sugar moiety may play an important role in the biosynthesis, secretion, plasma clearance, biological activity and immunoreactivity of the pituitary hormone.

We purified GPRL isoform from porcine pituitary and investigated hormone biological activity modified by glycosylhydrolases. The treatment of GPRL by α-mannosidases increases the hormone biological activity. The endoglycosidase H and N-glycanase treated GPRL samples recover the biological activity of nonglycosylated prolactin.

We examined the pituitary prolactin isoforms from various species on the Western blots. Surprisingly, we have found glycosylated prolactin in chicken pituitary. The molecular weight of this isoform, its susceptibility for endoglycoside H and interaction with lectins have strongly suggested N-glycosylation. Because known amino acid sequence of chicken pituitary prolactin does not contain typical N-glycosylation site (Asn-X Ser/Thr) the chicken prolactin cDNA was isolated and sequenced. There were 4 sites in PRL coding sequence where predicted amino acid sequence varied from the one in GenBank. However the changes described did not introduce the Asn-X Ser/Thr linkage into PRL amino acid sequence. We have hypothesized Asn-XCys as a potential alternative N-glycosylation site.

EXPRESSION THE PROTEIN OF THE THYROID DIFFERENTIATION MARKERS: THYROID PEROXIDASE AND SODIUM-IODIDE SYMPORTER IN NON- AND AUTOIMMUNE THYROID DISEASE AND DIFFERENTIATED THYROID TUMORS

Project co-ordinator: Barbara Czarnocka

To study thyroid peroxidase (TPO) protein expression and its antigenic properties in thyroid cancer, TPO was captured from a solubilizate of thyroid microsomes by a panel of murine anti-TPO monoclonal antibodies and

detected with a panel of anti-human TPO IgGκ Fab. Virtual absence of TPO expression was observed in 8 out of 30 cases of cancer. In the remaining 22 malignant thyroid tumors the TPO protein level varied considerably. When expressed TPO displayed similar epitopes, recognized by the Fabs to TPO from Graves' disease tissue. The results were confirmed by SDS-PAGE and Western blot analysis. The present results show that in about two-thirds of differentiated thyroid carcinomas, TPO protein is expressed, albeit to a more variable extent than normal; when present, TPO in malignant tissues is immunologically normal.

GANGLIOSIDES OF THE DIFFERENTIATED THYROID CANCER AND BENIGN THYROID NEOPLASMS – INFLUENCE OF THE HORMONAL STATUS AND TREATMENT

Supported by the State Committee for Scientific Research: grant # 4.P05B.042.15

Project leader: Jacek Kiljański
Contributors: Zbigniew Bartoszewicz, Barbara Czarnocka

We studied anti-gangliosides humoral responses accompanying thyroid malignancies. Using ELISA we have occasionally found antiganglioside autoantibodies of the IgG and IgM class (anti-GM3, anti-GD1b and anti-GM1) in the patients with differentiated thyroid cancer and in the patients with benign tumors and autoimmune thyroid disorders. Their prevalence and titre in the patients with cancer did not differ significantly from other studied groups of subjects. Contrary to reports of other groups we did not find anti-FucGM1 antibodies in sera of patients with thyroid cancer.

Publications:

Bartoszewicz Z.P., Jaffe H., Sasaki M., Möller J.R., Stebbins J.W., Gebrekristos H., Quarles R.H.: Prominent 85-kDa oligomannosidic glycoproteins of rat brain are signal regulatory proteins and include the SHP substrate-1 for tyrosine phosphatases. *J Neurochem* 1999, 72, 1688-1693.

Bednarczuk T., Stolarski C., Pawlik E., Słoń M., Rowiński M., Kubota S., Hiromatsu Y., Bartoszewicz Z., Wall J.R., Nauman J.: Autoantibodies reactive with extracellular matrix proteins in patients with thyroid-associated ophthalmopathy. *Thyroid* 1999, 9, 289-295.

Czarnocka B., Pastuszko D., Janota-Bzowski M., Weetman A.P., McIntosh R.S., Asghar M.S., Watson P.F., Kemp H.E., Łyczkowska A., Jarząb B., Gubała E., Włoch J., Lange D.: Thyroid peroxidase protein in differentiated thyroid carcinomas. *Endokrynol Pol* 1999, 50, 165-180.

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Kiljański J.I.: Treatment of patients with thyroid-associated ophthalmopathy. *Terapia* 1999, 5, 11-15 (in Polish).

