POLISH ACADEMY OF SCIENCES
MEDICAL RESEARCH CENTRE

ANNUAL REPORT
1997

WARSZAWA - 1998

Editors:
Halina Weinrauder
Wanda Dziedzic

Scientific Consultants:
Jan Albrecht
Krystyna Cedro-Ceremużyńska
Mieczysław Pokorski

Supported by the State Committee for Scientific Research

Polish Academy of Sciences
Medical Research Centre (MRC)
5 Pawińskiego St., 02-106 Warsaw, Poland
Telephones: (48-22) 668 52 50; 668 52 64; 608 65 50
Fax (48-22) 668 55 32

This year was a special year in the history of our Institute. By a happy coincidence the 30th anniversary of its foundation was celebrated in a newly acquired (and how long awaited) accommodation, a complex of modern facilities which is hoped to give new stimulus to research and educational activities. Prompted by this unusual moment the Editors decided to modify the content and layout of the Annual Report to what we assume will become more informative and better promote cooperation with partners around the world.

CONTENTS

MRC SCIENTIFIC COUNCIL

Chairman:
Adam Nowosławski

Vice-Chairman:
Janusz Nauman

Secretary:
Bogna Szereda-Przestaszewska

Members:

Jan Albrecht
Stefan Angielski
Włodzimierz Bicz
Krystyna Cedro-Ceremużyńska
Zbigniew Czernicki
Maria Dąmbska
Krystyna Domańska-Janik
Kazimierz Dux
Anna Fidziańska-Dolot
Barbara Gajkowska
Bogusław Halikowski
Irena Hausmanowa-Petrusewicz
Wojciech Hilgier
Janusz Jeljaszewicz
Jerzy Jurkiewicz
Hanna Kaciuba-Uściłko
Leszek Kaczmarek
Józef Kałuża
Andrzej Kapuściński
Witold Karczewski
Maria Kopeć
Wojciech Kostowski
Andrzej Kukwa
Bohdan Lewartowski
Andrzej Lipkowski
Jerzy Łazarewicz
Jerzy Maj
Witold Mazurowski
Mirosław J. Mossakowski
Olgierd Narkiewicz
Krystyna Nazar
Waldemar Olszewski
Kazimierz Ostrowski
Edmund Przegaliński
Janina Rafałowska
Urszula Rafałowska
Wojciech Rowiński
Witold Rudowski
Jan Ryżewski
Janusz Sadowski
Joanna Strosznajder
Andrzej Szutowicz
Andrzej Trzebski
Jerzy Vetulani
Michał Walski
Mieczysław Wender
Ewa Wójcik-Ziółkowska
Andrzej Ziemba
Bogusław Żernicki

EXECUTIVE BOARD

Director:
Professor Mirosław J. Mossakowski, M.D., Ph.D., D.Sci., D.h.c.
Member of the Polish Academy of Sciences

Scientific Directors:
Professor Jan Albrecht, M.Biol., Ph.D., D.Sci.
Professor Jerzy W. Łazarewicz, M.D., Ph.D., D.Sci.

Managing Director:
Eugeniusz Kaczmarczyk, M.C.L.

STAFF LIST

DEPARTMENT OF NEUROPHYSIOLOGY

Head: Professor Mieczysław Pokorski

Scientific Staff

Krystyna Budzińska, M. Pharm., Ph.D.
Leszek Czerwosz, M. Phys.
Nargiman Dziadosz, M. Biol.
Sylwia Elwich
Lidia Faff, M. Pharm., Ph.D. (since June 1997)
Henryk Gromysz, M. Biol., Ph.D.
Beata Kopczyńska, M.Biol.
Andrzej Kukwa, M.D., Ph.D., D.Sci., Prof. of Medical Sciences
Anna Lendzion-Radajewska, M. Biol.
Małgorzata Musielak, M. Biol. (since June 1997)
Anna Nowakowska, M.Biol.
Mieczysław Pokorski, M.D., Ph.D., D.Sci., Professor of Neurophysiology
Beata Sokołowska, M. Phys.
Robert Strosznajder, M. Zoot., Ph.D.
Bogna Szereda-Przestaszewska, M.D., Ph.D., D.Sci., Assoc. Prof. of Neurophysiology
Ewa Wojtal, M. Biol., Ph.D.

Technical Staff

Karina Goldsztajn, Kinga Sroczyńska, Teresa Warnawin, Ewa Wielochowska

Administrative Staff

Renata Kawka, secretary (until June 1997), Alicja Arent, secretary,
Ludmiła Haczyńska, secretary

LABORATORY OF EXPERIMENTAL PHARMACOLOGY

Head: Assoc. Prof. Paweł Grieb

Scientific Staff

Stanisław J. Chrapusta, M.Sci. Chem., Ph.D.
Paweł Grieb, M.Biol., Ph.D., D.Sci., Assoc. Prof. of Natural Sciences
Wiktor Janczewski, M.Sci.Eng., Ph.D.
Mirosław Ryba, M.D., Ph.D., D.Sci., Assoc. Prof. of Neurophysiology

Technical Staff

Monika Janisz

DEPARTMENT OF NEUROCHEMISTRY

Head: Professor Jerzy W. Łazarewicz

Laboratory of Pharmaconeurochemistry

Head: Professor Jerzy W. Łazarewicz

Scientific Staff

Mohd Alaraj, Eng., Ph.D.
Wanda Gordon-Krajcer, M. Pharm., Ph.D.
Irena Kosińska, Eng.
Jerzy W. Łazarewicz, M.D., Ph.D., D.Sci., Professor of Medical Sciences
Elżbieta Salińska, M.Biol., Ph.D.
Aleksandra Stafiej, M.Chem.

Technical Staff

Halina Nowińska, Anna Sobczuk, Apolonia Ziembowicz

Administrative Staff

Maria Izak, secretary

Laboratory of Pathobiochemistry of Central Nervous System

Head: Professor Urszula Rafałowska

Scientific Staff

Beata Dąbrowska-Bouta, M.Biol.
Urszula Rafałowska, M.Biol., Ph.D., D.Sci., Professor of Natural Sciences
Lidia Strużyńska, M.Anal. Med., Ph.D.
Jolanta Waśkiewicz, M.Biol., Ph.D.

Technical Staff

Aleksandra Lenkiewicz

Laboratory of Molecular Neuropathology

Head: Assoc. Prof. Krystyna Domańska-Janik

Scientific Staff

Krystyna Domańska-Janik, M.D., Ph.D., D.Sci., Assoc. Prof. of Medical Sciences
Joanna Sypecka, M.Biol., Ph.D.
Barbara Zabłocka, M.Biol. Ph.D.
Teresa Zalewska, M.Pharm., PhD.

Technical Staff

Teresa Czachmańska, Halina Zając

DEPARTMENT OF NEUROTOXICOLOGY

Head: Professor Jan Albrecht

Scientific Staff

Jan Albrecht, M.Biol., Ph.D., D.Sci., Professor of Biomedical Sciences
Hanna Borkowska, M.Agr.Sci.
Monika Dolińska, M.Sci.
Anna Dybel, M.Biol.
Lidia Faff-Michalak, M.Pharm, Ph.D. (until May 1997)
Wojciech Hilgier, M.Pharm., Ph.D.
Magdalena Wojda, M.Sci.
Magdalena Zielińska, M.Sci., Eng.

Technical Staff

Inez Fręśko, Gertruda Kaplińska, Mirosława Poławska

DEPARTMENT OF CELLULAR SIGNALLING

Head: Professor Joanna Strosznajder

Scientific Staff

Małgorzata Chalimoniuk, M.Chem.
Bronisław Głód, M.Chem, Ph.D.,
Maciej M. Łałowski, M. Biol., Ph.D.
Marek Samochocki, M.Chem., Ph.D.
Joanna Strosznajder, M.D., Ph.D., D.Sci., Professor of Medical Sciences

Technical Staff

Danuta Kacprzak, Stanisława Kuciak, Dorota Pazikowska, Magdalena Skorupka

Administrative Staff

Maria Izak, secretary

DEPARTMENT OF NEUROPATHOLOGY

Head: Professor Andrzej Kapuściński

Scientific Staff

Jolanta Baraniecka, M.Chem. Eng
Maria Barcikowska-Litwin, M.D., Ph.D., D.Sci.
Dorota Dziewulska, M.D., Ph.D. (since July 1997)
Roman Gadamski, M.Vet., Ph.D.
Marek Gołębiowski, M.D., Ph.D. (since September 1997)
Adam Gołąbek, M.Biol., Ph.D.
Andrzej Kapuściński, M.D., Ph.D., D.Sci., Professor of Nuclear Medicine
Elżbieta Kida, M.D., Ph.D., D.Sci.
Ewa Koźniewska-Kołodziejska, M.Phys., Ph.D., D.Sci.
Stanisław Krajewski, M.D., Ph.D., D.Sci., Assoc. Prof. of Neuropathology
Ewa Matyja, M.D., Ph.D., D.Sci.
Mirosław J. Mossakowski, M.D., Ph.D., D.Sci., Profesor of Neuropathology
Elżbieta Paplińska, M.Biol.
Anna Pfeffer-Baczuk, M.D.
Piotr Piotrowski, M.D.
Ryszard Pluta, M.D., Ph.D., D.Sci., Assoc. Prof. of Neuropathology
Robert Ostrowski, M.D.
Grażyna Szumańska, M.Biol., Ph.D.
Mieczysław Śmiałek, M.D., Ph.D., D.Sci., Assoc. Prof. of Neuropathology
Anna Taraszewska, M.D., Ph.D.
Halina Weinrauder-Semkow, M.Biol., Ph.D.
Irmina B. Zelman, M.D., Ph.D., D.Sci., Assoc. Prof. of Neuropathology

Technical Staff

Hanna Chrzanowska, Anna Dubiel, Irena Dybkowska-Anc, Jolanta Gębarowska (since July 1997), Elżbieta Grzywaczewska, Sławomir Januszewski, Jadwiga Kędzierska, Małgorzata Knejczuk-Wesoła, Małgorzata Kobryś, Zdzisława Kowalska, Wanda Ogonowska, Teresa Pańkowska, Izabella Przekop, Barbara Śliwińska (until May 1997), Lidia Radomska, Krystyna Wierzbicka, Renata Wojda, Maria Zielińska

Administrative Staff

Teresa Miodowska, secretary

DEPARTMENT OF DEVELOPMENTAL NEUROPATHOLOGY

Head: Assoc. Prof. Danuta Maślińska

Scientific Staff

Maria Dąmbska, M.D., Ph.D., D.Sci., Professor of Neuropathology
Lech Iwanowski, M.D., Ph.D., D.Sci., Assoc. Prof. of Neuropathology
Agnieszka Kaliszek, M.D. (since September 1997)
Izabela Kuchna, M.D., Ph.D.
Milena Laure-Kamionowska, M.D., Ph.D.
Danuta Maślińska, M.D., Ph.D., D.Sci., Assoc. Prof. of Medical Sciences
Mariusz Muzylak, M.Vet., Ph.D. (until June 1997)

Technical Staff

Jolanta Opertowska, Barbara Raczkowska, Jolanta Toborowicz, Elżbieta Wanacka, Halina Winiarska

Administrative Staff

Danuta Krysztofiak, secretary, Lidia Wąsowska, secretary

LABORATORY OF THE ULTRASTRUCTURE OF THE NERVOUS SYSTEM

Head: Assoc. Prof. Barbara Gajkowska

Scientific Staff

Marcin Cholewiński, M.D. (since September 1997)
Małgorzata Frontczak-Baniewicz, M. Biol.
Barbara Gajkowska, M.Biol., Ph.D., D. Sci., Assoc. Prof. of Medical Sciences
Hanna Olszewska, M.D. (since May 1997)
Michał Walski, M.D., Ph.D., D.Sci., Assoc. Prof. of Medical Sciences

Technical Staff

Henryk Bilski, Wanda Ciesielska, Teresa Barszcz

Administrative Staff

Małgorzata Zawistowska-Kasiak

DEPARTMENT OF NEUROSURGERY

Head: Professor Zbigniew Czernicki

Scientific Staff

Jarosław Andrychowski, M.D.
Jacek Bogucki, M.D.
Zbigniew Czernicki, M.D., Ph.D., D.Sci., Professor of Neurosurgery
Jerzy Dubicki, M.D. (since February 1997)
Jacek Dziduszko, M.D., Ph.D., D.Sci.
Anatol Dowżenko, M.D., Ph.D., D. Sci.
Ewa Fersten, M.Psych., Ph.D.
Witold Grochowski, M.D., Ph.D.
Dariusz Horsztyński, M.D.
Jerzy Jurkiewicz, M.D., Ph.D., D.Sci., Assoc. Prof. of Neurosurgery
Nana Kuridze, M.Biol., Ph.D.
Elżbieta Łuczywek, M.Psych., Ph.D.
Piotr Marszałek, M.D.
Eugeniusz Mempel, M.D., Ph.D., D.Sci., Professor of Neurosurgery
Michał Mierzejewski, M.D.
Paweł Nauman, M.D.
Stefan Piechnik, M.Sc.Eng.
Wojciech Sapieja, M.D.
Waldemar Rataj, M.D. (since March 1997)
Grażyna Stępińska, M.D.
Barbara Witkiewicz, M.D.
Wojciech Zabołotny, M.Sci.Eng.
Jerzy Walecki, M.D., Ph.D., D.Sci., Professor of Radiology

Technical Staff

Urszula Borowska, Elżbieta Kunicka, Jacek Małolepszy,
Michał Małyska, Sławomir Matyjek

Administrative Staff

Elżbieta Kamińska, M.Tour., secretary

NEUROMUSCULAR UNIT

Head: Professor Irena Hausmanowa-Petrusewicz

Scientific Staff

Małgorzata Dorobek, M.D.
Hanna Drac, M.D., Ph.D.
Anna Fidziańska-Dolot, M.D., Ph.D., D.Sci., Professor of Neurology
Irena Hausmanowa-Petrusewicz, M.D. Ph.D., D.Sci., Professor of Neurology
Hanna Jędrzejowska, M.D., Ph.D., D.Sci, Professor of Neurology
Anna Kamińska, M.D., Ph.D.
Elżbieta Kowalska-Olędzka, M.D.
Irena Niebrój-Dobosz, M.D., Ph.D., D. Sci., Assoc. Professor of Medical Sciences
Aleksandra Podlecka, M.D.
Janina Rafałowska, M.D., Ph.D., D. Sci., Professor of Neurology
Katarzyna Rowińska-Marcińska, M.D., Ph.D., D.Sci.
Maria H. Strugalska-Cynowska, M.D., Ph.D.

Technical Staff

Hanna Matz, Andrzej Stachurski, Ryszard Strzałkowski

Administrative Staff

Ewa Warelis-Witkowska, secretary

NEUROIMMUNOLOGICAL UNIT

Head: Professor Mieczysław Wender

Scientific Staff

Jacek Losy, M.D., Ph.D.
Grażyna Michałowska-Wender, M.Biol., Ph.D.
Elżbieta Tokarz-Kupczyk, M.D.
Mieczysław Wender, M.D., Ph.D., D.Sci., Professor of Neurology

Administrative Staff

Marianna Kłyś

DEPARTMENT OF APPLIED PHYSIOLOGY

Head: Professor Hanna Kaciuba-Uściłko

Scientific Staff

Barbara Bicz, M.Biol., Ph.D.
Zofia Brzezińska, M.Pharm., Ph.D.
Leszek Budohoski, M.Chem., Ph.D., D.Sci., Assoc. Prof. of Physiology
Jolanta Chwalbińska-Moneta, M.D., Ph.D., D.Sci., Assoc. Prof. of Physiology
Gerard Cybulski, M.Sci.Eng., Ph.D.
Anna Dubaniewicz, M.Biol.
Ilona Falęcka-Wieczorek, M.Biol., Ph.D.
Monika Górecka, M.Biol.
Ryszard Grucza, M.Sci., Ph.D., D.Sci., Assoc. Prof. of Physiology
Hanna Kaciuba-Uściłko, M.Zoot., Ph.D., D.Sci., Professor of Physiology
Alicja Kodrzycka, M.Phys.Ed. (since March 1997)
Barbara Kruk, M.Biol., Ph.D., D. Sci., Assoc. Prof. of Physiology
Hubert Krysztofiak, M.D.
Krzysztof Krzemiński, M.D., Ph.D.
Józef Langfort, M.Biol., Ph.D.
Krystyna Nazar, M.D., Ph.D., D.Sci., Professor of Medical Sciences
Wiktor Niewiadomski, M.D., Ph.D.
Marcin Synak, M.Chem.
Elżbieta Titow-Stupnicka, M.Biol., Ph.D. (until March 1997)
Andrzej W. Ziemba, M.Biol., Ph.D.
Ewa Żernicka, M.Biol.

Technical Staff

Maria Cisowska-Wiencław (since March 1997), Bożena Kurek, Wanda Radziszewska, Jadwiga Wężowska, Lidia Wiśnik

Administrative Staff

Barbara Tomczak, secretary, Eliza Szczęsna

LABORATORY OF RENAL AND BODY FLUID PHYSIOLOGY

Head: Professor Janusz Sadowski

Scientific Staff

Bożena Bądzyńska, M.Biol., Ph.D.
Leszek Dobrowolski, M.Biol., Ph.D.
Elżbieta Kompanowska-Jezierska, M.Biol., Ph.D.
Janusz Sadowski, M.D., Ph.D., D.Sci., Professor of Medical Sciences
Agnieszka Walkowska, M.Biol.

OUTPATIENT CARDIAC UNIT FOR DIAGNOSIS AND THERAPY

Head: Dr. Ewa Wójcik-Ziółkowska

Scientific Staff

Wiesława Pawłowska-Jenerowicz, M.D.
Magdalena Płachcińska-Bijak, M.D.
Celina Romiszowska, M.D.
Ewa Wójcik-Ziółkowska, M.D., Ph.D.

Technical Staff

Maria Koszutska, Wiesława Kowalska, Jolanta Wiśniakowska, Hanna Zduńczyk

CARDIOVASCULAR LABORATORY

Head: Professor Krystyna Cedro-Ceremużyńska

Scientific Staff

Krystyna Cedro-Ceremużyńska, M.D., Ph.D., D.Sci., Professor of Medical Sciences

Technical Staff

Stefania Słyk

DEPARTMENT FOR SURGICAL RESEARCH AND TRANSPLANTATION

Head: Professor Waldemar Olszewski

Scientific Staff

Marek Durlik, M.D., Ph.D.
Sergiusz Durowicz, M.D.
Andrzej Dworczyński, M.D.
Hanna Gałkowska, M.Sc., Ph.D.
Irena Grzelak, M.Sci., Ph.D.
Sława Janczewska, M. Anal. Med.
Urszula Kubicka, M.Sci., Ph.D.,
Barbara Łukomska, M.Vet., PhD., D.Sci., Assoc. Prof. of Surgery
Michał Maksymowicz, M.D.
Andrzej Namysłowski, M.D.
Waldemar L. Olszewski, M.D., Ph.D., D.Sci., Professor of Surgery
Ewa Orlewska, M.D., Ph.D.
Emilia Poreda, M.Biol.
Danuta Sadowska-Ryffa, M.Sci. (until March 1997)
Grzegorz Szczęsny, M.D.

Technical Staff

Ewa Cybulska, Kołakowska Bożena (since September 1997), Dorota Laszuk, Bożena Mecner, Alfred Nowotka, Ewa Paciorek, Krzysztof Plisak, Ewa Stelmach, Iwona Tyszer, Urszula Wojewódzka, Marzanna Zaleska, Anna Ziółkowska

Administrative Staff

Helena Kwaszczyńska, M.Law, secretary, Ewa Rytter, M.Arts., secretary (until September 1996), Anna Lendzion-Radajewska (since October 1996)

NEUROPEPTIDE LABORATORY

Head: Assoc. Prof. Andrzej Lipkowski

Scientific Staff

Przemysław Jakubowski, M.D.
Dariusz Kosson, M.D.
Barbara Kwiatkowska-Patzer, M.D., Ph.D.
Andrzej Lipkowski, M.Chem., Ph.D., D.Sci., Assoc. Prof. of Endocrinology
Iwona Maszczyńska, M.Pharm.
Anna Minich, M.Chem. (until July 1997)
Aleksandra Misicka-Kęsik, M.Chem., Ph.D.
Brunon Sagan, M.Biol. (until July 1997)

DEPARTMENT OF ENDOCRINOLOGY

Head: Professor Janusz Nauman

Scientific Staff

Piotr Bagiński, M.D.
Zbigniew Bartoszewicz, M.Chem.
Tomasz Bednarczuk, M.D.
Jacek Kiljański, M.D., Ph.D.
Dorota Krzeska, M.D.
Janusz Nauman, M.D., Ph.D., D. Sci., Professor of Medical Sciences
Monika Puzianowska-Kuźnicka, M.D., Ph.D.

Administrative Staff

Maria Falacińska-Bojakowska, secretary

RESEARCH REPORTS

DEPARTMENT OF NEUROPHYSIOLOGY

Head: Professor Mieczysław Pokorski
5 Pawińskiego St., 02-106 Warsaw
Telephones: 668 54 16, 608 65 20
Fax: 668 54 16
E-mail: mpokorski@medres.cmdik.pan.pl

STUDIES ON THE MECHANISMS OF FUNCTION AND INTERACTION OF PERIPHERAL CHEMORECEPTORS

Research team

M. Pokorski, H. Gromysz, W.A. Janczewski, B. Kopczyńska, B. Sokołowska, R. Strosznajder, M. Szereda-Przestaszewska

The studies were concerned with the subcellular mechanisms of carotid body function. The hypothesis was tested that an endogenous reductant, like e.g. vitamin C – ascorbate, if present in the carotid body, could be engaged in its response to hypoxia, which would lead to ascorbate concentration changes in the organ. Such changes would, in turn, be reflected in the level of ascorbyl radical. The carotid bodies excised from anesthetized cats exposed to normoxia, hypoxia, and hyperoxia were used. The technique used was that of the electron spin resonance spectroscopy. The peak-to-peak amplitude of the ascorbyl radical signal is proportional to its concentration and also reflects the level of ascorbate in the tissue. It was found that ascorbate was present in the carotid body and its concentration decreased in hypoxia. These results indicate that ascorbate is consumed in the free radical reactions generated by hypoxia in the carotid body. These studies have been performed in collaboration with the Pomeranian School of Medicine.