Kiljański J., Makowska A., Bar-Andziak E., Pietraszek A., Feltynowski T., Cygler B., Królicki L., Żukowska M., Dowżenko A., Olszewski W., Nauman J.: Cushing's syndrome due to ectopic ACTH production by a pulmonary carcinoid complicated by onset of rheumatoid arthritis following successful treatment of hypercortisolism – case report. *Endokrynol Pol* 1999, 50, 429-438.

Krasnodębska M., Kiljański J., Bar-Andziak E., Górnicka B., Bogdańska M., Faryna J., Skórski M., Demkow T., Nauman J.: Renal cell carcinoma metastases to the thyroid gland – report of two cases). *Endokrynol Pol* 1999, 50, 285-294.

Madej A., Puzianowska-Kuźnicka M.: Molecular mechanism of activity of the vitamin D nuclear receptor. *Endokrynol Pol* 1999, 50, 277-284 (in Polish).

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Nauman A., Ambroziak M., Pachucki J., Puzianowska-Kuźnicka M., Nauman J.: Effect of gold on 5-deiodination of iodothyronines in human thyroid, liver, kidney and adipose tissue. *Endokrynol Pol* 1999, 50, 239-247.

PROMOTIONS

HABILITATION THESIS

Bronisław Głód
Ion-exclusion chromatography: theory, influence of physicochemical parameters and applications.

Aleksandra Misicka-Kęsik
Application of medicinal chemistry methods for elaboration of new ligands of δ opioid receptors.

DOCTOR'S THESIS

Jacek Bogucki
Anatomical aspects of a surgical approach through the floor of the fourth ventricle.

Małgorzata Frontczak-Baniewicz
Influence of experimental focal brain damage on capillary vessels in rat brain

Michał Maksymowicz
Impairment of lymphocyte migration by cyclosporin A.

Grzegorz Szczęsny
The role of the lymphatic system in the pathomechanism of the posttraumatic edema of lower limbs.

Wojciech Zabołotny
Methods for estimation of maximum frequency in Transcranial Doppler Signal

Ewa Żernicka
Effect of experimental hypo- and hyper-thyroidism on lipoprotein lipase activity and triacylglycerol content in muscles of sedentary and exercising rats.

ORGANIZATION OF SYMPOSIA AND CONFERENCES

Symposium: Skin immunity in lymph stasis and after trauma (I), Warsaw, February 26, 1999

Conference on genetics of facio-scapulo-peroneal dystrophy. Prof. R. Griggs for Rochester (N. York, USA), was the speaker. Warsaw, March 29, 1999.

International Symposium: „Glutamate-Glutamine Homeostasis in the CNS: Physiological and Pathophysiological Aspects", Wierzba , June 19-23, 1999. Organizers: J. Albrecht (Warsaw), A. Schousboe (Copenhagen).

Conference on Behcet disease with participation of 4 scientists from Turkey. The audience consisted of neurologists, dermatologists and internists. Warsaw, July 8, 1999.

Session "Neurophysiology" on the XXIst Congress of the Polish Physiological Society, Poznań, September 9, 1999.

Symposium: Reaction to tumor in liver and peritoneal cavity, Bydgoszcz, September 22-24,1999

1st Italian-Polish-Hungarian Symposium on Medicinal Chemistry, Camerino, Italy, September 26 - October 1, 1999. Neuropeptide Laboratory was initiator of the organization of the first Italian-Polish-Hungarian Symposium on Medicinal Chemistry, under auspices of the national chemical societies. The major goal of the symposium was to present the scientific potential of three countries in the field of medicinal chemistry, and initiate new collaborative projects within the European Community. Over hundred lectures and communications have been presented on the symposium. The success of the symposium brought about a joint decision to organize such symposia every two years.

Symposium: Inflammatory aspects of arterial atherosclerosis, Warsaw, October 13, 1999

Symposium: Immunity in skin in lymphedema, tumors and after trauma (II), Warsaw, November 4, 1999

Symposium: Regulation of immune processes after trauma, Warsaw, November 25,1999

Polish-Finnish Symposium: „Neurotransmitters in models of brain pathology", Warsaw, November 29, 1999. Organizers: J. Albrecht (Warsaw), S. S. Oja (Tampere).

VIth Neurosurgical Meeting in Pultusk "Advances in hydrocephalus treatment", Pułtusk, December 2-4, 1999. Topics: "Hydrocephalus resulting from SAH"and "Diagnosis and treatment of intracranial arachnoid cysts". 89 participants including 3 foreign quests. 24 oral presentations including 1 presented by the worker of the Department of Neurosurgery.