Studies on pulmonary chemoreflex induced by an intravenous injection of nicotine in cats have shown that the expiratory apnoea is significantly

reduced by bilateral midcervical vagotomy. Short-lived hyperventilation was mainly due to increased tidal volume which suggests contribution of peripheral chemoreceptors. During stimulated breathing, the peak amplitude of inspiratory muscles increased three times while the activity of rib cage expiratory muscle was depressed, which occurred independent of integrity of the vagal pulmonary feedback.

The scientific training (Dr. Lidia Faff) including tissue culture methodology for chemoreceptor cells from carotid body took place in the John Hopkins University in Baltimore, USA. We are going to set up such a culture system in the Department of Neurophysiology in the near future.

Cooperating unit

Laboratory of the Ultrastructure of the Nervous System, MRC, PASci, Warsaw, Poland (M. Walski).

CONTROL MECHANISMS OF THE RESPIRATORY ACTIVITY

Research team

K. Budzińska, H. Gromysz, W.A. Janczewski, M. Pokorski, B. Sokołowska

We investigated the mechanisms of early respiratory compensation of partial paralysis of the diaphragm due to an unilateral or bilateral section of the C_5 rootlet of the phrenic nerves in anesthetized cats. Compensatory effects were evaluated during quiet breathing and during loading the system with hypercapnic stimulus. The results demonstrated that successive C_5 denervation of the diaphragm caused a decrease in the ipsilateral diaphragmatic electromyogram. Bilateral C_5 section evoked up to a 10% decrease in minute ventilation. Neural mechanisms of compensation of the unilateral and then bilateral partial impairment of the muscle function involve a general increase in the respiratory drive and specifically to the contralateral diaphragm. The ability of the respiratory system to compensate for the partial diaphragmatic paralysis is also preserved during the increased chemical drive.

A previous study on the mechanism of short-term potentiation (STP) of hypoglossal nerve activity of laryngeal origin has shown that arachidonic acid caused an inhibition of this process. Arachidonic acid is known to

activate the protein kinase C (PKC). In the current study, we investigated whether a change in PKC activity can modulate hypoglossal STP. It was found that applied PKC inhibitor did not affect either the amplitude or the duration of hypoglossal STP.

Publications

Gromysz H: Changes in the central respiratory rhythm following pharmacological blockade of the nucleus parabrachialis medialis in the rabbit. *Acta Neurobiol Exp* 1997, 57, 339-343.

Janczewski WA: Muscle relaxation attenuates the reflex response to laryngeal negative pressure. *Respir Physiol* 1997, 107, 219-230.

Pokorski M: Peripheral arterial chemoreceptors: function and mechanisms. *Pneumonol Alergol Pol* 1997, 65, 521-527 (in Polish).

Pokorski M, Gonet B: Hypoxia depletes ascorbate in the cat carotid body. *Respir Physiol* 1997, 107, 213-218.

Pokorski M, Gromysz H: Involvement of the motor trigeminal nucleus in respiratory phase-switching in the cat. *Acta Neurobiol Exp* 1997, 57, 31-39.

Pokorski M, Strosznajder R: ATP activates phospholipase C in the cat carotid body *in vitro*. *J Physiol Pharmacol* 1997, 48, 443-450.

Sokołowska B, Budzińska K, Pokorski M: Diaphragmatic electromyograms and respiratory pattern after unilateral and bilateral partial denervation of the cat diaphragm. *Pneumonol Alergol Pol* 1997, 65, 500-507 (in Polish).

Strosznajder RP: Effect of hypoxia and dopamine on arachidonic acid metabolism in superior cervical ganglion. *Neurochem Res* 1997, 22, 1193-1197.

Szereda-Przestaszewska M, Kopczyńska B: Action of serotonin on the laryngeal airway in anaesthetized cats. *Acta Neurobiol Exp* 1997, 57, 209-216.

LABORATORY OF EXPERIMENTAL PHARMACOLOGY

Head: Associate Professor Paweł Grieb
5 Pawińskiego St., 02-106 Warsaw
Telephone: 608 65 23
Fax: 608 65 27
E-mail: pgrieb@ibb.waw.pl

TREATMENT OF ISCHEMIC STROKE WITH CDP-CHOLINE (CITICOLINE)

Research team
P. Grieb, S. Chrapusta, M. Ryba

A double-blind placebo-controlled study of efficacy of CDP-choline (citicoline) in acute phase of ischemic stroke has been initiated. The drug, 1 gram once daily for 14 consecutive days, is given intravenously. Patients are admitted to the study provided that they are conscious and the therapy may be started with 48 hrs from the onset of symptoms. Primary end-point will be the neurological status at four months (therapy failure defined as score 3 or more in the Rankin scale). The first five patients have been treated.

CLADRIBINE IN THE TREATMENT OF MULTIPLE SCLEROSIS (MS)

Research team
P. Grieb, S. Chrapusta, M. Ryba

The two-year double-blind placebo-controlled crossover study of cladribine (5 mg daily sc. for 5 consecutive days, courses repeated 6 times at monthly intervals) in relapsing-remitting MS has been concluded. Eighty four patients participated in the trial. In the first year, the relapse rate in the group on active treatment was reduced by 73%, compared to the pre-trial relapse rate, while the corresponding relapse rate in the group receiving placebo was reduced by 41%. In the second year, the relapse rate in patients crossovered to placebo was markedly higher than in the previous year, but it still remained 30% below the average pre-study relapse rate.

The corresponding relapse rate in patients crossovered to the active treatment was 43% lower than the average pre-study relapse rate. During the first year of the study, the average EDSS score improved slightly in the actively treated group, while it did not change in the placebo group. The improvement seen in the actively treated group disappeared following crossover to placebo, while the average EDSS score in the group crossovered to active treatment did not change. In general, the very encouraging results of the first year have not been fully reproduced in the second year. It is likely that during the first year the patients in both groups, for psychological reasons, did not report relapses unless they were severe, whereas in the second year they reported less severe relapses as well. In line with this explanation, the requirement for steroid therapy in the group crossovered to the active treatment was reduced by half in the second year of the study, compared to the first year. It is concluded that treatment with cladribine modestly reduces the relapse rate and decreases the severity of relapses. In the second study, 20 MS patients were treated with cladribine (dose 2.1 mg/kg iv. over six months) given in monthly (0.07 mg/kg per day for 5 days), or in weekly intervals. Lymphocyte phenotyping was performed with flow cytometry at 3-month intervals during and after the treatment. Both dosing schedules produced similar reduction in lymphocyte counts, indicating that long exposure of cells to the drug is not a prerequisite of its lymphotoxicity. The reduction of B-cells was the deepest, the number of CD4+ T-cells were also markedly reduced, while CD8+ T-suppressor cell count remained unchanged and the number of NK cells was increased in the post-treatment period.

Cooperating unit

Neurology Clinic, Medical School, Lublin, Poland (Z. Stelmasiak).

FUNCTIONAL ASSESSMENT OF THE IMMUNE SYSTEM IN PATIENTS SUFFERING FROM SUBARACHNOID HEMORRHAGE

Research team

M. Ryba, S. Chrapusta, P. Grieb

It has been found that, compared to healthy blood donors, patients suffering from subarachnoid hemorrhage (SAH) present the signs of depression of the immune system (likely consequential to sympathetic activation, stress, and dexamethasone administration in some cases). However, SAH patiens who developed the "delayed vasospasm" (the most dangerous and often fatal complication of SAH), compared to patients who did not develop this condition, show the indices of immune activation including increased T-lymphocyte helper/suppressor (CD4+/CD8+) ratio and enhanced costimulatory response of T-cell to some proteins of extracellular matrix (eg., collagen IV). The data are in accordance with the hypotheses that the "delayed vasospasm" is immunologically mediated, and that immunosuppressants may prevent its occurrence.

Cooperating units

Neurology Clinic, Medical School, Lublin, Poland (Z. Stelmasiak)
Department of Cytophysiology, Medical Centre of Postgraduate Education, Warsaw, Poland (J. Kawiak)

INDICES OF OXIDATIVE STRESS IN BRAIN TISSUES FOLLOWING REVERSIBLE CIRCULATORY ARREST AND THE EFFECTS OF THE FREE RADICAL SCAVENGER IDEBENONE

Research team

P. Grieb, S. Chrapusta, G. Dębicki, M. Ryba

Rats were subjected to circulatory arrest for 7.5 minutes and resuscitated by external heart massage and artificial ventilation with air. Brains were sampled prior to, and at 15 and 60 min, 24 and 72 h after resuscitation. Conjugated diens (CDB), thiobarbituric acid-reactive material (TBAR), and total and protein-bound reduced thiols (-SH) were assayed in whole brain

homogenates. CDB and TBAR rose approximately 4-fold at 60 min. At the same time, protein– and non-protein–SH dropped by 2/3 and 9/10 of the preischemic level, respectively. Pretreatment with idebenone, a free radical scavenger capable of penetrating the intact blood-brain barrier, markedly attenuated CDB rise, virtually abolished TBAR accumulation, and significantly reduced the drop of protein– and non-protein–SH. The magnitude of –SH depletion observed in the present study was bigger than reported in other models of total or partial brain ischemia, which might have been related to transient increase of cerebral blood flow and oxygen delivery to the brain during resuscitation following cardiac arrest. The data indirectly points out that pretreatment with idebenone reduces the production of free radicals in brain tissues during postischemic reperfusion-reoxygenation.

BIODEGRADABLE POLYMERS CONTAINING NUCLEOSIDES FOR THE TREATMENT OF BRAIN TUMORS

Supported by the State Committee for Scientific Research: grant # 4 05F 02 412

Research team

P. Grieb, M. Ryba

A nucleoside analog (E)-2'-fluoro-methylene-deoxycytidine (FMdC), which is not available commercially, has been synthesized and its chemical structure has been confirmed with proton NMR. Cytotoxic properties of FMdC have been verified by *in vitro* studies on malignant cell lines (murine leukemia L-1210, human leukemia HL-60 and colorectal carcinoma COLO-205) with the use of DNA cell cytometry. The compound (known to be an irreversible inhibitor of rybonucleotide reductase) was found to be very active on these cell lines, with doses 10 mM and larger blocking cell cycle at the G_1 phase and/or causing apoptosis of cells in S and G_2+M phase following 24 hr incubation. An experimental model of rat glioma has been implemented by implanting cultured rat glioma cells (C6 line) into the *nucleus caudatus* of Spraque Dawley rats.

Cooperating unit

Department of Immunology, Transplantation Institute, Medical School, Warsaw, Poland.

CANCER AND BRAIN DISEASE CHARACTERIZATION AND THERAPY ASSESSMENT BY QUANTITATIVE MAGNETIC RESONANCE SPECTROSCOPY

Supported by BIOMED: grant # 1 PL-920432]

Research worker

P. Grieb

With the use of a Siemens Magnetom SP63 system with the spectroscopic option, the proton NMR spectra from 8 ml voxels in parietal cortex of 21 healthy volunteers have been recorded and the ratios of major NMR-visible compounds (N-acetylaspartate – NAA, choline compounds – Cho, and creatine compounds Cr+PCr) were determined. The results were NAA/Cr+PCr=2.52±0.71 (SD) and Cho/Cr+PCr=1.09±0.34 (SD), very close to those reported in a multicenter Siemens trial in 1993. Reproducible phosphorous spectra from human skeletal muscle were recorded during rest and following exercise, and marked decreases in phosphocreatine peak with concomitant increase in inorganic phosphate peak were observed (as expected). Phosphorous spectra from healthy human brains were collected and resolved into the major metabolites, phosphomonoesters (PME), phosphodiesters (PDE), phosphocreatine (PCr), and g, a and b nucleotides, superimposed on a broad peaks assigned to the membrane phospholipids with very short T_2 relaxation times. Acquiring phosphorous spectra from gliomas proved to be very difficult for technical reasons (most patients were unable to remain still for up to 1 hr required to collect the spectrum). However, in a patient with large intractable malignant glioma who was treated with cladribine (2-CdA), good quality spectra were recorded following repeated doses of the drug, and progressive changes were observed, including primarily a gradual disappearance of the broad signal from membrane phospholipids. This result suggests that treatment with 2-CdA may cause changes in membrane properties which can be detected with phosphorous spectroscopy *in vivo*.

Cooperating units

Laboratory of Cellular Physiology, Istitute Superiore di Sanita, Rome, Italy (Project Leader).

Department of Nuclear Medicine and Magnetic Resonance, Medical School, Warsaw, Poland (L. Królicki).

Laboratory of Cytometry, Institute of Drugs, Warsaw, Poland (J. Skierski)

Publications

Grieb P, Ryba M, Sawicki J, Chrapusta SJ: Oral coenzyme Q10 administration prevents the development of ischemic brain lesions in a rabbit model of symptomatic vasospasm. *Acta Neuropathol* 1997, 94, 363-368.

Grieb P: Biomedical applications of Magnetic Resonance Spectroscopy (MRS). *Rez Magn Med* 1997, 5, 5-7 (in Polish).

Robak T, Góra-Tybor J, Krykowski E, Walewski JA, Borawska A, Płużańska A, Potemski P, Hellmann A, Zaucha JM, Konopka L, Ceglarek B, Durżyński T, Sikorska A, Michalak K, Urasiński J, Opalińska J, Dmoszyńska A, Adamczyk-Cioch MB, Kuratowska Z, Dwilewicz-Trojaczek J, Boguradzki P, Dereń M, Maj S, Grieb P: Activity of 2-chlorodeoxyadenosine (cladribine) in 2-hour intravenous infusion in 94 previously treated patients with low grade non-Hodgkin's lymphoma. *Leukemia and Lymphoma* 1997, 26, 99-105.

Robak T, Błasińska-Morawiec M, Krykowski E, Skotnicki AB, Dmoszyńska A, Konopka L, Hellmann A, Hansz J, Komarnicki M, Kotlarek-Haus S, Płużańska A, Urasiński J, Zdziarska B, Kuratowska Z, Dwilewicz-Trojaczek J, Maj S, Grieb P: 2-Chlorodeoxyadenosine in the treatment of lymphoid malignancies – results of the Polish multicentre trials. *Acta Haematol Pol* 1997, 28, Suppl 2, 105-111 (in Polish).

DEPARTMENT OF NEUROCHEMISTRY

Head: Professor Jerzy W. Łazarewicz
5 Pawińskiego St., 02-106 Warsaw
Telephones: 668 54 23, 608 65 28
Fax: 668 54 23
E-mail: neurochemia@ibbrain.ibb.waw.pl

LABORATORY OF PHARMACONEUROCHEMISTRY

ROLE OF DISTURBANCES IN NEURONAL CALCIUM HOMEOSTASIS IN MECHANISMS OF EXCITOTOXIC AND ISCHEMIC BRAIN DAMAGE

Research team

J.W. Łazarewicz, M. Alaraj, W. Gordon-Krajcer, I. Kosińska, D. Makarewicz, H. Nowińska, E. Salińska*, A. Sobczuk, A. Stafiej, A. Ziembowicz, E. Ziemińska.

In vivo microdialysis of the rat dentate gyrus combined with the measurement of ^{45}Ca efflux from the pre-labelled endogenous calcium pool was used to continue studies on NMDA-evoked, calcium-induced calcium release *via* ryanodine receptors. To evaluate the role of dihydropyridine-sensitive voltage-dependent calcium channels (L channels) in the initiation of intracellular calcium mobilisation, we studied the effects of L channel agonist, Bay K 8644, and of L channel blocker, nimodipine on $^{45}Ca^{2+}$ release to dialysate, evoked by 1 mM and 5 mM NMDA, respectively. Since these studies failed to demonstrate any effect of L channel modulators on NMDA-evoked $^{45}Ca^{2+}$ release, we propose tentatively that NMDA receptors/channels, but not L channels, play the main role in this process in the rat dentate gyrus *in vivo*.

Pathological, excessive calcium influx to neurones is supposed to play a key role in the induction of neurodegenerative mechanisms in brain anoxia. Our collaborative studies with the Pavlov Institute of Physiology,

* On the leave of absence for the whole year 1997. A scholarship holder from the Royal Society for the postdoctoral training in the laboratory of Professor S. Rose, the Open University, Milton Keynes, Great Britain.

St. Petersburg, Russia, concern the mechanisms of post-anoxic disturbances in the cellular calcium homeostasis in brain. Slices of the rat olfactory cortex, submitted to *in vitro* superfusion with a control ion-balanced medium with glucose, saturated with O_2, were for 10 min treated with the N_2-saturated medium (*in vitro* anoxia). Changes in intracellular concentration of calcium ions ($[Ca^{2+}]_i$), and in the content of calcium bound to hydrophobic domains in intracellular membranes (Ca_b) were evaluated with spectrofluorimetric calcium probes, fura-2 and chlortetracycline, respectively. A Hitachi F-2000 spectrofluorimeter was utilised, supplemented with a house-made quartz optic fibre attachment. It was found that 10-min anoxia leads to a temporary 2.5-fold increase in $[Ca^{2+}]_i$ with simultaneous decrease in Ca_b by 8%. Reoxygenation resulted in return of $[Ca^{2+}]_i$ and Ca_b to basal levels within 10 min, which was followed during the subsequent 1-hr aerobic incubation by a gradual increase in $[Ca^{2+}]_i$ and Ca_b up to 200% and 120% of basal level, respectively. Both primary and secondary changes in intracellular calcium levels were inhibited by MK-801, a non-competitive NMDA receptor antagonist. Studies on the role of glutamate in the mechanism of post-anoxic disturbances in intracellular calcium homeostasis in the rat brain cortex are in progress.

We continued studies on possible links between excessive influx of calcium to neurones evoked by NMDA receptor stimulation and expression of changes in neurones resembling hallmarks of Alzheimer pathology. Our experiments were aimed at detecting immunoreactivities of abnormal epitopes of tau protein in the rat CA1 region of hippocampus after stereotaxic NMDA microinjection. Immunoblotting of proteins of the neurofilament fractions from the rat CA1 isolated 3 days after microinjection of 5 nmoles of NMDA demonstrated immunoreactivities with antibodies specific to phosphorylation-dependent tau epitopes PHF 1 and AT 8, comparable to observed in human foetal and Alzheimer brains, whereas in control rat hippocampus it was undetectable. Immunoreactivity with Tau 14, antibody specific to phosphorylation-independent tau epitope, was comparable to control rat brain. Studies on the mechanisms of tau pathology related to excitotoxic damage to hippocampal neurones are in progress.

There are indications that insulin plays a trophic role in the brain, and that modulation of $[Ca^{2+}]_i$ may be partially responsible for the central effects of insulin. In preparation to scheduled studies on trophic effects of

insulin on brain neurones, experiments were performed on the effect of insulin (2 units/kg, i.p.) on antiepileptic activity of carbamazepine against a maximal electroshock in mice. It was found that insulin increases ED50 of carbamazepine from 16.2 to 41.3 mg/kg. This decrease of antiepileptic activity of carbamazepine may be explained by the observed decrease of its concentration in brain and serum. These effects, probably unrelated to central effects of insulin, may have practical implication in antiepileptic treatment of diabetic patients.

Cooperating units

Department of Biophysics, High School of Agriculture, Warsaw, Poland.
Institute of Anatomy and Cell Biology, University of Göteborg, Sweden.
Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia.
New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York, USA.

MODULATION OF EICOSANOID RELEASE IN THE HIPPOCAMPUS *IN VIVO* BY NMDA RECEPTORS

Supported by the State Committee for Scientific Research: grant # 4P05A 042 08

Research team

J.W. Łazarewicz, I. Kosińska, A. Stafiej, A. Ziembowicz, E. Ziemińska

To assess the role of intracellular calcium stores in the mechanism of NMDA receptor mediated arachidonic acid release and eicosanoid production in brain neurones, we characterised the NMDA-evoked release of $^{45}Ca^{2+}$ in the rat hippocampus *in vivo*. Using pharmacological tools and microdialysis of the dentate gyrus it was demonstrated that this effect reflects a phenomenon of calcium release from the endoplasmic reticulum stores *via* ryanodine receptors. These data was utilised in studies on the role of ryanodine-sensitive calcium pool in the induction of NMDA-evoked prostaglandin D_2 release in the rat hippocampus. We also continue the studies on the cross-talk between two NMDA receptor mediated signal transduction mechanisms, namely on the role of nitric oxide in modulation of arachidonate metabolism *via* cyclo-oxygenase pathway in the hippocampus.

EFFECT OF ISCHEMIA AND EXCITATORY AMINO ACID RECEPTORS ON EXPRESSION AND METABOLISM OF BETA AMYLOID PRECURSOR PROTEIN (β-APP) IN BRAIN (INTERRELATIONSHIP)

Supported by the State Committee for Scientific Research: grant # 4P05A 059 08

Research team

W. Gordon-Krajcer, H. Nowińska

The effect of excitatory amino acids, glutamate and NMDA, on processing and accumulation of amyloid beta protein (βA) and its precursor β-APP was studied in the rat hippocampus CA1 *in vivo*. One ml of 2 μM or 5 mM NMDA, or 1 mM or 2 mM glutamate were applied *via* stereotaxic microinjection into the rat hippocampus CA1. In another experiment, MK-801 a non-competitive antagonist of NMDA receptors, was applied i.p. 30 min before intracerebral injections of NMDA or glutamate. Material (hippocampal CA1) was collected at a time 0 and 12 h, 24 h, 48 h, 3, 7 and 14 days after microinjections. Homogenates of this material were electrophoresed and electroblotted, then accumulation and metabolism of β-APP was studied utilising specific antibodies, against particular fragments of this protein. It was found that microinjection of NMDA, and to lesser extent of glutamate, results in the accumulation of βA (597-613 amino acid region) and of other fragments of β-APP and leads to altered processing of this protein. The immunoreactivities with antibodies to all β-APP domains were proportional to a dose of injected agonist and were more pronounced for C-terminal (cytoplasmatic) fragments of β-APP. The most pronounced immunoreactivity with antibodies against different β-APP fragments and against βA was observed 3 days after application of the excitotoxins. MK-801 almost completely cancelled this effect. These results indicate that stimulation of excitatory amino acid receptors, in particular of NMDA, leads to accumulation of different β-APP and βA fragments, which points to accelerated processing and disturbed metabolism of this protein.

LABORATORY OF MOLECULAR NEUROPATHOLOGY

POSTTRANSLATIONAL PROTEIN MODIFICATIONS IN THE RESPONSE TO CEREBRAL ISCHEMIA

Research team

K. Domańska-Janik, B. Zabłocka, T. Zalewska

Considering postsynaptic densities (PSD) as a functionally active zone intimately connected with an excitatory synaptic transmission, we have evaluated PSD-connected protein modifications after global, cerebral ischemia in rats. Attention was paid to the enzymes previously shown to be directly affected by ischemia, namely, a protein kinase C (PKC), a calcium, calmodulin-dependent kinase II (CaMK II), the neutral, calcium-activated proteases (calpains) were immunodetected in different subcellular fractions in control animals and compared to those after ischemia insult. The isolation of PSD by means of differential centrifugation was found to be an extremely low efficiency procedure (1 mg protein per 10 mg of tissue) but resulted in the high purity fraction as judged by electron microscopy and biochemical markers. Ischemia has affected all of the evaluated proteins but to differing extents and dynamics. For example, the ischemic translocation of CaMK II to PSD was so rapid that it has been almost accomplished in an interval between decapitation and homogenisation – less than 1 min. Only with *in situ* tissue freezing was it possible to preserve relatively low, control CaMK II level in PSD.

In contrast, the changes in calpains have been observed after 15 min ischemia and consisted in down-regulation of an active, 76 kDa subunit of μ isoform. This finding corresponds with previously reported decrease of μ calpain proteolytic activity in the bulk of cerebral membranes.

Completely new finding was the great enhancement of an intrinsic phosphorylation of the unidentified 85-90 kDa protein after denaturation-renaturation procedure of immunobloted PSD proteins. The related phosphokinase was much more active in PSD than in any other brain fraction (homogenate or P2) and has been greatly potentiated by ischemia. Identification of the kinase and its substrate will be a matter of further study.

We have evaluated immunochemically (Western blot) the 8 PKC isoenzymes belonging to classical as well as a new and atypical subfamilies. All of these isoforms, except of μ PKC, were present in PSD, however, compared to the other subcellular fractions (P2, synaptosomes, synaptic membranes or highly purified presynaptic membranes) the immunoreactions were not very strong in control PSD. After brain ischemia the classical, calcium-activated PKC isoenzymes (α, β and γ) were extensively translocated toward plasma membranes and particularly to PSD structure. A particularly strong reaction from the new isoforms of δ and ε PKC types was noticed. Interestingly, the activation of this last isoenzyme, together with γ type of phospholipase C and phosphoinositide 3-kinase was reported in several growth factors signalling pathways. The existence of the element of this pathway in the structure where an extensive synaptic transmission is localised, would have important functional implications.

MOLECULAR BASIS OF NEURONAL OR GLIAL DIFFERENTIATION IN MEDULLOBLASTOMA CELLS LINE

Supported partially by INSERM grant for East/West Cooperation: # GWO11A

Research worker

L. Bużańska

The human neuroblastoma cell line (DEV) has been used to initiate analyses of glial and neuronal development under influence of several genes encoding for the differentiation-determining transcriptional factors. In collaboration with Prof. B. Zalc from INSERM U-134, Paris, the molecular procedure for this experiment has been developed. This included:

a) Molecular construction of plasmid DNAs for genes of interest: Mash 1, Neurogenin 1, Neurogenin 2, Neurogenin 3, Neuro D, Lin 26 and TTK 69/88;
b) Establishment of cell growth conditions;
c) Setting up transfection procedure using GFP (Green Fluorescent Protein) as a reporter;
d) Immunochemical staining for differentiation marker proteins: MAP-2, GFAP and GalC.

The culture was successfully transported to our laboratory where transfection experiments are continuing and their effect on the expression of marker proteins is extensively evaluated.

MOLECULAR MECHANISMS OF APOPTOSIS/NECROSIS INDUCTION IN NEUROBLASTOMA (N-2A) CELL LINE

Research team

K. Domańska-Janik, L. Bużańska

The murine neuroblastoma cell line N-2A growing in the conditions of optimised serum concentration or induced to differentiate by deprivation of serum was prone to apoptosis by unspecific kinase inhibitor – staurosporine or by cytotoxic agents like glutamate. Apoptosis was evaluated by TUNEL together with Annexin-V-FLUOS and propidium iodide for discrimination between necrotic and apoptotic cells. Experiments have been designed to evaluate the mechanism of protein kinase C influence (protective vs. destructive) in execution of neuronal cell death. Particularly the following cross-links are of interest:

a) activation/phosphorylation of proteins connected with MAPK (Mitogen Activated Protein Kinase) signalling pathway;
b) The bcl2 gene expression.

Cooperating unit

Laboratory of Cellular, Molecular and Clinical Neurobiology, Hôpital Salpétriére, Paris, France (B. Zalc).

INTERACTIONS OF PROTEIN KINASE C AND CALPAIN UNDER BRAIN ISCHEMIA

Supported by the State Committee for Scientific Research: grant # 4P05A 026 08

Research team

T. Zalewska, T. Czachmańska, K. Domańska-Janik, B. Zabłocka, H. Zając

Excessive activation of calpains due to postischemic disruption of calcium homeostasis may be a crucial factor in calcium-mediated neuronal

degeneration. In order to clarify the role of calpain in ischemic damage, we have studied correlations among calpain activation, its subcellular distribution and degree of proteolysis of specific calpain substrates in the course of ischemia and recovery. Ischemia was induced by bilateral ligation of common carotid arteries in gerbils for 5 minutes. Our immunochemical studies show that transient ischemia leads to the rapid translocation of the cytosolic calpains toward the particular fraction with concomitant increase of the level of active (post-autolytic) μ-calpain isoform. These results fit well with the generally accepted mechanism of calpain activation at the plasma membranes, where the preferential substrates are located. The observed changes were specific only to the hippocampus, the most sensitive structure to the delayed neuronal damage. Our suggestion of the initial post-ischemic activation of calpain function deduced from its characteristic behaviour was additionally evidenced by proteolysis of the specific substrates: cytoskeletal protein – fodrin and regulatory enzyme, protein kinase C. Transient ischemia leads to the substantial reduction of both investigated calpain substrates only in hippocampus. No evidence of calpain autolysis and calpain-mediated substrates degradation was detected in the frontal brain cortex. In addition, the protective effect of calpains' inhibitor on PKC-dependent protein phosphorylation in hippocampus was demonstrated. The selectivity of the observed changes of calpain activity suggests the involvement of the investigated protease in the cascade of events leading to delayed neuronal degeneration.

LABORATORY OF PATHOBIOCHEMISTRY OF CNS

BIOCHEMICAL AND MORPHOLOGICAL INVESTIGATIONS ON MYELIN OF THE CENTRAL NERVOUS SYSTEM IN LEAD TOXICITY

Research team

U. Rafałowska, B. Dąbrowska-Bouta, A. Lenkiewicz, G. Sułkowski, L. Strużyńska

The aim of the investigations was to study whether prolonged consumption of lead (Pb)-enriched water, that imitates environmental exposure,

affects the structure of myelin in the central nervous system (CNS) in rats and whether observed destructions are reflected by biochemical changes. Up to now there has been no credible information concerning the effect of lead intoxication on the structure of myelin in CNS.

Our results indicated that during chronic Pb intoxication Pb level in myelin fraction increases significantly. The electron microscopic studies have shown that myelin from brains of Pb-treated rats is morphologically changed. The myelin in control experiments is built up of order layers, whereas in Pb-intoxicated rats this order of myelin was destroyed in the majority of preparations. Myelin sheaths were irregular and distant from one another. Observed morphological disturbances were reflected by the changes in myelin membrane fluidity measured by spectrofluorometric methods. We have observed the differences in the anisotropy of spin label – difenylohexatrien (DPH) incorporated into myelin obtained from Pb-treated rats. Chronic Pb treatment of rats significantly increased myelin membrane fluidity as indicated by the decrease of DPH anisotropy. We also examined the effect of Pb on myelin brain membranes using electron paramagnetic resonance (EPR) technique with fatty acid spin label. The spectral parameter measured was the order parameter S, an index of membrane fluidity. The values of S obtained from EPR spectra of myelin membranes were significantly lower in Pb-treated rats than in controls. This finding indicates that membrane fluidity is higher in Pb-treated rats.

In CNS, myelin in generated by oligodendrocytes. Prolonged Pb toxicity caused significant changes in morphological structure of oligodendrocytes. Comparing to the controls, we detected different dispersion of chromatin in the nucleus. Heterochromatin is more electron lucent and dispersed in the whole nucleus. In the cytoplasm of oligodendrocytes irregular structure of Golgi system and associated with it numerous vesicles was observed. Rough endoplasmic reticulum was also changed and comparing to control pictures was more destructed. In the cytoplasm, more free particles of ribosomes were seen. It is possible that changes in oligodendrocytes caused by Pb can be the first cause of destruction of myelin sheath.

Observed destruction in morphology of myelin and in its fluidity are partially reflected by biochemical changes. Protein and total phospholipid content in intoxicated myelin did not change as compared to the control values. Also the SDS-PAGE electrophoresis did not show significant

differences between myelin obtained from brains of control and Pb-treated rats in the pattern of total protein myelin. However, we observed significant increase in phosphatidylethanolamine, decrease in the level of protein-SH groups and an increase in the activity of 2'3'-cyclic nucleotide 3'-phosphodiesterase (CNPase). But Pb itself did not induce peroxidation in the myelin fraction. Also it did not accelerate peroxidation produced by iron in brain myelin. It seems that the changes in brain myelin produced by chronic intoxication with Pb in rats can be caused not only by the changes of oligodendrocytes, but also by the disturbances in the activity of CNPase, decrease in the level of protein-SH groups with simultaneous increase in the level of phosphatidylethanolamine, what may account for observed differences in fluidity of myelin membrane. It is also certain that peroxidation process under condition of Pb toxicity can not be the cause of changes in structure of brain myelin.

Cooperating units

Institute of Biophysics, University of Łódź, Łódź, Poland (G. Bartosz).
Laboratory of Electron Microscopy, MRC PASci, Warsaw, Poland (M. Walski)
Department of Neurotoxicology, MRC PASci, Warsaw, Poland.
Department of Biochemistry, University of Oklahoma, USA.

Publications

Domańska-Janik K, Sypecka J, Taraszewska A: Immunohistochemical study of myelin-specific proteins in pt rabbits. *Folia Neuropathol* 1997, 35, 1-7.

Domańska-Janik K, Zalewska T: Transient increase of calcium/calmodulin-dependent kinase II (CaMKII) in its autonomous state under brain ischemia. In: *Neurochemistry: Cellular, molecular and clinical aspects. The Proceedings of the 11th General Meeting of the European Society for Neurochemistry,* June 15-20, 1996, Groningen, The Netherlands. Eds: A.W. Teelken, J. Korf. Plenum Press, New York, London, Washington DC, Boston, 1997, pp. 369-373.

Łazarewicz JW, Gadamski R, Parsons CG, Danysz W: Protection against post-ischaemic neuronal loss in gerbil hippocampal CA1 by glycineB and AMPA antagonists. *J Neural Transm* 1997, 104, 1249-1254.

Łazarewicz JW, Rybkowski W, Salińska E, Gordon-Krajcer W, Ziemińska E, Ziembowicz A, Puka-Sundvall M, Hagberg H: Generation of Ca2+ signal in NMDA receptors of rat and rabbit hippocampus in vivo: Ca2+ influx and mobilisation. In: *Neurochemistry: Cellular, molecular and clinical aspects. The Proceedings of the 11th General Meeting of the European Society for Neurochemistry,* June 15-20, 1996, Groningen, The Netherlands. Eds: A.W. Teelken, J. Korf. Plenum Press, New York, London, Washington DC, Boston, 1997, pp. 361-367.

Strużyńska L, Dąbrowska-Bouta B, Rafałowska U: Acute lead toxicity and energy metabolism in rat brain synaptosomes. *Acta Neurobiol Exp* 1997, 57, 275-281.

Strużyńska L, Walski M, Gadamski R, Dąbrowska-Bouta B, Rafałowska U: Lead-induced abnormalities in blood-brain barrier permeability in experimental chronic toxicity. *Mol Chem Neuropathol* 1997, 31, 207-224.

Sypecka J, Domańska-Janik K: Major myelin specific genes expression in pt PLP mutant rabbit. In: *Neurochemistry: Cellular, molecular and clinical aspects. The Proceedings of the 11th General Meeting of the European Society for Neurochemistry,* June 15-20, 1996, Groningen, The Netherlands. Eds: A.W. Teelken, J. Korf. Plenum Press, New York, London, Washington DC, Boston, 1997, pp. 233-237.

Waśkiewicz J, Lenkiewicz A, Rafałowska U: Lead as an inductor of changes in the citrate transport to rat brain synaptic mitochondria. *Folia Neuropathol* 1997, 35, 191-195.

Zalewska T, Zabłocka B, Saido TC, Domańska-Janik K: On the mechanism of calpain activation under ischemia. In: *Neurochemistry: Cellular, molecular and clinical aspects. The Proceedings of the 11th General Meeting of the European Society for Neurochemistry,* June 15-20, 1996, Groningen, The Netherlands. Eds: A.W. Teelken, J. Korf. Plenum Press, New York, London, Washington DC, Boston, 1997, pp. 407-414.

DEPARTMENT OF CELLULAR SIGNALLING

Head: Professor Joanna Strosznajder
5 Pawińskiego St., 02-106 Warsaw
Telephones: 668 53 23, 608 64 14
Fax: 668 52 23
E-mail: joannas@ibb.waw.pl

FURTHER STUDIES ON MOLECULAR MECHANISM OF BRAIN AGING AND ALZHEIMER'S DISEASE AND ON DISTURBANCES OF SIGNAL TRANSDUCTION DURING REPERFUSION AFTER BRAIN ISCHEMIA

Research team

J. Strosznajder, M. Chalimoniuk, B. Głód, M. Łałowski, M. Samochocki, A. Zambrzycka

In 1997 the studies were continued on molecular processes involved in pathomechanism of brain aging and reperfusion injury after global ischemia. It has been known that reperfusion of previously ischemic brain is associated with exacerbation of cellular injury. Reperfusion occasionally potentiates release of intracellular enzymes, influx of Ca, breakdown of membrane phospholipids, accumulation of amyloid precursor protein and apo-lipoprotein E.

In this study, the effect of reperfusion injury on the activity of brain cortex membrane bound and cytosolic phospholipase C (cPLC) and cytosolic phospholipase A_2 (cPLA$_2$) was investigated. The recently indentified 85-Kda cPLA$_2$ is a receptor-regulated phospholipase A_2 (PLA$_2$) that associates with the phospholipid substrate in the presence of Ca^{2+} in activated cells and selectively liberates arachidonic acid (AA) from cellular membranes. Upon phosphorylation on Ser-505 by proline-directed kinases, the enzymatic activity of cPLA$_2$ is significantly enhanced. We studied cPLA$_2$ in the brain following transient global ischemia. Brain ischemia in gerbils (*Meriones unquiculatus*) was induced for 5 min and then the brain was perfused for 15 min, 2 h and 7 days. During short reperfusion time 15 min after ischemia, significant activation of cPLC and cPLA$_2$ involved in degradation of phosphatidyloinositol (PI) into diacylglycerol (DAG) and AA was observed. The highest activity of cPLC was found 2 h after ischemia. In brain cortex,

membrane PI was degraded mainly to DAG by membrane bound phospholipase C (PLC) which was quickly converted to phosphatidic acid (PA) by DAG-kinase and subsequently used for phospholipid biosynthesis. After prolonged reperfusion time of 7 days after ischemia, the level of DAG radioactivity in brain cortex and hippocampus membranes was again enhanced. Determination of ^{3}H inositol PI degradation indicated higher activity of cPLC form of PI-PLC till 7 days after short forebrain ischemia. Using the other labelled phospholipids, phosphatidylethanolamine (PE) or phosphatidylcholine (PC), very low activity of the enzymes degrading these substrates in brain cortex was found. Moreover, reperfusion injury had an insignificant effect on the activity of these enzymes. Brain reperfusion injury remained without effect on phosphatidylinositolo 4,5 bisphosphate (PIP_2)-PLC in brain cortex. It seems, however, that PIP_2 is degraded during short reperfusion time in hippocampus by PLA_2. We conclude that mainly cytosolic phosphoinositides cPLC and $cPLA_2$ may contribute to pathophysiology of delayed neuronal death following cerebral ischemia and that specific inhibitors of PI-PLC and PLA_2 may offer good therapeutic strategies to protect the brain from damage triggered by ischemia.

The product of PLA_2 action, AA plays an important role in modulation of membrane function and signal transduction as well as in the control of microcirculation. AA in brain must be synthesized from plasma-derived precursor fatty acid or taken up intact from plasma where it is normally bound to albumin. In our previous studies we demonstrated that AA is able to cross the blood-retina and blood-brain barrier (BBB). However, till now it was not known if BBB for AA is altered significantly during reperfusion after ischemia. Cardiac arrest in rats was chosen because it mimics the human condition of global ischemia. It was found that permeability-surface area product of [^{14}C] arachidonate significantly increases in cortex and striatum 1 and 6 hours after cardiac arrest. Additionally, significant increase of conjugated diene and carbonyl group levels and microsomal lysophosphatidylcholine acyltransferase activity were observed in cerebral tissue. These studies suggested that reactive oxyradicals were generated during the early phase of ischemic reperfusion process and that membrane repair mechanisms were at the same time activated. We also demonstrated ultrastructural evidence suggesting barrier leakiness predominantly in the cortex but almost absent in the retina microvessels. Our results indicate

that ischemia-reperfusion does affect an influx through BBB into regional structures of rat brain of arachidonate, a metabolic substrate and lipid mediator, rapidly incorporated into microcapillary and brain lipids. The studies were carried out in cooperation with dr. M. Alberghina from the Department of Biochemistry, University of Catania, Italy.

In continuation of our studies on regulation of Ca^{2+} homeostasis in aged brain we have confirmed significantly higher activity of Ca^{2+} ATP-ase of endoplasmic reticulum (SERCA) in aged brain comparing to adult brain. There was no difference between aged and adult brain in Ca^{2+} influx through voltage dependent Ca^{2+} channel and NMDA receptor operated Ca^{2+} channel.

In cooperation with the Department of Neuropathology, MRC, PASci in Warsaw, the frequency of APOE-e4 allele in persons with Alzheimer's disease (AD) was analysed. Among AD and multi infarct dementia (MID) patients, APOE-e4, e3, e2 allele frequency was 0.333, 0.650 and 0.017, respectively.

The further study on cytokine hepatin binding growth associated molecule (HB-GAM), performed in cooperation with dr. T. Wiśniewski from the Institute of Pathology, University of New York, indicated that HB-GAM is present not only in AD brain but also in other types of cerebral amyloidoses including prion amyloidosis, but is absent in control aged brain.

In 1997 the projects of 4 grants were realized. Two projects were finished and the other two are continuing in 1998.

THE ROLE OF CYTOKINE HB-GAM IN FIBRILLOGENESIS OF AMYLOID β IN AGED BRAIN AND ALZHEIMER DISEASE AND ITS ROLE AS A MARKER OF NEURONAL DEGENERATION

Prof. Joanna Strosznajder, promotor grant

Supported by the State Committee for Scientific Research: grant # 4 P05A 020 11

AMYLOID β PEPTIDES IN NORMAL AND DISEASED STATE

Dr. Maciej M. Lalowski

Supported by the Sandoz Foundation for Gerontological Research
Regional Committee for Europe

THE ROLE OF PHOSPHORYLATION–DEPHOSPHORYLATION PROCESSES IN MODULATION OF NITRIC OXIDE SYNTHASE ACTIVITY DURING REPERFUSION AFTER SHORT FOREBRAIN ISCHEMIA

Prof. Joanna Strosznajder, promotor grant

Supported by the State Committee for Scientific Research: grant # 4 P05A 070 12

MOLECULAR MECHANISM OF BIOCHEMICAL TRANSFORMATION OF NONTOXIC AMYLOID b TO TOXIC FORM AND ITS ROLE IN SIGNAL TRANSDUCTION IN BRAIN

Prof. Joanna Strosznajder

Supported by the State Committee for Scientific Research: grant # 4 P05A 073 10

The scope of the above grant projects converge with the statutory project. Accordingly, the progress of research within the projects and the statutory project have been described collectively (see previous section).

Cooperating units

Department of Biochemistry, University of Catania, Italy (M. Alberghina).
Neurological Department, Medical School, Warsaw, Poland.
Institute of Pathology, University of New York, N. York, USA (T. Wiśniewski).

Publications

Głód B: Ion exclusion chromatography: parameters influencing retention. *Neurochem Res*, 1997, 22, 1237-1248.

Głód B: Principles and applications of ion exclusion chromatography. *Acta Chromatographica* 1997, 7, 72-87.

Głód B: Retention of acidic aromatic compounds in ion exclusion chromatographic separations. *J Liq Chrom & Rel Technol*, 1997, 20, 1005-1013.

Głód BK, Perez G, Tomlinson AAG: Use of mordenite columns in ion-exclusion chromatography. *J Chromatography*, 1997, 760, 292-294.

Strosznajder J: Regulation of phosphatidylethanolamine degradation by enzyme(s) of subcellular fractions from cerebral cortex. *Neurochem Res*, 1997, 22, 1199-1204.

DEPARTMENT OF NEUROTOXICOLOGY

Head: Professor Jan Albrecht
5 Pawińskiego St., 02-106 Warsaw
Telephone: 668 53 23
E-mail: jalb@cmdik.pan.pl

NEUROACTIVE AND NONNEUROACTIVE AMINO ACIDS IN AMMONIA NEUROTOXICITY

Research team

J. Albrecht, H.D. Borkowska, M. Dolińska, W. Hilgier, M. Zielińska

A number of effects associated with acute liver failure (ALF) in rat in the thioacetamide (TAA) model were evaluated. TAA treatment in a variant leading to clinical symptoms of hepatic encephalopathy (HE) resulting from 3 TAA administrations at 24h intervals, increased the *in vitro* uptake to synaptosomes of α-ketoglutarate (α-KG), which is an astrocytic precursor of neurotransmitter glutamate. This effect may reflect a compensatory response of glutamatergic terminals to decreased astrocytic production of α-KG. HE in this model also increased potassium-induced, calcium-dependent release of newly taken up radiolabelled dopamine from cerebrocortical and striatal nerve endings. This result extends earlier observations that HE alters the modulation of dopaminergic tone by generic (high potassium) or specific (glutamate) stimuli. The same variant of TAA model served to characterise retinal changes associated with ALF. Morphological and ultrastructural changes were confined to retinal Müller glia, which in addition showed decreased glial fibrillary acidic protein and increased glutamine synthetase immunoreactivity. These changes render the model suitable for detailed investigations on the pathomechanisms of hepatic retinopathy.

Rats which received two TAA administrations, showed 21 days later no overt biochemical or pathophysiological deficits typical of HE. HPLC analysis revealed no changes in blood amino acids, but increased levels of most of the neuroactive and nonneuroactive amino acids in brain of these animals. In microdialysates of striatum, increased basal levels of excitatory amino acids (EAA), glutamate, aspartate, and decreased levels of inhibitory amino

acids (IAA), GABA, taurine, were noted. Stimulation *in vivo* with high potassium or N-methyl-D-aspartate produced a dramatic increase of extrastriatal taurine level in TAA-treated animals, indicating increased osmosensory response. The prevalence of EAA in the extrasynaptic space was consistent with morphological manifestations of excitotoxic nerve cell damage and activation of astrocytes. The observations point to durable subclinical HE in animals treated twice with TAA.

Acute ammonia treatment *in vivo* and *in vitro* was found to inhibit the synthesis of a neuroprotectant kynurenic acid (KYNA) from kynurenine in rat cerebral cortical slices. This result suggests that impaired neuroprotection by KYNA may aggravate the glutamate receptor-mediated component of ammonia neurotoxicity.

L-arginine (Arg) is a precursor of neuroactive compounds (nitric oxide, neurotransmitter amino acids). Analysis of kinetic of Arg uptake and of the effects of the potential competitors of the uptake in rat cerebral mitochondria revealed both similarities to, and discrepancies with the cell membrane $\gamma+$ system, the most profound among the latter being lack of competition by glutamate and glutamine.

Cooperating units

Paul Flechsig Institute for Brain Research, University of Leipzig, Leipzig, Germany (A. Reichenbach, H. Kuhrt).

Department of Physiology, University of Leicester, Leicester, UK (O. Law).

Department of Pharmacology and Toxicology, Medical School, Lublin, Poland (W.A. Turski, E.M. Urbańska, T. Saran, T. Kocki).

Tampere Brain Research Center, University of Tampere, Tampere, Finland (S.S. Oja, S. Saransaari).

Publications

Albrecht J, Waśkiewicz J, Dolińska M, Rafałowska U: Synaptosomal uptake of alpha-ketoglutarate and glutamine in thioacetamide-induced hepatic encephalopathy in rats. *Metabolic Brain Dis* 1997, 12, 281-286.

Borkowska HD, Oja SS, Saransaari P, Albrecht J: Release of [3H]dopamine from striatal and cerebral cortical slices from rats with thioacetamide--induced hepatic encephalopathy: different responses to stimulation by potassium ions and agonists of ionotropic glutamate receptors. *Neurochem Res* 1997, 22, 101-106.

Dolińska M, Albrecht J: Glutamate uptake is inhibited by L-arginine in mitochondria isolated from rat cerebellum. *NeuroReport* 1997, 8, 2365--2368.

Faff L, Reichenbach A, Albrecht J: Two modes of stimulation by ammonia of taurine release from cultured rabbit Müller cells. *Neurochem Int* 1997, 31, 301-305.

Saransaari P, Oja SS, Borkowska HD, Kostinaho J, Hilgier W, Albrecht J: Effects of thioacetamide-induced hepatic failure on the N-methyl-D--aspartate receptor complex in the rat cerebral cortex, striatum and hippocampus: binding of different ligands and expression of receptor subunit mRNA's. *Mol Chem Neuropathol* 1997, 32, 179-193.

Szumańska G, Albrecht J: Lectin histochemistry of the rat brain following thioacetamide-induced hepatic failure. *Mol Chem Neuropathol* 1997, 32, 163-177.

DEPARTMENT OF NEUROPATHOLOGY
Head: Professor Andrzej Kapuściński
5 Pawińskiego St., 02-106 Warsaw
Telephones: 668 53 69, 608 65 35
E-mail: kapuscinski@ibbrain.ibb.waw.pl

FURTHER STUDIES ON PATHOGENIC MECHANISMS OF POSTISCHEMIC ENCEPHALOPATHY WITH PARTICULAR EMPHASIS TO LATE DISTURBANCES OF BLOOD-BRAIN BARRIER

Research team

A. Kapuściński, D. Dziewulska, R. Gadamski, S. Januszewski, E. Kida, E. Koźniewska, E. Matyja, M.J. Mossakowski, R. Ostrowski, T. Pańkowska, P. Piotrowski, R. Pluta, G. Szumańska, M. Śmiałek, A. Taraszewska, H. Weinrauder, K. Wierzbicka, I. Zelman

In 1997 investigations have been continued on pathogenic mechanisms of postischemic encephalopathy by means of several experimental models. In the model of ischemic hypoxia, after injection of endothelin 1 (ET-1) and endothelin 3 (ET-3) into the rat lateral cerebral ventricle, metabolic disturbances manifested as lactate acidosis and decrease of ATP in brain were more profound after ET-1 as compared to ET-3. Therapeutic application of Coenzyme Q_{10} (Q_{10}) effected in more rapid normalization of lactate and ATP levels after ET-3 as compared to ET-1. Morphological brain examination after injection of ET-1 revealed foci of cellular loss in cerebral cortex with shrunken dark neurons, and glycogen deposits in glial cells and some of the surrounding blood vessels. Aplication of Q_{10} significantly decreased cellular pathology. In two experimental models of pathochemical and mechanical focal cerebral ischemia, changes in distribution of four different glucoconjugate receptors in neurons and glial cells of the rat brain have been evaluated. Studies are in progress and the results are summarized as the preliminary report. Lectin histochemistry of chosen glucoconjugate receptors was applied also in gerbils after 3 and 4 min of brain ischemia. Alterations in localization of glucoconjugates have been shown in neocortex, hippocampus and corpus callosum after ischemic insult. In collaboration with the Laboratory of Ultrastructure of the Central

Nervous System, injuries of the cortical vessels after photochemical rat brain ischemia have been evaluated by means of transmission and scanning electron microscopy. In collaboration with the Department of Neurochemistry of our Institute and the Department of Pharmacology in Frankfurt, protective effect of glycine$_B$ antagonists and AMPA on postischemic loss of CA1 neurons in hippocampus was evaluated in gerbils after 3 min bilateral carotid ligation. In collaboration with the Laboratories of Neurochemistry and Regulation of Transcription Processes of the M. Nencki Institute of Experimental Biology, the role of neurotrophic factors and cytokines in the central nervous system in ischemic conditions have been studied. In the model of global cerebral ischemia in rats, localization and immunoreactivity of subunit p 65 NF kappa, nerve growth factor (NGF), its receptor TrkA, Interleukin-1 (IL-1) beta and astrocytic marker GFAP in free swimming brain patches cut in cryostat have been evaluated. After ischemia, induction of astrocytic immunoreactivity of the above substances was observed. Astrocytic induction of IL-1 beta was observed during the first three days after ischemia preceding stimulation of NGF and TrkA in astrocytes. The degree of degeneration of CA1 pyramidal neurons correlated with the degree of astrocytic immunoreactivity of the substances studied. In the condition of brain ischemia, astrocytic NF kappa B participates in the regulation of NGF expression through IL beta in astrocytes. Besides intensive production of NGF, neurodegenerative process progressed, what can be explained by hyperproduction of IL-1 beta in astrocytes. Experimental model of clinical death in rats was modified by permanent ligation of the common carotid arteries preceding cardiac arrest. Morphological studies in light and electron microscope after 2, 7 and 12 months of survival after resuscitation have been performed. Investigations are in progress and the preliminary results have been published. The reactivity of cerebral vessels to papaverine (PP), acethylcholine (ACh) and the methyl ester of NG-nitro-L-arginine (NO synthase inhibitor) was also studied during reperfusion after 30 min occlusion of the medial cerebral artery. The results showed no reactivity of the vessels to ACh with an increased reactivity to PP. Ultrastructural features of astrocytes in organotypic cultures of rat hippocampus exposed to different excitotoxic aminoacids and anoxia showed phagocytic response. The results confirmed phagocytic role of astroglia after injury of neurons in different pathogenic conditions.

Accumulation of beta-amyloid in rat brain after resuscitation from 10-min cardiac arrest was evaluated up to the 7th day of survival. Immunohistochemical methods revealed protein amyloid precursor (APP) as extracellular and surrounding vessels deposits in the majority of brain regions. Studies with APP were supplemented by apolipoprotein E, J and A-1. The results have shown an increased permeability of the blood-brain barrier in this experimental model. They also confirmed the notion that APP could be the main risk factor in cerebral ischemia and Alzheimer's disease.

Investigations conducted beyond the statutory program relate to methodology of tissue culture, diagnostics of brain tumors and case reports.

Cooperating units

Institute of Brain Research, Russian Academy of Medical Sciences, Moscow, Russia (I. Victorov, I. Barskov).

New York State Institute for Basic Research in Developmental Disabilities, New York, USA (A.S. Lossinsky, K. Wiśniewski, H. Wiśniewski).

Department of Pharmacology, Merz+Co, Frankfurt/Main, Germany (W. Danysz).

Department of Neurochemistry, Medical Research Centre, PASci, Warsaw, Poland (J. Łazarewicz).

Laboratory of Neurochemistry of the M. Nencki Institute of Experimental Biology, PASci, Warsaw, Poland (B. Oderfeld-Nowak).

Laboratory of Regulation and Transcription Processes of the M. Nencki Institute of Experimental Biology, PASci, Warsaw, Poland (B. Kamińska-Kaczmarek).

Laboratory of Ultrastructure of the Nervous System, Medical Research Centre, PASci, Warsaw, Poland (B. Gajkowska).

REGULATION OF CEREBROCORTICAL MICROCIRCULATION DURING REPERFUSION FOLLOWING FOCAL CEREBRAL ISCHEMIA IN THE RAT

Supported by the State Committee for Scientific Research: grant # 4 P05A 088 09

Research team

E. Koźniewska, L. Radomska

This study was designed to find out what was the impact of transient focal cerebral ischemia of various duration on the endothelium-dependent and endothelium-independent regulation of cerebral microcirculation during early reperfusion. Ischemia was induced in the anesthetized Wistar rats using a modified method of intraluminal suture occlusion of the middle cerebral artery (MCA). Suture was introduced from the site of the common carotid artery through the internal carotid and advanced 17 and 19 mm above the carotid sinus. Reperfusion was initiated by pulling back the suture into the lumen of the common carotid artery. Severity of ischemia was assessed by a laser Doppler flowmetry using a probe placed above the intact dura in the territory supplied by the MCA. This probe was left for the duration of the experiment to control stability of ischemia and to measure changes of microflow (LDF) upon reperfusion as well as during various tests of the reactivity of cerebrocortical microcirculation at different times of reperfusion. MCA was occluded either for 30 minutes or for 2 hours. The responses of cerebral microcirculation to the administration of nitro-L--arginine methyl ester (L-Name), acetylcholine (ACh), carbon dioxide (CO_2) and papaverine as well as to blood pressure changes were studied at 15 min or 2 hours during subsequent reperfusion. The effect of the pretreatment with free radical scavenger – superoxide dismutase (SOD) on the response of LDF to ACh and CO_2 during reperfusion after 30 min MCA occlusion was also tested. Sham-operated rats (the same surgery as in the rats with MCA occlusion and placement of the suture in the internal carotid artery without occluding MCA) served as controls.

Upon occlusion of MCA, LDF decreased to 24% of control value and remained at this low level until the end of ischemia. Changes of LDF during reperfusion depended upon the duration of ischemia. Thirty minutes ischemia was followed by immediate hyperperfusion which lasted 10 to 15 minutes. Next, LDF was stabilized at about 85% of the preischemic control. In the

case of reperfusion after 2 hours of ischemia, hyperperfusion occurred usually more slowly and in some instances lasted longer than an hour. Delayed hypoperfusion was rarely observed. The response of LDF to the inhibition of basal release of nitric oxide (NO) during reperfusion after 30 minutes or 2 hours of ischemia was not different than in sham-operated, control animals whereas the responses which required the mobilization of additional amounts of NO (ACh and CO_2) were severely impaired. Moreover, 2 hours of ischemia resulted in the transient loss of the reactivity of LDF to papaverine which indicates the impairment of the myogenic relaxation. This lack of response of LDF to papaverine after 2 hours of ischemia contrasted with the preservation of the response after 30 minutes MCA occlusion. Pretreatment of animals with SOD (30000 units/kg) improved the response of LDF to ACh without significantly affecting the reactivity of CO_2 during reperfusion after 30 minutes of MCA occlusion.

In conclusion, the impairment of the regulation of cerebrocortical microcirculation during reperfusion following transient focal ischemia is related to the duration of an insult. Endothelial control is disturbed after relatively short period of ischemia and may be partially caused by the enhanced production of free radicals. Loss of endothelium-dependent vasodilation to ACh suggests that some of the endothelium-dependent agonists may result in vasoconstriction and a decrease of microflow during early reperfusion which might in turn increase the risk of reperfusion injury of the affected brain tissue.

Cooperating unit

Max-Delbrück-Centre for Molecular Medicine (MDC), Berlin-Buch, Germany (R. Reszka).

MALIGNANT NEOPLASTIC PROCESSES AND INFLAMMATORY CHANGES IN THE BRAINS OF AIDS PATIENTS

Research team

I.B. Zelman, M.J. Mossakowski

In the collection of 160 neuropathologically verified brains of patients who had died due to AIDS between 1987-1997, there were 16 with malignant

proliferative processes: 2 with Kaposi's sarcoma, 1 with metastatic carcinoma and 15 (9.4%) with malignant non-Hodgkin's central nervous system lymphomas (CNSL). The latter neoplasms are oficially included into the group of AIDS-associate pathology (CDC, 1982). The age of patients with CNSL ranged from 25 to 61 years. In 10 cases lymphomas affected only CNS (primary – PCNSL), in 4 cases they were associated with systemic lymphomatous proliferation (secondary – SCNSL). In one case lack of detailed clinical and general autopsy data did not permit the definition of its primary or secondary nature. Dynamics and aggresiveness of the proliferative process and its neuropathological expression showed remarkable variability in particular cases. In 5 cases, lymphomas were already recognised at the brain section, 5 brains cutling autopsy did not exhibit any macroscopically detectable lesions, in 5 cases with additional extensive pathological changes, the presence of CNSL was established histologically. The lymphomatous proliferation was uni- or multifocal, bilateral and asymmetric and exhibited meningeal perivascular and diffuse growth pattern. In advanced stages of lymphoma, it was often simultaneously meningeal, perivascular and diffuse in character. In the brain, parenchymal foci of lymphomatous proliferation were localized in the white matter of cerebral hemispheres, in subcortical nuclei, in cerebellar hemispheres, being rather uncommon in brain stem structures. In the case of tumor infiltrations, the typical features was the presence of coagulative necrosis in the central part of lymphomatous foci.

Among our cases with CNSL, 11 were B cell lymphomas, 2 were of T cell origin. In 2 cases, immunophenotype of lymphoblastic cells was not established because of technical reasons. Admixture of reactive lymphocytes (CD45RO+) among neoplastic cells varied to a great extent, in particular cases ranging from 5 to 30%.

Characteristic feature of our material was the coexistence of CNSL with wide spectrum of other pathological processes: specific HIV-related changes and various opportunistic infections, many of them coinciding in the same case. In 8 cases, CNSLs were associated with specific HIV-related changes, in 6 cytomegalovirus (CMV) infections were present, in 3 – progressive multifocal leucoencephalopathy (PML), in 3 – toxoplasmosis and in 2 – cryptococcosis. It is to be stressed that the high frequency of HIV-related changes and opportunistic infections in cases with CNSL markedly exceeded

their frequency in the collection of AIDS cases without lymphomatous proliferation. This does not seem to be incidental, although no reciprocal pathogenic relations have been yet established.

Another typical feature of our cases was the coexistence of the exponents of different pathological processes within the same altered tissue areas for example toxoplasmic trophozoits and the end cysts with diffuse, lymphoblastic proliferation, HIV-specific multinucleated giant cells with perivascular lymphoblastic cuffs or CMV intranuclear inclusions within lymphoblastic cells. Concomitance of blastomatous and reactive infiltrates within the same area was not uncommon. In cases of CNSLs coexisting with HIV-related processes, great accumulation of multinucleated giant cells and activated microglia in the vicinity of lymphomatous foci was observed. This may indicate direct connection of HIV-virus with blastomatous transformation of lymphocytic cells within CNS.

Cooperating unit

Institute of Oncology, Medical Academy, Łódź, Poland (H. Nowakowska).

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

Research team

M. Barcikowska-Litwin, M. Gołębiowski, A. Pfeffer

Alzheimer's disease (AD) is the leading cause of dementia in the elderly. Recent studies have shown a strong association between the apolipoprotein E (APO E) ε4 allele in both sporadic, early and late onset of familial AD in different populations. Isoforms of APO E have been examined in a cohort of patients from an outpatient's clinic for Alzheimer's disease. The APO E genotyping has been performed according to the protocol of restriction isotyping by Chapman. APO E frequencies have been calculated by using gene counting method. Genotypes have been identified in persons with clinically diagnosed AD on the basis of DSM IV and NINCDS/ADRDA and NINDS/AIREN criteria. Our results did not differ from the results obtained elsewhere and we observed a clear tendency to more frequent presence of isoform ε4 within AD when compared with non demented cases. Allele ε4

of APO E appears in 40-50% of AD patients as compared with 10-20% in control cases. There has been no escalation in numbers of disease cases in 2/4 pairs alleles – which could suggest protective features of alleles 2. The females without APO E4 alleles progressed more rapidly (4.8 points per year on the Mini Mental Status Examination (MMSE). The rate of decline for females with other genotypes was slower at 1.7 points per year on the MMSE ($p<0.01$). We suggest that APO E ε4 allele does not influence the age of onset and is not associated with more rapid course of AD. Indeed, it may even suggest a better prognosis in female AD patients. Our data indicate significant correlation between decreased total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) ratio in a group of AD patients versus non-demented individuals, regardless of APO E genotype and increased TC and LDL-C ratio in group of AD patients homozygous for APO E ε3 versus their children of the same genotype. The age factor has implication in serum lipid profile that is independent of APO E genotype status in AD patients. Patients homozygous for ε3/3 also presented better memory and learning capacity than homozygous 4/4.

These promising preliminary results still need to be confirmed in a progressive study on a larger number of subjects. More elaborate statistical analysis may show further correlations.

Cooperating units

Department of Neurosurgery, MRC, PASci, Warsaw, Poland (E. Łuczywek).
Department of Cellular Signalling, MRC, PASci, Warsaw, Poland (M. Łałowski).
Neurological Clinic, Medical Academy, Warsaw, Poland (K. Czyżewski).

Publications

Barcikowska M: Levy body disease. *Pol J Pathol* 1997, 49, Suppl 1, 79-93 (in Polish).
Barcikowska M: Role of growth factors and cytokines in Alzheimer's disease. *Neurol Neurochir Pol* 1997, Suppl 1, 85-92 (in Polish).
Barcikowska M, Gołębiowski M, Pfeffer A: The significance of cerebral white matter changes in CT images in Alzheimer's disease. *Neurol Neurochir Pol* 1997, 32, 237-245 (in Polish).

Barcikowska M, Kwieciński H: Alzheimer's disease from beta-amyloid to helpless patient. *Medipress Psychiatria–Neurologia* 1997, 1, 16-21 (in Polish).

Barcikowska M, Liberski PP: Alzheimer disease. *Pol J Pathol* 1997, 49, Suppl 1, 51-78 (in Polish).

Friedman A, Barcikowska M: Parkinson disease. *Pol J Pathol* 1997, 49, Suppl 1, 121-129 (in Polish).

Gołębiowski M, Łuczywek E, Pfeffer A, Barcikowska M: MR based hippocampal volumetry in patients with dementia of Alzheimer type. *Pol Przegl Radiol* 1997, 62, 190-194 (in Polish).

Jesionek-Kupnicka D, Majchrowska A, Krawczyk J, Wedorff J, Barcikowska M, Łukaszek S, Liberski PP: Krabbe disease. Ultrastructural study of globoid cells and reactive astrocytes at the brain and optic nerves. *Folia Neuropathol* 1997, 35, 155-162.

Kroh H, Figols J, Sobieraj A: Intracranial hemangiopericytomas. Histological and immunohistochemical study. *Folia Neuropathol* 1997, 35, 121-127.

Liberski PP, Mossakowski MJ: Non-Alzheimer dementia. *Pol J Pathol* 1997, 49, Suppl 1, 93-108 (in Polish).

Matyja E: Intracellular calcium overload in a model of quinolinic acid neurotoxicity in organotypic culture of rat hippocampus; inhibited by nimodipine. *Folia Neuropathol* 1997, 35, 8-17.

Mossakowski MJ, Zelman IB: Neuropathological syndromes in the course of full blown acquired immune deficiency syndrome (AIDS) in adults in Poland (1987-1995). *Folia Neuropathol* 1997, 35, 133-143.

Papierz W, Barcikowska M, Liberski PP: Amyotrophic lateral sclerosis. *Pol J Pathol* 1997, 49, suppl 1, 109-116 (in Polish).

Pluta R: Experimental model of neuropathological changes characteristic for Alzheimer's disease. *Folia Neuropathol* 1997, 35, 94-98.

Pluta R, Barcikowska M, Kida E, Zelman I, Mossakowski MJ: Late extracellular deposits of β-amyloid precursor protein in ischaemic rat brain show different immunoreactivity to the N- and C-terminal. *Alzheimer's Res* 1997, 3, 51-57.

Pluta R, Barcikowska M, Dębicki G, Ryba M, Januszewski S: Changes in amyloid precursor protein and apolipoprotein E immunoreactivity following ischemic brain injury in rat with long-term survival: influence of idebenone treatment. *Neurosci Lett* 1997, 232, 95-98.

Pluta R, Misicka A, Januszewski S, Barcikowska M, Lipkowski AW: Transport of human β-amyloid peptide through the rat blood-brain after cerebral ischemia. *Acta Neurochirurg (Wien)* 1997, 70, Suppl, 247-249.

Śmiałek M, Gajkowska B, Ostrowski P, Piotrowski RP: Experimental squalene encephaloneuropathy in the rat. *Folia Neuropathol*, 1997, 35, 262-264.

Śmiałek M, Gajkowska B, Piotrowski P, Ostrowski RP: Experimental tellurium encephaloneuropathy in the rat. I. Effect of intoxication with sodium tellurite. *Pol J Environment Studies* 1997, 6, Supl, 186-188.

Śmiałek M, Gajkowska B, Piotrowski P, Ostrowski RP: Experimental tellurium encephaloneuropathy in the rat. II. Does squalene play an important role in pathogenesis of tellurium encephaloneuropathy? (Electron microscopic studies). *Pol J Environment Studies* 1997, 6, Suppl, 189-191.

Taraszewska A, Kroh H, Majchrowski A: Subependymal giant cell astrocytoma: clinical, histologic and immunohistochemical characteristic of 3 cases. *Folia Neuropathol* 1997, 35, 181-186.

Wisniewski KE, Kida E, Kuchna I, Wierzba-Bobrowicz T, Dąmbska M: Regulators of neuronal survival (Bcl-2, Bax, c-Jun) in prenatal and postnatal human frontal and temporal lobes in normal and Down syndrome brain. In: *Normal and abnormal development of cortex*. Eds: AM Galaburda, Y Christen. Springer-Verlag, Berlin, Heidelberg, New York, 1997, 179-195.

Wiśniewski KE, Zhong N, Kida E, Kaczmarski W, Kaczmarski A, Connell F, Sklower-Brooks S, Brown WT: Atypical late infantile and juvenile forms of neuronal ceroid lipofuscinosis and their diagnostic difficulties. *Folia Neuropathol* 1997, 35, 73-79.

DEPARTMENT OF DEVELOPMENTAL NEUROPATHOLOGY

Head: Associate Professor Danuta Maślińska
5 Pawińskiego St., 02-106 Warsaw
Telephone: 668 54 34

DEVELOPMENT OF THE NERVOUS SYSTEM IN NORMAL AND PATHOLOGIC CONDITION (QUALITATIVE AND QUANTITATIVE EVALUATION)

Research team

M. Dąmbska, M. Laure-Kamionowska

The dependence of structure and topography of changes within the central nervous system (CNS) on its developmental age was examined.

It has been found that this dependence is evident in degenerative syndromes in infant brains. Five cases of poliodystrophy of Alpers type belonging to three families were neuropathologically examined and brains of two siblings with degenerative syndrome with demyelination and calcifications. In both syndromes it was seen that the stage of CNS maturation influences the type and topography of degenerative changes. Similar dependence was observed in six microencephalic brains of infants 14-34 months old with congenital HIV infection. In addition to encephalopathic changes, the retardation of myelination was seen in all brains, particularly evident within U-fibres.

Cooperating unit

Institute for Basic Research in Developmental Disabilities, New York, USA

ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDIES OF THE BRAIN DEVELOPMENTAL DISORDERS

Research team

D. Maślińska, I. Kuchna, M. Muzylak

Ultrastructural changes found in the rabbit brain following prolonged treatment with vincristine induced nuclear inclusions in the brain cells,

degeneration of some axons and myelin sheath. The drug also affected the basement membrane of numerous brain vessels. This membrane was often multiplied in similar way as it was previously described in the course of diabetes or aging. Following acute intoxication, vincristine induced apoptosis in numerous cells in the brain. Ultrastructure of apoptotic cells and engulfment of apoptotic bodies were documented. The results described in our previous publications led to the conclusion that side effects of vincristine treatment may be concomitant with irreversible morphological changes in the brain parenchyma.

Immunohistochemical studies of mast cells in different forms of meningiomas were performed. The tumors were studied in children (6-14 years of age) and in adults. In all tumors mast cells were localized in the close vicinity of the vessels. In some tumors, capillary network was very developed and mast cells were immunopositive with tryptase and chymase monoclonal antibodies. In meningiomas with poor capillary network mast cells were immunopositive only with tryptase antiserum. The results showed that different immunoreactivity of mast cells is related to the morphological maturity of the tumor and is independent of the patient's age. The hypothesis that mast cells are involved in the mechanism of the tumor cysts formation has not been confirmed.

In cooperation with the Medical Academy and the Institute of Rheumatology in Warsaw the studies were undertaken on mast cells and biologically active substances released from these cells in the course of fetal and newborn development.

Studies performed on human placentas have shown that histamine released from placental mast cells influences the labour activity of the uterus in healthy and diabetic pregnant women.

In cooperation with Department of Gynecological Pathology, Medical Academy, Warsaw and with Department of Biogenic Amines, PASci, Łódź, pathomorphological studies were performed on mammary gland of mice. The results showed mast cells involved in the development of lactating function of the gland.

Cooperating units

Department of Pathophysiology, Medical Academy, Warsaw, Poland.
Department of Biogenic Amines, Polish Academy of Sciences, Łódź, Poland.
Department of Gynecological Pathology, Medical Academy, Warsaw, Poland.
Departments of Pathomorphology and Pathophysiology, Institute of Reumathology, Warsaw, Poland.

Publications

Dąmbska M, Anzil AP, Wrzołek M, Patxot OF: The degenerative diseases or syndromes in maturing central nervous system. *Folia Neuropathol* 1997, 35, 205-208.

Dąmbska M, Kozłowski PB, Brudkowska J, Rao C, Anzil AP, Laure--Kamionowska M: The white matter changes in microencephalic HIV infected infants. A preliminary report. *Folia Neuropathol* 1997, 35, 145--148.

Iwanowski L, Kupryjańczyk J, Kuchna I, Matyja E, Wierzba-Bobrowicz T, Ząbek M: Embryonal carcinoma of the pineal gland with yolk sac tumor differentiation. A case report. *Folia Neuropathol* 1997, 35, 128-132.

Lossinsky AS, Wisniewski HM, Dąmbska M, Mossakowski MJ: Ultrastructural studies of PECAM-1/CD31 expression in the developing mouse blood-brain barrier with the application of a pre-embedding technique. *Folia Neuropathol* 1997, 35, 163-170.

Maślińska D: Apoptosis in rheumatic diseases. *Reumatologia* 1997, 3, 350--352 (in Polish).

Maślińska D, Derçgowski K, Kierska D, Fogel WA, Maśliński C: Mast cells in the different functional periods of mouse mammary gland. *J Physiol Pharmacol* 1997, 48, Suppl. 2, 14-21.

Maślińska D, Gujski M, Wagner T, Górnicka M: Expression of histamine in tumours of human mammary gland. *J Physiol Pharmacol* 1997, 48, Suppl 2, 27-33.

Maślińska D, Kuchna I, Szukiewicz D, Wątroba M: Localization of mast cells in cystic meningiomas. *J Physiol Pharmacol* 1997, 48, Suppl 2, 34-42.

Maślińska D, Muzylak M: Ultrastructure of myelinated fibers of sciatic nerve in acute vincristine intoxication. *Folia Neuropathol* 1997, 35, 18--23.

Muzylak M, Maślińska D: Ultrastructure of nuclear inclusions of rabbits central nervous system cells after intoxication with vincristine. *Medycyna Wet* 1997, 53, 613-617 (in Polish).

Szukiewicz D, Szukiewicz A, Maślińska D, Gujski M, Poppe P, Markowski M: Influence of endogenous and exogenous histamine on the placental vascular resistance in diabetes complicated pregnancy. *J Physiol Pharmacol* 1997, 48, Suppl 2, 86-93.

Szukiewicz D, Szukiewicz A, Maślińska D, Poppe P, Gujski M, Piórkowski R: Influence of dopamine on vascular resistance – human placenta perfusion study. *J Physiol Pharmacol* 1997, 48, Suppl 2, 80-85.

LABORATORY OF THE ULTRASTRUCTURE OF THE NERVOUS SYSTEM

Head: Associate Professor Barbara Gajkowska
5 Pawińskiego St., 02-106 Warsaw
Telephones: 668 52 77, 608 64 45
E-mail: gajk@cmdik.pan.pl

ELECTRON MICROSCOPY INVESTIGATION: MORPHOLOGICAL, IMMUNOCYTOCHEMICAL AND HISTOCHEMICAL STUDY OF VASCULAR-TISSUE INTERPHASE IN BARRIER-COMPETENT AND BARRIER-FREE REGIONS OF ANIMALS IN EXPERIMENTAL, PATHOLOGICAL CONDITIONS

Research team

B. Gajkowska, M. Frontczak-Baniewicz, M. Walski

The aim of the study was to investigate the effects of 10 min total, experimental brain ischemia on the vascular-tissue interphase in barrier-competent and barrier-free regions of rat brain (cerebral cortex, hippocampus, hypothalamus and neurohypophysis). The major achievements are as follows:

Immunocytochemical studies using the antibody against e-NOS showed presence of this enzyme not only in the endothelial cells (in the barrier-competent and the barrier-free region) but for the first time also in perivascular mast cells. The expression of e-NOS in mastocytes is in normal circumstances higher than that in endothelial cells and further increases by the faster 10-30 min after ischemia. Early expression of e-NOS in mast cells may be an important mechanism alleviating the deleterious effects of ischemia on brain tissue.

Histochemical studies with NADPH-diaphorase confirmed the presence of NO in endothelial cells, pericytes and pituicytes and additionally in some neurons of hippocampus. This technique enabled to investigate the localization and role of NO in the development of pathological changes in the vessels and perivascular space 10 min after total brain ischemia.

Morphologic studies of brain vasculature in electron microscopy (both transmission and scanning) enabled to describe the participation of basal

membrane in the processes of damage repair caused by ischemia or photochemical injury.

These statutory investigations were covered by Medical Research Centre PASci.

Apart from planned investigation, the following studies were performed:

1) Electron microscopy-based histochemistry showing localization of calcium in murine skin. We showed for the first time that topical treatment with an active analog of vitamin D_3, KH1060 (20-epi-22-oxaderivative) causes major alterations in epidermal calcium distribution.

2) Electron microscopy and immunofluorescent microscopy studies on the effects of activated vitamin D_3 on keratinocytes *in vitro* revealed that this hormone stimulates assembly of adherens junctions in cultured cells. The likely biochemical mechanism is activation of the species of protein kinase C.

3) Electron microscopy-based on immunocytochemical studies using the antibody against LH-RH showed their presence in some neurons and synapses of preoptic area of the female rat. The decreasing influence of ovariectomy on content of Gn RH immunoreactivity in synapses of preoptic area region was detected.

4) Morphologic studies of experimental tellurium and squalene encephaloneuropathy were performed to investigate the mechanisms of the demyelination processes in central and peripheral nervous system.

5) Electron microscopy histochemical studies were performed to evaluate whether prolonged drinking of lead acetate-containing water by adult rats, affects some morphological properties of brain microvessels. In lead exposed rats, the breakdown of the blood-brain barrier was determined using horseradish peroxidase.

6) Electron microscopy morphological and biochemical study of carotid body, a chemosensory organ was performed using phenyl-methylsulfonyl fluoride (PMSF), an inhibitor of lipases and proteases. This study indicates that different subcellular organs play different roles in chemoreception.

Apart from these investigation studies on "Regeneration of the liver after anatomic resection in humans" were supported by the State Committee for Scientific Research: grant No III C/0, 4P05 C 047 09.

During surgery liver tissue with a tumor was resected within anatomic segments. The study was done in the course of the surgical treatment of 13

adults. Biopsy taken on the seventh postoperative day showed regenerative changes. Electron-microscopic examination has shown hepatocyte mitosis. Presence of hepatocytes in the mitotic phase proves the regenerative process.

Cooperating units

University of Copenhagen, Bispebjerg Hospital, Department of Dermatology, Copenhagen, Denmark (R. Gniadecki, M. Hansen, J. Serup).

Kielanowski Institute of Animal Physiology and Nutrition PASci, Jabłonna near Warsaw, Poland (K. Kochman).

3rd Department of Surgery, Medical School, Warsaw, Poland (J. Polański, J. Dziuk, D. Bobilewicz).

Institute of Biochemistry and Biophysics, PASci, Warsaw, Poland (Z. Matysiak).

Department of Neuropathology, MRC PASci, Warsaw, Poland (R. Gadamski, M. Śmiałek, R.P. Ostrowski, P. Piotrowski).

Department of Neurochemistry, MRC PASci, Warsaw, Poland (B. Dąbrowska-Bouta, U. Rafałowska, L. Strużyńska).

Department of Neurophysiology, MRC PASci, Warsaw, Poland (M. Pokorski, M. Musielak).

Publications

Chomicz L, Cielecka D, Walski M, Czubaj A: Ultrastructural studies on the encospheral envelopes of hymenolepidid cestode, *Wardium aequabile* (Rudolphi, 1810) Spassky et Spasskaya, 1954, in the final stage of infective egg formation. *Acta Parasitol* 1997, 42, 40-45.

Dul B, Gajkowska B: The influence of the calcium channel antagonist, verapamil and ethanol on myocardial ultrastructure in the rat. *Exp Toxic Pathol* 1997, 49, 493-496.

Gajkowska B: Endothelin-like immunoreactivity in hippocampus following transient global cerebral ischemia. I. Neuronal and glial cells. *Folia Neuropathol* 1997, 35, 36-48.

Gajkowska B, Frontczak-Baniewicz M, Gadamski R, Barskov I: Photochemically-induced vascular damage in brain cortex. *Acta Neurobiol Exp* 1997, 57, 203-208.

Gajkowska B, Kochman K: The GmRH-containing synapses in the preoptic area of normal and ovariectomized female rat: immunocytochemical study. *Neuroendocrinol Lett* 1997, 18, 153-159.

Gajkowska B, Mossakowski MJ: Endothelin-like immunoreactivity in hippocampus following transient global cerebral ischemia. II. The blood-brain interphase. *Folia Neuropathol* 1997, 35, 49-59.

Gajkowska B, Mossakowski MJ: Endothelial nitric oxide synthase in vascular endothelium of rat hippocampus after ischemia: evidence and significance. *Folia Neuropathol* 1997, 35, 171-180.

Gajkowska B, Viron A: Protracted elevation of endothelin immunoreactivity in hypothalamo-neurohypophysial system after ischemia. *Folia Neuropathol* 1997, 35, 107-114.

Gniadecki R, Gajkowska B, Hansen M: 1,25-Dihydroxyvitamin D3 stimulates the assembly of adherens junctions in keratinocytes: involvement of protein kinase C. *Endocrinology* 1997, 138, 2241-2248.

DEPARTMENT OF NEUROSURGERY

Head: Professor Zbigniew Czernicki
16 Barska St., 02-315 Warsaw
Telephone: 822 36 43
Fax: 822 35 87
E-mail: neuro9@polbox.com

CONTINUATION OF RESEARCH IN THE FIELD OF THE DIAGNOSIS OF INTRACRANIAL HOMEOSTASIS DISTURBANCES

Research team

Z. Czernicki, J. Andrychowski, J. Bogucki, J. Dubicki, J. Dziduszko, A. Dowżenko, E. Fersten, W. Grochowski, D. Horsztyński, J. Jurkiewicz, N. Kuridze, E. Łuczywek, P. Marszałek, E. Mempel, M. Mierzejewski, P. Nauman, S. Piechnik, W. Rataj, W. Sapieja, G. Stępińska, B. Witkiewicz, W. Zabołotny, J. Walecki

Studies performed concerned three main topics: cerebrovascular reactivity, disturbances of the cerebrospinal fluid circulation and electrophysiological activity of the brain evaluated by the somatosensory evoked potentials analysis.

Cerebrovascular reactivity was a subject of experimental and clinical research. In the continued laboratory study, the influence of the calcium channel blocker Dotarizine upon the vascular reactivity was investigated. It was found that compared to basilar artery, middle cerebral artery is more affected by the orally administered Dotarizine. In the experimental studies on rabbit, a model to evaluate cerebrovascular autoregulation while increasing systemic blood pressure by Dopamine infusion under normal conditions and after global brain ischemia was developed. Laboratory data are currently collected. Five experiments have already been conducted.

In the clinical studies, measurements of blood flow velocity in middle cerebral and basilar arteries in patients after severe head trauma before and during Dopamine infusion were performed. It was found that blood flow in the infratentorial region (basilar artery) is better preserved than in the supratentorial region (middle cerebral artery). A new method of analysis and processing of data obtained by transcranial Doppler sonography, developed

in collaboration with the Engineering School of Warsaw, was used in the studies.

Cerebrovascular reactivity was also studied during cognitive activities in humans. Based on the observed changes in flow velocity, lateralisation of the hemispheric activity and type of tasks activating both cerebral hemispheres were found. An experimental model of the growing intracerebral hematoma by intraparenchymal silicone infusion was elaborated. Exhaustion of the intracranial reserve was evaluated by applying physiological loadings. The study is in an initial stage. An experimental model has been elaborated and experimental procedures started.

Concerning hydrocephalus studies, a new method of ventriculo-lumbar drainage to treat the disturbances of CNS circulation was introduced into the clinical practice and the clinical data are being collected.

Diagnosis and treatment of post-traumatic hydrocephalus was an important subject in clinical research. Diagnostic procedures developed in the Department of Neurosurgery were presented. In the other study, in a group of 58 patients with normotensive hydrocephalus the disturbances in cognitive activities were investigated by the complex neuropsychological analysis. On the basis of significant differences in the tasks performance, 5 types of disturbances were found. In the study which is a subject of doctor's dissertation and has been started, the results of treatment of 44 hydrocephalic patients are evaluated.

Electrophysiological studies on the clinical material as well as on experimental animals (cats) in collaboration with the Afferent Systems Laboratory of the Neurophysiology Department of the Nencki Institute of Experimental Biology were conducted. The picture of somatosensory evoked potentials (SEP) in cerebellar lesions was studied in particular. Both clinical and experimental studies showed the occurrence of significant oscillations in cerebral lesions. Results of SEP evaluation in cases of cervical discopathia, brain stem lesions and focal brain lesions were accumulated. Altogether, 38 patients were examined.

Cooperating units

Department of Neurophysiology, Nencki Institute of Experimental Biology, PASci, Warsaw, Poland.

Engineering School of Warsaw, Warsaw, Poland.

Publications

Andrychowski J, Nauman P, Czernicki Z, Dowżenko A, Bogucki J, Sapieja W, Mierzejewski M: Transcranial Doppler sonography evaluation of the changes in cerebral circulation after intravascular surgery and angiography. In: *Therapeutic concepts in spontaneous intracerebral hemorrhage*. Eds: D.-K. Böker, W. Deinsberger, M. Winking. Biermann Verlag, Zülpich, 1997, pp. 156-159.

Bogucki J, Gielecki J, Czernicki Z: The anatomical aspects of a surgical approach through the floor of the fourth ventricle. *Acta Neurochir (Wien)* 1997, 139, 1014-1019.

Czernicki Z, Kuridze N, Jurkiewicz J, Cervos-Navarro J: The effect of dotarizine (Ca^{2+} channel blocker) on cerebral vessel reactivity in animals subjected to hyperventilation and anoxia. *Acta Neurochir (Wien)* 1997, Suppl., 70, 209-211.

Fersten E, Łuczywek E, Jurkiewicz J, Dowżenko A: Assessement of perceptive activity in patients with low-pressure hydrocephalus treated surgically. *Neurol Neurochir Pol* 1997, 31, 77-88 (in Polish).

Jurkiewicz J: Treatment of selected cases of obstructive hydrocephalus without using shunts. *Neurol Neurochir Pol*, 1997, 31, 415-416 (in Polish).

Jurkiewicz J: Ventriculo-lumbar drainage in the treatment of obstructive hydrocephalus. Technical note. *Eur J Pediatr Surg* 1997, 7, Suppl I, 61-62.

Jurkiewicz J, Marszałek P, Mierzejewski M, Bogucki J, Czernicki Z: Hydrocephalus following severe head injury. The method of differential diagnosis. In: *The pathophysiological basis and controversy in neuro intensive care*. W. Zuckschwerdt Verlag, München, Bern, Wien, New York, 1997, pp. 132-136.

Kuridze N, Czernicki Z, Jurkiewicz J, Cervos-Navarro J: Influence of the calcium channel blocker – Dotarizine on regional differences of the cerebrovascular reactivity in rabbits. *J Cerebral Blood Flow Metabol* 1997, 17, Suppl 1, S295.

Łuczywek E: The object of paliative treatment in degenerative cerebral diseases – the patients or the care provider. In: *The incurable patient*. Eds: B. Block, W. Otrębski. WNS KUL, Lublin, 1997, pp. 224-234 (in Polish).

Marszałek P, Jurkiewicz J, Fersten E, Łuczywek E, Czernicki Z, Gielecki J, Bogucki J: Multistage method for diagnosis of low-pressure hydrocephalus. *Neurol Neurochir Pol* 1997 31, 523-535 (in Polish).

Sapieja W, Kuridze N, Czernicki Z: Regional differences in the cerebrovascular reserve following acute global ischemia in rabbits. *Acta Neurochir* 1997, Suppl., 70, 59-61.

Tarnecki R, Mempel E, Kołodziejak A, Witkiewicz B: Somatosensory evoked potentials (SEP) in a patient with Parkinson's disease before and after cryothalamotomy. *Neurol Neurochir Pol* 1997, 31, 357-366 (in Polish).

NEUROMUSCULAR UNIT

Head: Professor Irena Hausmanowa-Petrusewicz
1a Banacha St., 02-097 Warsaw
Telephone: 658 45 01
Fax: 658 45 01
E-mail: neurmyol@cmdik.pan.pl

STUDIES ON STRUCTURE AND FUNCTION OF MUSCLE AND NERVE DURING DEVELOPMENT, AGING AND IN GENETIC AND ACQUIRED DISEASES

The main subjects of studies in 1997 were: muscle dystrophy, muscle spinal atrophy and inflammatory myopathies. The investigations on the features of motor unit potential abnormalities in neuromuscular disorders and on the peripheral nerves abnormalities were also continued.

Research team

I. Hausmanowa-Petrusewicz, A. Fidziańska-Dolot, A. Kamińska, I. Niebrój-Dobosz, K. Rowińska-Marcińska

A biochemical analysis was performed of human dystrophic muscle and muscle of mdx mice (hind and diaphragm).

In comparative studies on the pattern of amino acids in skeletal muscles and in the diaphragm of mdx mice and in the skeletal muscles of Duchenne dystrophy patients, an increased proteolysis was confirmed. An increased efflux of amino acids from Duchenne dystrophy skeletal muscles and the mdx mice diaphragm and also the evident decrease of the branched amino acid content may be, at least in part, responsible for more profound morphological and biochemical changes in these muscles. Unchanged amino acid release and branched amino acid content in the mdx skeletal muscles are possible factors in preventing the overt dystrophic clinical symptoms in mdx mice.

Ultrastructural study on nuclear abnormalities was conducted in biopsied muscles from 7 patients with Emery-Dreifuss muscular dystrophy of 3 not related families and in 2 sporadic cases. The diagnosis was based on clinical data and molecular findings. We detected abnormalities of different degree in sarcolemmal nuclei ranging from marked condensation of chromatin to

complete damage of nuclear components. Other nuclei in the same muscle cell very often appeared normal. The extrusion of nuclear chromatin into sarcoplasm as a consequence of nuclear membrane disintegration was observed in numerous nuclei.

All these nuclear changes are considered to be the cytological indicators of nuclear dysfunction evoked by emerin deficiency.

Research team

I. Hausmanowa-Petrusewicz, A. Fidziańska-Dolot

Analysis of clinical data of 569 patients was done in two combined series with childhood and juvenile proximal spinal muscular atrophy (SMA). This cohort included only the patients who had achieved the ability to sit unaided (type II and III SMA). The survival rate among 240 type II patients (who sit but never walked) was 98.5% at 5 years and 68.5% at 25 years.

A group of SMA III (n – 329), those who walked and had the symptoms before 30 years of age, was subdivided into those with an onset before and after 3 years of age (type IIIa, n – 195; type IIIb, n – 134). In the patients with SMA III, life expectancy is not significantly less than that of a normal population. In SMA IIIa the probability of being able to walk at 10th year after onset was 70.3%, and at 40th year, 22%. In SMA IIIb, 96.7% were able to walk 10 years after onset and 58.7% at 40th year. The subdivision of type III SMA was based on the ability of the patients to walk at the age of onset; the prognosis differed for those with an onset before or after the 3rd year of age. The data provides a reliable basis for the natural history of proximal SMA and supports a classification system that is based primarily on the age of onset and the achievement of motor milestones.

Mild to moderately elevated creatine kinase (CK) activity is a frequent biochemical finding in proximal SMA. In a collaborative study on all types of childhood and juvenile onset SMA, we analyzed CK activity of 504 patients (138 type I, 127 type II, 144 type IIIa, and 95 type IIIb). Under an assumption of a lognormal distribution of CK activity as the most appropriate statistical model, CK levels were transformed into logarithms and compared by standard deviation scores = CK-SDS (log). CK activity was statistically different between an early and later onset of SMA: in SMA I and II, about one-third of the patients showed CK-SDS (log) > 2SD, the

analysis of the means did not show significant differences. In SMA III, CK-SDS (log) was significantly higher ($p<0.01$) than in the two groups, which was the most pronounced in SMA IIIb. Over 90% of SMA IIIb patients showed CK-SDS (log) values > 2 vs. 57% in SMA IIIa. As similar values were obtained for a subgroup of 100 patients in whom the diagnosis of autosomal recessive SMA was confirmed by a deletion of the telomeric copy of the survival motor neuron gene, our results can be considered representative for SMA I-III. There was no correlation between CK level and disease duration. The fact that the patients were ambulatory or chair-bound had no influence on CK activity in type III SMA. There was no sex influence in SMA I, II and IIIa. The observed higher male values in the group of SMA IIIb are most likely the result of a lack of female patients with an onset after puberty.

Cooperating unit

Military Medical Academy, Warsaw, Poland (J. Borkowska).

Research team

E. Kowalska-Olędzka, H. Drac, I. Hausmanowa-Petrusewicz

Clinical, serologic, and immunogenetic correlations were determined in patients with idiopathic inflammatory myopathies (IIM) and evaluated useful grouping of some diseases for practical clinical purposes.

Patients with IIM were categorized according to clinical presentation as compared with autoantibody specificity. Serum samples from 84 patients were screened for myositis-specific autoantibodies (MSAs) by indirect immunofluorescence and double immunodiffusion. All sera were also studied by protein A-assisted immunoprecipitation. Genomic DNA was isolated from peripheral blood mononuclear cells, and HLA-DQA1 and DRB1 alleles were determined. The patients were seen and followed up for many years in the same center.

MSAs were present in 19% of patients. The most common MSAs were antisynthetases in 13% of patients (Jo-1 10.7%, PL-12 1.2%, and EJ 1.2%), associated with the antisynthetase syndrome. Anti-SRP was found in 1.2% patients and associated with polymyositis, anti-Mi-2 in 4.9% was found exclusively in patients with dermatomyositis. The most frequent MSA was

PM-Scl in 23.8% patients and associated with scleromyositis, Ku was present in 9.6% patients with overlap syndromes. The alleles found with significantly increased frequency were HLA-DRB1 0301 (59.4%) and DQA1 0501 (71.6%), which are in linkage disequilibrium. DQA1 0501 was present in 85.7% patients with antisynthetases, and in 100% patients with PM-Scl and Ku.

The HLA-DRB1 0301; DQA1 0501 haplotype was found to be significantly increased in this population and in those myositis patients with antisynthetase, anti-PM-Scl, and anti-Ku antibodies. The results of this study confirm that IIM are heterogeneous syndromes but can be divided into more useful groups on the basis of clinical, serologic, and immunogenetic features.

Research team

K. Rowińska-Marcińska, I. Hausmanowa-Petrusewicz

The abnormalities of the motor unit (MU) and their pathomechanism were evaluated. The analysis of MU potential shape and pathomechanism of the satellite components of MU and the repetitive discharges (RDs) in MU were studied.

It seems that RDs of the voluntary activated MU reflect hyperexcitability of MU and are the signs of the membrane functional changes which can lead to MU degeneration. The RDs can originate from different sites along MU and in some instances the upper motor neuron can play a role in their generation. The shape analysis may contribute to resolving the problem of their origin.

Cooperating unit

Center of Science and Research in Railway Medicine (E. Zalewska).

GENETIC AND CLINICAL STUDIES ON INFANTILE AND JUVENILE SPINAL MUSCULAR ATROPHY (SMA)

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

Research team

I. Hausmanowa-Petrusewicz, A.Fidziańska-Dolot, H. Jędrzejowska, I. Niebrój-Dobosz, K. Rowińska-Marcińska

The grant started in May 1997. Since that time the new equipment has been prepared, laboratories organised and training of the staff for genetic analysis started. The data base of more than 600 SMA cases is prepared, the questionnaires and instructions mailed to all children health centers in Poland offering help in the diagnosis, advice and counselling the families at risk.

Cooperating units

Institute of Psychiatry and Neurology, Warsaw, Poland (J. Zaremba).
Institute of Mother and Child, Warsaw, Poland (T. Mazurczak).
Military Medical Academy, Warsaw, Poland (J. Borkowska).
Neurological Clinic, Medical Academy, Warsaw, Poland (B. Ryniewicz).

N-CAM AS A MARKER OF SOME CONGENITAL MUSCULAR DISORDERS IN CHILDREN

Supported by the State Committee for Scientific Research: grant # 4 S405 005 06

Research team

A. Fidziańska-Dolot, A. Kamińska

Human fetal muscle cells at various stages of development were analysed using electron microscopic and immunohistochemical techniques. In the muscle maturation, three main steps have been documented. The primary myotube, the earliest form of muscle fiber is characterised on cross section by a centrally located nucleus and scant sarcoplasm with peripherally distributed myofibrils. The primary myotubes are rich in desmin and neural cell adhesion molecule (N-CAM).

The next stage involves side to side fusion of a primary myotube with undifferentiated cells forming mature myotube. In the cross section,

mature myotube appears as two or three cells at different stages of maturation, in close contact, enveloped by a common basement membrane. Mature myotube, N-CAM decorated also shows high desmin activity. At the last stage of maturation immature muscle fibers appear as a cluster of the cells at the same stage of differentiation which are closely connected and enveloped by a common basement membrane. Immature muscle fibers devoid of N-CAM show a low desmin activity.

Similar structural pattern of muscle fiber maturation and differentiation was observed in experimentally induced regeneration using Bupivacaine. It seems that this model may be useful in studying structural and immunocytochemical features in neuromuscular disorders.

Cooperating unit

Neurological Clinic, Medical Academy, Warsaw, Poland (Z. Glinka).

CLINICAL-GENETIC CORRELATIONS IN RECENTLY DESCRIBED DYSTROPHIES

Supported by the State Committee for Scientific Research: grant # 4 P05B 113 08

Research team

I. Hausmanowa-Petrusewicz, M. Dorobek, A. Fidziańska-Dolot, I. Niebrój-Dobosz, K. Rowińska-Marcińska,

A series of Becker dystrophy patients was examined with the use of biochemical, morphological, electrophysiological and molecular genetics methods as related to the clinical features and of laboratory results. There was a considerable clinical variability in this group. In some patients, the symptoms were only cramps, myalgia or cardiomyopathy. The most common deletion was located at egzons 45-53 and 2-18. The deletion was found in 68% of Becker patients versus 54% of Duchenne patients. Interestingly, the frequency of deletion was higher in sporadic than familial cases of DMD. The reevaluation of the group of patients with limb-girdle diagnosis permitted also to find the Becker patients within this group, misdiagnosed before the era of molecular genetics.

An application of all mentioned tests to the group of women – dystrophy (Duchenne or Becker) carriers showed that 15% of carriers were clinically

manifesting carriers; these carriers either demonstrated clinical symptoms of the disease or being symptom free have had high activity of creatine kinase (CK) and dystrophin deficiency evaluated qualitatively (immunofluorescence) and quantitatively (Westernblot). The qualitative changes consisted of s.c. mosaics of dystrophinpositive and dystrophinnegative fibers; quantitative changes consisted mostly of the decrease in the amount of dystrophin but preservation of its normal molecular weight.

Among the carriers we distinguish the group of familial cases, easily identified due to their affected male relatives (brothers or sons) and sporadic cases mostly with scewed, asymmetric X-chromosome inactivation.

The electrophysiological macroelectrode technique permits the demonstration of defined abnormalities in the motor unit in the carriers.

Cooperating unit

Institute of Psychiatry and Neurology, Warsaw, Poland (J. Zaremba).

ANGIOGENESIS AND MYELINATION OF THE CENTRAL NERVOUS SYSTEM

Supported by the State Committee for Scientific Research: grant # 4 S402 071 06

Research team

J Rafałowska, D. Dziewulska, Z. Jamrozik, A. Podlecka

1) Cervical and lumbo-sacral segments of the spinal cord in 1, 3, 6, 9, 12, 15, 18, 21 and 25 day-old Wistar rats were examined by light and electron microscopy. For light microscopy examination, Klüver-Barrera method and antibodies to myelin basic protein (MBP) and for ultrastructural study, glial fibrillary acidic protein (GFAP), conventional procedures were used.

Rat spinal cord during first 25 days of postnatal life shows two types of blood vessels. One type consists of capillaries and other small vessels with narrow endothelial cells, few organelles and wide lumen which correspond to morphologically mature blood vessels. Another type is characterized by hypertrophied, thick and rich in organelles endothelial cells. Their luminal surfaces show invaginations and microvilli. These features represent immature blood vessels.

From birth to 25th postnatal day, intensive myelination of the spinal cord was observed, numerous axons were covered by myelin sheaths of various thickness. The immature vessels (angiogenesis) appeared in the period of fast progressing myelination. Myelination is associated with active metabolic processes, caused both by proliferation of oligodendroglial cells and development of myelin components.

The nutrition of a very active metabolically tissue certainly becomes a necessity. It is not known which trophic and growth factors act in myelination in the course of angiogenesis.

PARTICIPATION OF ASTROCYTES IN RAT SPINAL CORD MYELINATION

Research team

J. Rafałowska, D. Dziewulska, A. Podlecka

Astrocytes play an important role in the development of the central nervous system, in neuron migration and blood-brain barrier formation but still little is known about their role in the process of myelination. The aim of our investigation was to establish the relationship between astrocytes and myelin formation. Our study has revealed that myelination in rat spinal cord tracts begins between 6th and 9th postnatal day involving initially anterior funiculi, later lateral ones and then posterior. The process of myelination ended about 25 postnatal day. GFAP immunoreactivity of astrocytes, increased in paralell with increasing MBP reactivity. It is suggested that the temporary increase of GFAP positive cells accompanying the process of myelination is linked to the astroglial local secretion of growth factors necessary for the development of normal myelin.

Cooperating unit

Neurological Clinic, Medical Academy, Warsaw, Poland (Z. Jamrozik).

Publications

Drac H, Jamrozik Z: Axonal form of Guillain-Barré syndrome?. *Neurol Neurochir Pol* 1997, 31, 343-348.

Hausmanowa-Petrusewicz I, Kowalska-Olędzka E, Miller FW, Jarząbek-Chorzelska M, Targoff IN, Błaszczyk-Kostanecka M, Jabłońska S: Clinical, serological and immunogenetic features in Polish patients with idiopathic inflammatory myopathies. *Arthritis & Rheumatism* 1997, 40, 1257-1266.

Jamrozik Z, Przybyłowski T, Zakrzewska-Pniewska B, Rafałowska J: Obstructive sleep apnea syndrome in patient with Creutzfeld-Jakob disease. Clinical and morphological report. *Folia Neuropathol* 1997, 35, 150-154.

Rowińska-Marcińska K, Ryniewicz B, Hausmanowa-Petrusewicz I, Karwańska A: Diagnostic value of satellite potentials in clinical EMG. *Electromyogr Clinic Neurophysiol* 1997, 37, 483-489.

Zerres K, Rudnik-Schöneborn S, Forrest E, Łusakowska A, Borkowska J, Hausmanowa-Petrusewicz I: A collaborative study on the natural history of childhood and juvenile onset proximal spinal muscular atrophy (type II and III SMA): 569 patients. *J Neurol Sci* 1997, 146, 67-72.

Zimowski JG, Bisko MU, Fidziańska EJ, Fidziańska AZ, Badurska B, Niebrój-Dobosz I, Kozłowska M, Hausmanowa-Petrusewicz I, Zaremba JS: Detection of deletions within the gene of dystrophin in Polish families affected with Duchenne/Backer muscular dystrophy. *Europ J Neurol* 1997, 4, 138-142.

NEUROIMMUNOLOGICAL UNIT

Head: Professor Mieczysław Wender
Przybyszewskiego St., 60-355 Poznań
Telephones: 67 98 87, 67 68 41
Fax: 867 12 32

EFFECT OF THERAPY ON DISEASE ACTIVITY IN PATIENTS WITH MULTIPLE SCLEROSIS (MS)

Research team

M. Wender, J. Losy, G. Michałowska-Wender, E. Tokarz-Kupczyk

Immunological markers [tumor necrosis factor alpha – TNF-α in cerebro--spinal fluid (CSF) and serum, myelin basic protein (MBP) and MBP antibodies in CSF, interleukin 2 soluble receptor (sRIL-2) in serum], IgG index, MBP reactive CD2 lymphocytes in peripheral blood, kappa light chain to creatinine ratio in urine were studied in patients with multiple sclerosis (MS) in the course of steroid (methylprednisolone or prednisone) treatment. Immunological studies were correlated with magnetic resonance imaging (MRI) of the brain. The obtained results have proven the markers to be helpful for therapy monitoring in patients with MS.

Cooperating units

Institute for Basic Research in Developmental Disabilities, Staten Island, New York, USA.

Neurological Clinic, Medical Academy, Poznań, Poland (M. Śniatała-Kamasa).

VALUE OF BETA 2-MICROGLOBULIN AND SOLUBLE VASCULAR CELL ADHESION MOLECULE-1 (sV CAM -1) EVALUATION IN PATIENTS WITH MS

Research team

M. Wender, J. Losy, A. Niezgoda

Levels of beta 2-microglobulin and sV CAM-1 in serum and CSF were studied in MS patients. The values of studied markers did not differ signifi

cantly in MS patients in comparison with control group. In the course of cladribine treatment, the level of beta 2-microglobulin decreased significantly. The results indicate that evaluation of beta 2-microglobulin may serve as a marker of immunological activity in the course of MS treatment. Treatment of MS with different steroids (methylprednisolone or prednisone) has no significant effect on sV CAM-1 level in CSF and serum.

COMPARISON OF T CELL RECEPTOR (TCR) GENES IN TWO AUTOIMMUNE DISEASES: MS AND MYASTHENIA GRAVIS (MG)

Research team

G. Michałowska-Wender, M. Wender

In view of the recent evidence that in addition to α/β T lymphocytes also γ/δ T cells may have autoreactive potentials, TCR delta repertoire in peripheral blood was compared in MS and MG. Rearrangement of TCR Vδ-Jδ genes has been analysed in 20 MS and 20 MG patients using the polymerase chain reaction (PCR) technique. Oligoclonal primers specific for six Vδ regions and for Jδ1 genes were used for amplification of Vδ-Jδ junctional region, responsible for the diversity of γ/δ TCR. As opposed to healthy subjects the majority of MS and MG patients demonstrated mono- or oligoclonal character of rearrangement of TCR Vδ1-6 to Jδ1 genes, but more frequently MS cases demonstrated the rearrangement of Vδ1-Jδ1 and Vδ2-Jδ1. On the basis of the similarity of the bonding pattern in MS and MG it can be suggested that clonal expansion of γ/δ T lymphocytes *in vivo* represents a general reaction in autoimmune diseases.

Cooperating unit

Institute of Human Genetics, PASci, Poznań, Poland (J. Nowak).

GENETIC AND CLINICAL STUDIES OF ALZHEIMER'S AND PARKINSON'S DISEASES

Research worker

M. Wender

a) The apolipoprotein E (APOE) gene has in many studies been identified as a susceptibility factor in Alzheimer's disease (AD). The APOE association is rather strong, but other not yet identified genetic factors could be involved in the pathogenesis of AD as well. Recently, an association between the intronic polymorphism in presenilin-1 (PS-1) gene and late--onset of AD has been postulated. In order to confirm this observation we studied a sample of Polish patients with sporadic AD. However, our results did not confirm the existence of an association between the intronic polymorphism in the PS-1 gene and late-onset of Alzheimer's disease.

b) SPECT studies have established that pathomechanism of dementia in Alzheimer's disease is not connected with the decrease of regional cerebral blood flow.

Cooperating units

Institute of Human Genetics, PASci, Poznań, Poland (a) (A. Kowalska).
Department of Nuclear Medicine, Medical School, Poznań, Poland (b) (D. Lenart-Jankowska, R. Junik, J. Sowiński, M. Gembicki).

Publications

Geppert A, Losy J: Significance of TNF in pathogenesis of multiple sclerosis. *Post Psychiatr Neurol* 1997, 6, 73-77 (in Polish).

Lenart-Jankowska D, Junik R, Sowiński J, Gembicki M, Wender M: Cognitive decline and regional cerebral blood flow in SPECT pattern in Alzheimer disease. *Nowiny Lekarskie* 1997, 66, 288-293 (in Polish).

Lenart-Jankowska D, Wender M: Contribution to the problem of posttraumatic apalic syndrom. *Neurol Neurochir Pol* 1997, 31, 587-591.

Losy J: Oligoclonal IgG in immunological diseases of the nervous system. Significance in diagnostics and in evaluation of treatment results. *Post Psychiatr Neurol* 1997, 6, 23-28.

Losy J, Geppert A: TNFα in patients with multiple sclerosis treated with steroids. Correlation with MRI studies. *Nowiny Lekarskie* 1997, 66, 413--417 (in Polish).

Losy J, Wender M: IgG subclasses in patients with syphilis of the nervous system. *Neurol Neurochir Pol* 1997, 31, 19-26.

Michałowska-Wender G, Nowak J, Wender M: Presence of mono-oligoclonal rearrangement of Vδ-Jδ TCR genes in myasthenia gravis. *Basic Appl Myol* 1997, 7, 271-274.

Nowak J, Michałowska-Wender G, Januszkiewicz D, Wender M: Limited junctional diversity of Vδ5-Jδ1 rearrangement in multiple sclerosis patients. *Mol Chem Neuropathol* 1997, 30, 95-100.

Wender M, Losy J: Update paper of the scientist panel on demyelinating diseases: Is multiple sclerosis still a diagnostic and therapeutic enigma? *Europ J Neurol* 1997, 4, 429-430.

DEPARTMENT OF APPLIED PHYSIOLOGY

Head: Professor Hanna Kaciuba-Uściłko
5 Pawińskiego St., 02-106 Warsaw
Telephones: 668 54 45, 608 65 18
Fax: 668 54 45
E-mail: kaciuba@cmdik.pan.pl

FACTORS MODIFYING HEMODYNAMIC, METABOLIC AND NEUROHORMONAL RESPONSES TO PHYSIOLOGICAL STIMULI. EARLY PHYSIOLOGICAL EFFECTS OF MODERATE AEROBIC TRAINING

Research team

K. Nazar, B. Bicz, J. Chwalbińska-Moneta, H. Kaciuba-Uściłko, B. Kruk, K. Krzemiński, A. Ziemba

The aim of this work was to follow-up changes in physiological responses to incremental graded exercise during three weeks of moderate endurance training. Twelve healthy, sedentary men (22.0±0.7 yrs, VO_2max 2.89±0.111·min^{-1}) volunteered for this study. The subjects were submitted to 4 cycloergometer exercise tests of increasing intensity until volitional exhaustion: twice before training with one week interval (C1 and C2), and then after one (T1) and three (T3) weeks of training. In the first week the subjects completed 4 sessions, and in the following period 3 sessions per week. The training session included 45 min of bicycle exercise at HR corresponding to the blood lactate threshold. During exercise tests HR, VO_2 and VCO_2, blood pressure (BP) and electrical activity (EMG) of *rectus femoris*, *triceps femoris*, *soleus* and *trapezius* muscles were recorded. After each load venous blood samples were taken for blood lactate (LA), plasma ammonia (NH_4), and catecholamine (CA) determinations. Before exercise and after the last load, plasma growth hormone (hGH) and cortisol concentrations were measured. The exercise loads associated with a rapid increase in the amplitude of EMG, blood LA, NH_4, and CA concentrations were defined as the thresholds of these variables.

The data showed that 10 sessions of moderate training led to improvement of exercise tolerance, a significant increase in VO_2max, a decrease in submaximal HR and shifts the plasma catecholamine and EMG thresholds

towards higher work intensities. However, the comparison of physiological responses to exercise in the two control trials performed before training suggests that a part of early changes attributed to training may be caused by familiarisation of sedentary subjects to exercise testing protocol. This is also indicated by a decline of pre-exercise plasma hGH and cortisol levels during the experiment.

EFFECT OF THREE-DAY BED-REST ON HEMODYNAMIC, METABOLIC AND HORMONAL RESPONSES TO PHYSIOLOGICAL STIMULI IN SEDENTARY MEN AND IN ATHLETES

Research team

B. Bicz, G. Cybulski, H. Kaciuba-Uściłko, A. Kodrzycka, K. Nazar

Continuing the study on the effect of habitual physical activity on bed-rest deconditioning, an effect of three-day bed-rest on hemodynamic, metabolic and hormonal responses to graded exercise and hand cooling (cold pressor test) was investigated in 12 healthy, sedentary men. It was found that short term hypokinesia results in an elevation of plasma renin activity (PRA) with accompanying increase in heart rate and blood pressure at rest in supine position. During exercise after bed-rest, the subjects were unable to attain the same work loads as before bed-rest. Their pulmonary and respiratory exchange ratio tended to be higher, whilst no differences were noted in blood lactate, plasma catecholamines, and PRA responses to exercise. There was no influence of bed-rest on hemodynamic and sympatho-adrenal reactions to cold-pressor test.

Cooperating unit

Department of Sport Medicine, Academy of Physical Education, Poznań (J. Smorawiński, E. Kamińska).

COMPARISON OF THE AUTONOMIC NERVOUS SYSTEM ACTIVITY IN SEDENTARY MEN AND IN ELITE SWIMMERS

Research team

A. Kodrzycka, W. Niewiadomski

The effect of physical training on the tonic activity of the autonomic nervous system and its reactivity to physiological stimuli is not fully recognised, although it is commonly believed that increased physical activity increases the parasympathetic tonus. In the present study, an attempt was made to obtain more information on this problem by analyzing heart rate variability (HRV) in the subjects in supine position and after standing up. An original, own computer program, containing indices not included in commercial programs, was used for this purpose. In addition, heart rate and blood pressure responses to Valsalva maneuver and static handgrip were examined. Twenty young sedentary men and 11 elite swimmers participated in this study. Each test was performed twice under identical conditions. Comparison of indices characterising the HRV of high frequency in the supine position did not reveal differences between the two groups of subjects. The study did not support, therefore, the hypothesis of enhanced tonic activity of the parasympathetic innervation of the heart in athletes. On the other hand, the amplitude of slow HR fluctuations tended to be diminished in swimmers, which suggests reduction of the sympathetic activity in trained individuals. After standing up, increases in HR and in the amplitude of slow HR fluctuations were greater in swimmers than in sedentary subjects indicating greater reactivity of the sympathetic nervous system to orthostatic stimulus. No differences between groups were found in the responses to Valsalva maneuver and static exercise.

APPLICATION OF HOLTER TYPE REOMONITOR FOR CENTRAL HEMODYNAMICS EVALUATION IN HEALTHY SUBJECTS AND CARDIAC PATIENTS

Research team

G. Cybulski, A. Kodrzycka, W. Niewiadomski

The 4-channel recorder enabling simultaneous collection of ECG and impedance cardiography (ICG) signals from a built-in miniaturised ICG device on 20 MB PCMCIA Flash Memory Card was used for off-line beat-to-beat automatic measurement of heart rate, cardiac output, stroke volume, ejection time and pre-ejection period. This device was constructed in this Department in cooperation with the Technical University of Warsaw. Forty healthy subjects and 10 patients with arrhythmia participated in the study. It was proven that the applied system records with acceptable quality ICG signals during sleep time, normal office activity, static and moderate dynamic exercise on cycloergometer, tilt test and Valsalva maneuver. It was also shown that ambulatory ICG may be applied to follow the beat-to-beat variations in hemodynamics during arrhythmia events, such as atrial fibrillation, supraventricular and ventricular ectopy.

Cooperating unit

Outpatients Cardiac Unit for Diagnosis and Therapy, MRC PASci, Warsaw, Poland (E. Wójcik-Ziółkowska).

EFFECT OF GINSENG EXTRACT ON WORKING ABILITY, METABOLIC AND HORMONAL RESPONSES TO EXERCISE AND PSYCHOMOTOR PERFORMANCE

Research team

B. Kruk, H. Kaciuba-Uściłko, K. Nazar, A.W. Ziemba

Ginseng extracts are commonly used as ergogenic acids, but the data on their effectiveness are controversial. In the present study, 7 subjects were given 200 mg daily of Ginseng (Krka, Slovenia) and 8 subjects received placebo for 6 weeks, in double blind manner. All the subjects were submit

ted to identical training regime during the period of the study. Before and after the treatment, the subjects performed graded incremental exercise until volitional exhaustion. During each exercise load, HR, VO_2 and VCO_2 were recorded, multiple choice reaction time (RT) was measured and venous blood samples were taken for lactate (LA) determinations. Besides, before exercise and after the last load, plasma growth hormone (hGH), cortisol and testosterone concentrations were measured. It was shown that Ginseng extract exerts a significant influence on psychomotor performance evaluated on the basis of RT. The subjects who received Ginseng demonstrated shorter choice RT at rest ($p<0.05$) and during all exercise loads ($p<0.01$) than before the treatment. No such differences were found in the placebo group. The data indicate that Ginseng increases the athletes alertness and improves their integrated sensory-motor function during fatigueing exercise. No effect of Ginseng on working capacity, blood lactate threshold, plasma hGH, cortisol and testosterone concentrations was noted.

Cooperating unit

Department of Physiology, Academy of Physical Education, Katowice, Poland (J. Chmura).

NEURO-HORMONAL CONTROL OF LIPID AND CARBOHYDRATE METABOLISM IN SKELETAL MUSCLE. THE RELATIONSHIP BETWEEN UPTAKE OF NON-ESTERIFIED FATTY ACIDS BY SKELETAL MUSCLE OF THE RAT AND THEIR CELLULAR UTILISATION

Research team

L. Budohoski, I. Falęcka-Wieczorek, M. Synak

Two experimental techniques, the perfused hindlimb preparation and *in vitro soleus* muscle incubation were compared to find out whether fatty acid uptake depends on the availability of fatty acids in a free unbound form (UFA). It was shown that incorporation of palmitate into the *soleus* muscle triacylglycerol (TG) stores closely correlates with the concentration of non-esterified fatty acids (NEFA) in the incubation ($r=0.97$) media. However, when concentration of UFA was maintained at a constant level, despite increasing concentration of NEFA, the incorporation of fatty acids

into TG did not change either in the incubated or perfused *soleus* muscle. This finding strongly supports the hypothesis that uptake of fatty acids by skeletal muscles depends on their availability in the unbound form. It was also shown that the rate of palmitate incorporation into acylglycerol store is almost identical in both procedures used.

THE EFFECT OF ELECTROSTIMULATION ON LIPOPROTEIN LIPASE (LPL) ACTIVITY AND TRIACYLGLYCEROL (TG) CONTENT IN TENOTOMIZED AND DENERVATED SKELETAL MUSCLES OF THE RAT

Research team

A. Dubaniewicz, E. Żernicka

Tenotomy leads to muscle immobilisation which results in a variety of metabolic changes. A significant decrease (50%) in the activity of two forms (extra- and intra-cellular) of LPL was noted in muscles following tenotomy and tenotomy with simultaneous denervation. Electrostimulation of intact muscles caused a two-fold increase in the activity of both forms of LPL but had no effect on tenotomized muscles. When tenotomized and denervated muscles were stimulated, the activity of LPL, especially of its intracellular form, was enhanced. It was, however, lower than in the stimulated intact muscles.

There was no influence of tenotomy and denervation on TG content in the muscles examined. Electrostimulation of intact muscles had no effect on TG content, but it caused a marked decrease in TG content in muscles after tenotomy followed by denervation. The increase in LPL activity after electrostimulation of damaged muscles indicates that changes in activity of this enzyme after tenotomy and denervation are reversible.

Cooperating unit

Department of Physiology, Academy of Physical Education, Katowice, Poland (E. Smol).

LIPID METABOLISM IN SKELETAL MUSCLE OF PERFUSED RAT HINDLIMB

Supported by the State Committee for Scientific Research: grant # 6 P04C 033 10

Research team

L. Budohoski, M. Synak, E. Żernicka

A new technique allowing measurements of intracellular lipid pool was used to determine an influence of 24 hrs of fasting on the rate of incorporation of labeled fatty acids into intracellular triacylglycerol pool in the perfused rat hindlimb muscles. Primary results failed to demonstrate any effect of fasting on this process in skeletal muscles of different fibre types (*soleus*, red and white portion of *quadriceps*, red and white portion of *gastrocnemius* and *plantaris*). It seems possible that 24 hrs of fasting is too short to induce changes in muscle lipid metabolism.

THE EFFECT OF INSULIN LIKE GROWTH FACTOR ON SKELETAL MUSCLE SENSITIVITY TO INSULIN

Supported by the State Committee for Scientific Research: grant # 4 P05B 103 10

Research team

L. Budohoski, A. Dubaniewicz, I. Falęcka-Wieczorek, E. Żernicka

Insulin-like growth factor (IGF-I) stimulates the rate of glucose transport and utilization in skeletal muscles independently of insulin. On the contrary, IGF-II seems to enhance these processes in the presence of low concentration of insulin. Possible contribution of adenosine in regulation of glucose metabolism by IGF I and II was investigated in the *soleus* muscle incubated in the presence of adenosine deaminase which lowers adenosine concentration and 8-phenyltheophylline, the adenosine receptor antagonist.

A new procedure allowing a simultaneous measuring of glucose transport and the rate of glycogen synthesis in the isolated preparation of the rat skeletal muscles was established.

Publications

Cybulski G, Ziółkowska E, Kodrzycka A, Niewiadomski W, Sikora K, Książkiewicz A, Łukasik W, Pałko T: Application of impedance cardiography ambulatory monitoring system for analysis of central hemodynamics in healthy man and arrhythmia patients. *Computers in Cardiology* 1997, 24, 509-512.

Grucza R, Pekkarinen H, Timonen K, Titov E-K, Hänninen O: Physiological responses to cold in relation to the phase of the menstrual cycle and oral contraceptives. *An NY Acad Sci* 1997, 697-701.

Kaciuba-Uściłko H, Langfort J, Zarzeczny R, Nazar K: Influence of low-carbohydrate diet on hormonal responses to graded exercise in relation to lactate threshold. In: *Sport science in a changing world of sports*. Eds: J. Bangsbo, B. Saltin, H. Bonde, Y. Hellsten, B. Ibsen, M, Kjaer, G. Sjogaard. University of Copenhagen, Copenhagen, 1997, pp. 556-557.

Kaciuba-Uściłko H, Nazar K: Diet and exercise metabolism. *Medicina Sportiva* 1997, 1, 19-26.

Kaciuba-Uściłko H, Nazar K, Chmura J, Kruk B, Krzemiński K, Ziemba A, Wiśnik P, Konarska M: Effect of ambient temperature on psychomotor performance during physical exercise. In: *Thermal physiology 1997*. Eds: B. Nielsen-Johannsen, R. Nielsen. The August Krogh Institute, Copenhagen, 1997, pp. 3-6.

Kruk B, Brzezińska Z, Nazar K, Kaciuba-Uściłko H, Falęcka-Wieczorek J, Greenleaf JE: Muscle metabolism during exercise in dogs after 8-week confinement. *J Gravit Physiol* 1997, 3, 13-15.

Kubala P, Smorawiński J, Kaciuba-Uściłko H, Nazar K, Bicz B, Greenleaf JE: Effect of three day bed-rest on circulatory and hormonal responses to active orthostatic test in endurance trained athletes and untrained subjects. *J Gravit Physiol* 1997, 3, 40-41.

Langfort J, Zarzeczny R, Pilis W, Nazar K, Kaciuba-Uściłko H: The effect of a low-carbohydrate diet on performance, hormonal and metabolic responses to a 30-s bout of supramaximal exercise. *Eur J Appl Physiol* 1997, 76, 128-133.

Langfort J, Żernicka E, Mayet-Sornay MH, Dubaniewicz A, Desplanches D: Effects of acute and chronic hindlimb suspension on sensitivity and responsiveness to insulin in the rat soleus muscle. *Biochem Cell Biol* 1997, 75, 41-44.

Nazar K, Kaciuba-Uściłko H, Chmura J, Kruk B, Ziemba AW, Krysztofiak H: Psychomotor performance during fatiguing exercise: effects of work intensity, environmental and nutritional factors. In: *Sport science in a changing world of sports*. Eds: J. Bangsbo, B. Saltin, H. Bonde, Y. Hellsten, B. Ibsen, M,.Kjaer, G. Sjogaard. University of Copenhagen, Copenhagen, 1997, pp. 470-471.

Nazar K, Kaciuba-Uściłko H, Ziemba AW, Krysztofiak H, Wójcik-Ziółkowska E, Niewiadomski W, Chwalbińska-Moneta J, Bicz B, Stupnicka E, Okinczyc A: Physiological characteristics and hormonal profile of young normotensive men with exaggerated blood pressure response to exercise. *Clin Physiol* 1997, 17, 1-18.

Smorawiński J, Kubala P, Kaciuba-Uściłko H, Nazar K, Titow-Stupnicka E, Greenleaf JE: Effects of three day bed-rest on circulatory, metabolic and hormonal responses to oral glucose load in endurance trained athletes and untrained subjects. *J Gravit Physiol* 1997, 3, 44-45.

Smorawiński J, Kubala P, Klamecka E, Kaciuba-Uściłko H, Nazar K, Greenleaf JE: Hormonal responses to oral glucose load and active orthostatic test in trained and untrained subjects after three days of bed-rest. In: *Sport science in a changing world of sports*. Eds: J. Bangsbo, B. Saltin, H. Bonde, Y. Hellsten, B. Ibsen, M. Kjaer, G. Sjogaard. University of Copenhagen, Copenhagen, 1997, pp. 554-555.

Torlińska T, Langfort J, Maćkowiak P, Hryniewiecki T, Kaciuba-Uściłko H, Nogowski L: Effect of hypothermia on 2-deoxy-glucose transport, insulin binding and insulin sensitivity of the rat soleus muscle. *Pathophysiology* 1997, 4, 205-212.

LABORATORY OF RENAL AND BODY FLUID PHYSIOLOGY

Head: Professor Janusz Sadowski
5 Pawińskiego St., 02-106 Warsaw
Telephones: 668 53 77, 608 65 46
E-mail: renal@cmdik.pan.pl

STUDIES OF THE MECHANISM OF VASOPRESSIN NATRIURESIS

Research team

J. Sadowski, E. Kompanowska-Jezierska, A. Walkowska

In addition to the best known effect of vasopressin which increases permeability of the renal collecting duct to water, natriuresis was repeatedly reported after administration of pharmacologic doses of the hormone. In this study, renal effects of physiological amounts of vasopressin were examined in conscious dogs during servo-controlled overhydration (2% body wt).

During infusion of vasopressin (50 pg/min·kg body wt.) plasma vasopressin concentration increased to 2.30±0.20 pg/ml compared with 0.12±0.03 pg/ml during control (water diuresis). With vasopressin infusion, urine flow was significantly lower (0.30±0.10 ml/min) and sodium excretion ($U_{Na}V$) significantly higher (58.0±15.8 μmol/min) than without vasopressin (4.6±0.4 ml/min and 14.4±4.1 μmol/min, respectively, dDAVP, a V_2 receptor agonist (4 pg/min·kg) mimicked the antidiuretic response (0.20±0.03 ml/min) without changing $U_{Na}V$ (9.7±4.4 μmol/min).

Indomethacin given during AVP infusion suppressed prostaglandin E_2 excretion, intensified the antidiuresis (0.10±0.02 ml/min) and abolished the natriuresis (13.4±3.7 μmol/min). During AVP infusion, $U_{Na}V$ was highly correlated (r=0.85) with prostaglandin E_2 excretion.

Blood pressure, glomerular filtration rate, plasma atrial natriuretic peptide concentration, and the rate of proximal tubular reabsorption (derived from lithium clearance) were similar in all series.

The data indicate that in the dog physiological amounts of vasopressin can induce natriuresis, probably through activation of V_1 receptors and an increase in intrarenal synthesis of prostaglandins. This action could contribute,

in concert with the vasopressin dependent water retention, to the defence of the organism against hypernatremia.

Cooperating unit

Laboratory of Body Fluid Homeostasis, The Panum Institute, University of Copenhagen, Denmark (P. Bie, C. Emmeluth, L. Grove).

ROLE OF RENAL HEMODYNAMICS IN MODULATION OF THE CORTICO-PAPILLARY ELECTROLYTE GRADIENT

Supported by the State Committee for Scientific Research: grant # 4 P05A 013 08

POST-INDOMETHACIN NATRIURESIS AND DIURESIS IN THE HYPERTONIC SALINE LOADED RAT KIDNEY

Research team

J. Sadowski, B. Bądzyńska, L. Dobrowolski, E. Kompanowska-Jezierska, A. Walkowska

The usual renal response to blockade of prostaglandin biosynthesis with indomethacin includes depression of renal hemodynamics, antinatriuresis and an increase in urine osmolality (Uosm). In our control anesthetized rats (n=8) given suprarenal aortic infusion of 0.9% NaCl (2 ml/h), indomethacin (5 mg/kg i.v.) decreased GFR (^{3}H-inulin clearance) by 27% ($p<0.08$) and urine flow (V) by 36% whereas urine [Na^+] increased by 60% and sodium excretion ($U_{Na}V$) did not change.

When 5% rather than isotonic saline was infused, indomethacin injection increased V from 14.7±3.3 to 40.8±6.7 µl/min ($p<0.005$) and $U_{Na}V$ from 2.0±0.6 to 7.6±1.3 µmol/min ($p<0.001$). The renal perfusion pressure was maintained constant by means of a suprarenal aortic snare and no changes in GFR and cortical blood flow (CBF; laser Doppler probe) were observed. Urine [Na^+] increased from 123±11.4 to 183±9.1 mM ($p<0.005$) whereas urine osmolality did not change. Indomethacin injection did not affect CBF but decreased the medullary blood flow (needle laser-Doppler probe) by 36 and 35% in isotonic and in hypertonic saline infused rats, respectively ($p<0.001$), indicating tonic vasodilator influence of prostaglandins on the medullary circulation.

The electrical admittance of medullary tissue, an index of ion concentration, increased significantly after indomethacin, both in isotonic and in 5% saline infused rats.

Sustained sodium excretion despite decreasing GFR and V, as seen after indomethacin in the isotonic saline infused kidney, suggested some drug dependent inhibition of salt transport. This effect was probably greatly amplified in the hypertonic saline infused kidney, leading to pronounced natriuresis. We interpret this unexpected post-indomethacin natriuresis as follows. The blockade of cyclooxygenase shifts arachidonic acid metabolism in the kidney to other pathways, possibly to cytochrome P450 route, resulting in enhanced biosynthesis of certain natriuretic and diuretic eicosanoids (McGiff, *Ann Rev Pharmacol Toxicol*, 1991).

Publications

Dobrowolski L, Endlich K, Sadowski J, Steinhausen M: Cardiovascular and renal effects of endothelin receptor blockade with PD 145065 and interaction with urodilatin. *Acta Physiol Scand* 1997, 159, 7-13.

Kompanowska-Jezierska E, Walkowska A, Sadowski J: Reinvestigation of water diuresis in the rat: role of anesthesia and acute surgery. *Archiv Physiol Bioch* 1997, 105, 1-4.

Sadowski J, Kompanowska-Jezierska E, Dobrowolski L, Walkowska A, Bądzyńska B: Simultaneous recording of tissue ion content and blood flow in rat renal medulla: evidence on interdependence. *Am J Physiol* 1997, 273 (*Renal Physiol* 42), F658-F662.

OUTPATIENT CARDIAC UNIT FOR DIAGNOSIS AND THERAPY

Head: Dr. Ewa Wójcik-Ziółkowska
5 Pawińskiego St., 02-106 Warsaw
Telephone: 658 46 43

SIGNIFICANCE OF ANGINA PECTORIS IN THE COURSE OF CORONARY HEART DISEASE LONG TERM AFTER MYOCARDIAL INFARCTION

Research team

E. Wójcik-Ziółkowska, W. Pawłowska-Jenerowicz, M. Płachcińska-Bijak, C. Romiszowska

In 1974 Stern and Tzivoni published the first report on the incidence of silent myocardial ischemia detected in ECG recorded by the Holter method. Since that time, the term "angina pectoris" has not been used as a synonym of coronary heart disease (CHD) and the syndrome of the "silent myocardial ischemia" was introduced. The incidence of this syndrome is approx. 5% in general population and silent myocardial ischemia occurs in 40 to 60% of patients with CHD. It is still not clear (1) which form of CHD is more severe, especially in patients after myocardial infarction, (2) what is the prognostic value of the presence of coronary chest pain and (3) whether the lack of pain in patients with silent ischemia reflects their diminished pain perception. Among a number of publications on these problems only in few studies the patients were examined after several years following myocardial infarction.

The aims of the present study were as follows:

1. Comparison of coronary insufficiency in patients with silent and painful ischemia at rest and during exercise;

2. Comparison of left ventricular function in patients with silent and painful ischemia;

3. Evaluation of significance of angina pectoris presence for treatment strategy in patients with silent and painful ischemia.

The study involved 78 men (mean age 61.3±4.8 years) being 5 or more years after myocardial infarction. According to the absence or presence of angina pectoris, the patients were assigned into two groups: "silent group"

consisting of 37 patients (47% of the total group) and "angina group" including 41 patients (52.5% of the total group).

The following examinations were made in all patients:

– 24 hour ECG monitoring by Holter method for determination of the ST segment depression;

– echocardiography for evaluation of left ventricular function;

– cardiopulmonary exercise test for assessment of exercise capacity;

– evaluation of myocardial perfusion by sestamibi single photon emission – computed tomography (SPECT) at rest and during exercise (number of persistent and transient ischemic incidents).

The results of the study indicated that irrespectively of location and extent of infarction area, nearly half of the patients had silent ischemia. No differences between the groups were found in the degree of coronary insufficiency determined by exercise tests and SPECT results. The groups did not differ in the range of left ventricular dysfunction estimated by echocardiography.

It is concluded that angina pectoris is not a criterion of CHD severity and a symptom of more advanced disease even in the long-term follow-up after myocardial infarction. Thus, the patients with silent ischemia should be diagnosed, supervised and treated similarly to those with angina pectoris.

CARDIOVASCULAR LABORATORY

Head: Professor Krystyna Cedro-Ceremużyńska
5 Pawińskiego St., 02-106 Warsaw
Telephones: 668 52 43, 608 64 11
E-mail: ceremuzynska@cmdik.pan.pl

CLINICAL STUDIES ON L-ARGININE IN CORONARY HEART DISEASE AND CONGESTIVE HEART FAILURE

Research worker
K. Cedro-Ceremużyńska

Effect of L-arginine supplementation on exercise capacity in patients with congestive heart failure

Endothelium-dependent vascular dilation is depressed in congestive heart failure. Since nitric oxide (NO) contributes to exercise-induced vasodilation, we aimed to investigate whether supplementation with NO substrate, L-arginine enhances exercise-induced vasodilation and, in consequence, improves exercise capacity in patients with congestive heart failure. We have recently documented increased exercise capacity in stable angina pectoris after L-arginine supplementation (Am. J. Cardiol. 1997, 80, 331-333).

The protocol includes males with congestive heart failure in NYHA class II-IV with ejection fraction <40%, in stable clinical condition, on treatment with diuretic, ACE inhibitor, digoxin. Patients are randomized to L-arginine (9 g/day) orally or placebo for one week. After one week of the wash-out period, the treatment is crossed-over for the final week of the study. Exercise test is performed at the beginning of each week and on the last day of the whole study period. The study is in progress.

Effect of amiodarone on oxygen free radicals production by isolated leucocytes *ex vivo*

Amiodarone is a class III antiarrhythmic agent with adrenoreceptor blocking and vasodilator properties, effective in the treatment of supraventricular and ventricular arrhythmias. Biochemical mechanisms underlying its com

plex pharmacological properties are not fully elucidated. The concept that oxygen free radicals are involved in generation of arrhythmias has recently received considerable support in experimental and clinical studies. Activated neutrophils are effective source of oxygen free radicals in ischemic/reperfused myocardium. Amiodarone was shown *in vitro* to inhibit neutrophil oxygen free radicals production (Basic Res. Cardiol. 1991, 86, 335-362). If this effect occurs *in vivo*, it may contribute to antiarrhythmic and cardioprotective action of the drug. In the present study we investigate the effect of amiodarone infused i.v. (10 mg/kg) on oxygen free radicals production by isolated leucocytes *ex vivo*. The protocol includes the patients with ventricular and/or supraventricular tachycardia in the past, subjected to programmed ventricular stimulation to evaluate the effect of amiodarone on electrophysiological parameters indicating risk of recurrent serious arrhythmias. Blood is sampled before and 30 min after amiodarone infusion. Oxygen free radical production by isolated, arachidonic acid-stimulated leucocytes is measured by lucigenin-amplified chemiluminescence. The study is in progress.

Cooperating unit

Department of Cardiology, Postgraduate Medical School, Warsaw, Poland (L. Ceremużyński).

Publication

Ceremużyński L, Chamiec T, Herbaczyńska-Cedro K: Effect of supplemental oral L-arginine on exercise capacity in patients with stable angina pectoris. *Am J Cardiol*, 1997, 80, 331-333.

DEPARTMENT OF SURGICAL RESEARCH AND TRANSPLANTOLOGY

Head: Professor Waldemar L. Olszewski
5 Pawińskiego St., 02-106 Warsaw
Telephone: 668 53 16
Fax: 668 53 34
E-mail: wlo@cmdik.pan.pl

LOCAL IMMUNE REACTION IN ALLOGRAFTS

Research team

W.L. Olszewski, M. Maksymowicz, A. Namysłowski, E. Poreda

The immunosuppressive drug Cyclosporin A is administered to allograft recipients in two preparations: as suspension in oil (Sandimmune), and as emulgated suspension (Neoral). The first is administered intramuscularly or intravenously, whereas, the other orally. We studied the effect of both preparations on the migration process to the heart allograft of the *in vivo* CsA-pretreated lymphocytes. Although the given dose of CsA was the same in both situations, the serum concentration of Neoral was on the average 2–3-times higher than after administration of Sandimmune. The accumulation of pretreated lymphocytes in the allograft was decreased in both groups compared to controls, however, no differences between the Sandimmune and Neoral were observed: there was similar accumulation rate of pretreated lymphocytes in the mesenteric lymph nodes in both groups. In contrast, Neoral brough about stimulation and Sandimmune inhibition of the response of lymphocytes to mitogens. The results point to various effects of CsA on lymphocytes function depending on preparation and route of administration.

CYTOKINES AND HEMOPOIETIC CELLS IN PERIPHERAL BLOOD IN PATIENTS AFTER SURGICAL TRAUMA

Research team

W.L. Olszewski, I. Grzelak

The response of human body to trauma is multifactorial. One of the observed effects of trauma is the appearance in peripheral blood of leukocyte-produced cytokines concomitantly with certain subsets of hemopoietic cells. We studied the effect of medium and low degree surgical trauma (open and laparoscopic cholecystectomy) on serum cytokine level and appearance in peripheral blood of immature bone marrow cells. The surgical trauma produced an increase in serum concentration of IL6, IL6sR, IL8 and GM CSF. Among the blood cell population, the CD34 (stem cell) and early myeloid cells with CD13, CD14 and CD33 phenotypes were found. Open cholecystectomy evoked more intensive changes than the laparoscopic procedure.

Another study was devoted to the immune deficiency developing in malnourished patients with protracted infected wound healing and fistulae of the upper part of the gastrointestinal tract. Parenteral nutrition brought about in this group of patients recovery of basic immune parameters as lymphocytosis, CD4/8 ratio and *in vitro* response of blood lymphocytes to PHA and ConA.

Cooperating units

Department of Transplantation and General Surgery, Medical School, Warsaw, Poland.

Clinic of Gastroenterological Surgery and Nutrition, Medical School, Warsaw, Poland.

RECONSTITUTION OF BONE MARROW CAVITIES AND LYMPHOID TISSUES AFTER VASCULARIZED BONE MARROW TRANSPLANTATION

Research team
B. Łukomska, M. Durlik, S. Janczewska, W.L. Olszewski

We have reported previously that bone marrow transplantation in an orthotopic hind limb graft (vascularized bone marrow transplantation – VBMT) to lethally irradiated syngeneic recipients brings about complete hemopoietic repopulation of bone marrow cavities within 10 days. The purpose of this study was to investigate the lymphopoietic reconstitution of peripheral blood and lymph nodes of irradiated rats after VBMT in comparison to i.v. bone marrow cell (BMC) infusion. Lewis rats were irradiated and repopulated with syngeneic BMC introduced intravenously or in orthotopic hind limb graft. Vascularized bone marrow transplantation was more effective in repopulation of bone marrow cavities of lethally irradiated rats than BMC infusion. Orthotopic hind limb graft promoted rapid lymphocyte replenishment of peripheral blood (PB) and lymph nodes (MLNL) of lethally irradiated syngeneic recipients. The population rate of BMC, PBL and MLNL was higher after VBMT than BMC injection in suspension. The percentage of T and B lymphocytes in PB and MLNL on day 10 after VBMT was comparable with control values. Reconstituted PBL showed two subsets of CD4+ cells: "bright" and "dull". All $CD4^+$ cells in PBL of i.v. BMC infused recipients expressed low level of this molecule ("dull" subset). The population of $CD4^{+dull}$ cells may represent immature cells. The $CD4^{+dull}$ were not found in MLNL of VBMT or i.v. BMC infused rats. All MLNL $CD4^+$ cells represented the $CD4^{+bright}$ subset, what suggests that the process of cell maturation takes place in the lymphoid organs. The results of our studies indicate that the presence of stromal cells in their close relationship with stem cells is essential for the fast hematopoietic repopulation of lymphoid tissues of irradiated recipients.

KINETICS OF IMMUNE CELL ACCUMULATION IN INFLAMMED SKIN

Research team

W.L. Olszewski, H. Gałkowska, I. Grzelak

Extravasation of lymphocytes, macrophages and dendritic cells in skin depends on the expression of adhesion molecules and their ligands, and is related to fenotype changes and activation of genes for cytokines. We observed, in studies on leg chronic dermatitis in patients with lymph stasis and in patients with arthritis, in the extravascular space, memory T lymphocytes (CD45RO, CD4) with IL2 receptor (CD25) and MHC class II antigens (HLADR). The percentage of $CD25^+DR^+$ lymphocytes correlated with clinical intensity of inflammation. Moreover, we observed that lymphocytes from extravascular space expressed more intracellular cytokines as IL1, IL6, IL8, TNFα and GM-CSF, than lymphocytes from the peripheral blood of the same patients. It remains an open question whether changes of phenotypes and cytokine expression are due to extravasation of these cell populations or such activation of genes occurs after transmigration of cells to the tissue space. Especially important observation concerned the different levels of cytokines in tissue fluid and serum of our patients. Concentration of IL6, IL8, TNFα, IL1Ra was several times higher in tissue fluid of patients with chronic dermatitis and 40× higher in acute inflammation than in controls. This observation points out that in the inflammed tissues the local production and release of these cytokines takes place, whereas the cytokines influx from blood capillaries is insignificant.

LOCAL IMMUNE RESPONSE IN THE LIVER TO PROLIFERATING TUMOR CELLS AND IMMUNOMODULATION FOR PREVENTION OF LIVER METASTASES FORMATION

Supported by the State Committee for Scientific Research: grant # 4 P05B 020 08

Research team

B. Łukomska, W.L. Olszewski

Liver is a principal barrier preventing systemic dissemination of tumor cells migrating from splanchnic organs with the portal blood stream. There

is evidence that lymphocytes marginating in the liver sinusoids play a principal role in local destruction of tumor cells tending to form metastases in the liver. Tumor burden can modify the responses of potentially reactive lymphocytes. In our previous studies we showed that liver sinusoidal lymphocytes adjacent to adenocarcinoma metastases (LSL-2) expressed lower cytotoxic activity against K-562 (NK-related) and Raji cells (LAK-related) than liver sinusoidal lymphocytes isolated from remote healthy areas (LSL-1) of liver. The present study was designed to investigate the proliferative ability of liver sinusoidal lymphocytes in patients with benign and malignant liver tumors. Thirty five patients undergoing partial hepatectomy for benign, primary and metastatic colorectal adenocarcinoma tumors were included in the study. Liver sinusoidal lymphocytes isolated from the peritumoral tissue (LSL-2) expressed 3-4 fold higher proliferation response to PHA than lymphocytes from normal liver fragments (LSL-1). There were no statistically significant differences between LAL-2 isolated from benign, primary and metastatic tumors. It seems that focal tumor growth within the liver results in an increase of peritumoral LSL proliferation. Extensive lymphocyte proliferation may limit quantitatively other cell function (e.g. cytotoxicity).

Cooperating units

3rd Department of Surgery, 2nd Faculty of Medicine, Medical School, Warsaw, Poland (J. Polański).

Groupe de Recherches pour l`Etude du Fois, Université de Bordeaux II, Bordeaux, France (M. Winnock).

Services de Chirurgie Digestive, Hôpital Saint-André, Bordeaux, France (J. Saric).

HUMAN PERITONEAL IMMUNE CELLS IN INFLAMMATION AND GASTROINTESTINAL TUMORS

Supported by the State Committee for Scientific Research: grant # 4 S402 128 07

Research team

U. Kubicka, W.L. Olszewski

From our previous studies we know that colon tumors evoke recruitment of myelomonoidal cells into human peritoneal cavity, whereas gastric cancer elicit accumulation of lymphocytes. The process of recruitment of immune cells is regulated by cytokines. The aim of the study was to evaluate the cytokine profile of peritoneal cells in patients with gastric and colon cancer. Intracellular cytokines IL1β, TNFα, IL6, IL8 were identified on cell smears with immunocytochemical staining using specific antibodies. The level of cytokines in peritoneal fluid was evaluated with ELISA. Studies were carried out on patients with adenocarcinoma of stomach (n=20, gr. I), and of colon (n=30, gr. II). Patients undergoing elective cholecystectomy served as controls (n=36). There was a decreased frequency of peritoneal macrophages with intracellular $TNF\alpha^+$ and $IL1\beta^-$ in the whole peritoneal cell population in group II, in comparison with control, and an increased percentage of macrophages with $IL6^+$ and $IL8^+$ in both groups of patients. The cytokine expression in peritoneal lymphocytes was weak. The level of cytokines in the peritoneal fluid was correlated with their intracellular deposition. In summary, adenocarcinoma of colon evokes local immune response in the peritoneal cavity, leading to cytokine release, cell recruitment and their activation. Less evident changes were observed in the peritoneal cavity of patients with adenocarcinoma of the stomach.

Cooperating unit

Clinic of General Surgery, Medical Centre of Postgraduate Education, Warsaw, Poland.

PATHOMECHANISM OF PROTRACTED WOUND HEALING – DISORDERS IN THE PRODUCTION OF GROWTH FACTORS REGULATING FIBROBLAST AND KERATINOCYTE PROLIFERATION PROPOSED THERAPY

Supported by the State Committee for Scientific Research: grant # 4 P05C 030 10

Research team

H. Gałkowska, W.L. Olszewski

We analysed intracellular cytokines in epidermal and infiltrating cells in patients with: a) lower leg varicous ulcer and b) incisional surgical wound. Studies were carried out using specific antibodies and immunohistochemical techniques. The presence of IL1α, IL1β, TNFα, IL6, IL8, TGFβ, PDGFa, FGFb, EGF, GM-CSF, VEGF and receptors for TGFβ and EGF was followed. In group a) at the edge of ulcer the presence of IL1α and β in keratinocytes and infiltrating cells was observed. IL8, absent from cells of surgical incisional wound, appeared in endothelial and infiltrating cells at the edge of ulcer. In contrast, TNFα, present in normal epidermis, was seen in group a) only in cornified layer. PDGFa, GM-CSF and FGFb, present in epidermal and endothelial cells of surgical wound, were seen at the edge of ulcer in infiltrating cells. VEGF was observed in endothelial cells at the edge of ulcer. TGFβ, present in the perivascular space of normal wound, was seen in keratinocytes and infiltrating cells, at the edge of ulcer. The infiltrating cells expressed receptors for TGFβ, not detected in normal skin. EGF, present in epidermis and endothelial cells of normal skin, was found at the edge of ulcer only in the cornified layer. Keratinocytes of the basal layer expressed EGF receptor, normally not detected in epidermis. The observations suggest that basic differences in expression of cytokines in the skin ulcer take place not in dermis but in epidermis. At the edge of ulcer, where the proliferation and migration of keratinocytes takes place, cytokine expression is depressed.

PATHOMECHANISM OF POSTTRAUMATIC EDEMA IN THE LOWER EXTREMITIES

Supported by the State Committee for Scientific Research: grant # 4 P05C 037 10

Research team

W.L. Olszewski, G. Szczęsny

The aim of our study was to study an influence of standardised mechanical trauma on lymphatic outflow from the lower extremity. Mechanical trauma applied to lower extremity caused a severe local soft tissue injury with puncture skin wound and bleeding from disrupted blood vessels. Moreover, edema distally to the site of injury occurred, what signs of structural lymphatic disruption. Analysis of the time of the first visualisation of lymphatics revealed an increased dynamic of lymph outflow from traumatised extremities in comparison with control animals, where lymphatics became visible after 60-90 minutes. Comparison of the summarised relative lymphatic vessels diameters revealed after trauma higher values at each observation level in comparison with controls. In summary, direct mechanical trauma affecting of the lower extremity does not produce structural damage of the lymphatic vessels draining distal parts of the extremity. An increased lymph outflow from traumatised extremity has been observed, mostly likely to injury of veins and subsequent venous blood stases.

Cooperating unit

Institut für Chirurgische Forschung, Klinikum Großhadern, Ludwig-Maximilians Universität, Münich, Germany (D. Nolte, A. Veihelmann, K. Messmer).

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

Research team

W.L. Olszewski, J. Dłużniewska, S. Durowicz, B. Łukomska

Studies strongly suggest that liver plays an important immunoregulatory role in systemic and local immunity. The local immune function of liver is associated with a specific lymphocyte population marginating in liver sinusoids. They seem to be responsible for destruction of malignant cells, which have reached liver *via* the bloodstream. In the present study we investigated if the presence of colon cancer metastases (evoked by inoculation of CC351 colon adenocarcinoma cells, into the portal vein of syngeneic WAG rats) had influence on types and cytotoxic properties of cells trapped in liver sinusoids. Monoclonal antibody analysis showed a prevalence in sinusoids of liver with metastases compared to control liver, of helper/inducer W3/25+, class II+OX6+, NK cells 3.2.3.+, monocyte/macrophage ED1+, whereas fewer of T cells OX19+. After 1 h perfusion with syngeneic blood, livers with metastases trapped in sinusoids cells, which displayed the same characteristic as before perfusion, with an evidently higher than in normal liver percentage of W3/25, OX6+, ED1+ and 3.2.3.+ cells. In normal rats, sinusoidal cells revealed higher cytotoxic activity against CC531 and K562 cells than blood cells. Cells from liver sinusoids with metastases revealed lower cytotoxicity compared to cells of normal liver. Interestingly, the cytotoxic activity of cells trapped in liver sinusoids during 1 h perfusion with syngeneic blood was significantly higher compared to cells residing in neighbourhood of cancer cells for longer time. In conclusion, rat liver with metastases of colon adenocarcinoma retains in sinusoids a population of blood cells enriched in CD4+, NK, monocyte/macrophage, MHC class II+ cells. Under normal conditions liver-associated lymphocytes express higher cytotoxic activity against tumor cells than blood isolated mononuclear cells. Long lasting liver tumor metastases decrease cytotoxic activity of liver sinusoidal lymphocytes.

Publications

Durlik M, Łukomska B, Namysłowski A, Cybulska E, Janczewska S, Olszewski WL: The kinetics of seeding of syngeneic cells from vascularized bone marrow grafts. *Transplant Proc* 1997, 29, 2008-2009.

Durlik M, Łukomska B, Namysłowski A, Janczewska S, Cybulska E, Olszewski WL: Long-term survival of limb allografts after weaning of cyclosporine: possible role of microchimerism. *Transplant Proc* 1997, 29, 1226-1227.

Durowicz S, Olszewski WL: Liver associated lymphocytes – adherence and release mechanism. In: *Cells of the hepatic sinusoid*, vol 6. Eds: E. Wisse, D.L. Knook, C. Balabaud. Kupffer Cell Foundation, 1997, pp. 218-220.

Durowicz S, Olszewski WL: Mechanism of trapping of intravenously transplanted lymphocytes in the liver. *Transplant Proc* 1997, 29, 2010-2011.

Gałkowska H, Olszewski WL, Interewicz B, Porębska A, Ziółkowska A, Wojewódzka U: Transplantation biology – how to control the activation of cutaneous dendritic cells. *Central-Europ J Immunol* 1997, 22, 20-26.

Grzelak I, Olszewski WL, Zaleska M, Ziółkowska A, Durlik M, Łagiewska B, Muszyński M, Rowiński W, Słotwiński R: Surgical trauma evokes changes in the frequency of mononuclear cell populations in blood circulation. *Central-Europ J Immunol* 1997, 22, 298-304.

Grzelak I, Zaleska M, Olszewski WL: Immune response after syngeneic and allogeneic blood cell transplantation. *Transplant Proc* 1997, 29, 2181-2182.

Jaskłowska-Englisz M, Olszewski WL, Maksymowicz M, Poreda E, Ziółkowska A: Humoral mechanism of split tolerance to lymphocyte and heart allografts after donor-specific transfusion. *Transplant Proc*, 1997, 29, 2185-2188.

Łukomska B, Garcia-Barcina M, Gawron W, Winnock M, Interewicz B, Saric J, Balabaud Ch, Olszewski WL: Expression of cell adhesion molecules on liver-associated lymphocytes and their counter-receptors on sinusoidal lining cells in human livers promotes margination of blood cells in the liver microvasculature. *Central-Europ J Immunol* 1997, 22, 305-312.

Łukomska B, Winnock M, Balabaud Ch, Saric J, Polański J, Olszewski WL: Human liver passengers cells suppress autologous and allogeneic blood lymphocytes proliferation. *Transplant Proc* 1997, 29, 1105-1107.

Łukomska B, Winnock M, Balabaud Ch, Saric J, Polański J, Olszewski WL: Liver sinusoidal lymphocytes in humans: their potential role in the suppression of host alloresponse. *Central-Europ J Immunol* 1997, 22, 27-32.

Łukomska B, Winnock M, Balabaud Ch, Saric J, Polański J, Olszewski WL: Passenger lymphocytes in human liver graft and their potential role in the suppression host alloresponse. In: *Cells of the hepatic sinusoid*, vol 6. Eds: E. Wisse, D.L. Knook, C. Balabaud. Kupffer Cell Foundation, 1997, pp. 221-224.

Olszewski WL: La linfangiologia in Europa e nel mondo 1994. *Minerwa Cardioangiol* 1997, 45, 7-8.

Olszewski WL: The recurrent bacterial inflammation of skin, lymphatics and lymph nodes causes progressive lymphedema. *Flebolimfologia* 1997, 5, 11-15 (in Polish).

Olszewski WL, Grzelak I, Ziółkowska A, Engeset A: Adhesion molecules on immune cells trafficking through the normal human skin. *Central--Europ J Immunol* 1997, 22, 292-297.

Olszewski WL, Jamal S, Manokaran G, Pani S, Kumaraswami V, Kubicka U, Łukomska B, Dworczyński A, Swoboda E, Meisel-Mikołajczyk F: Bacteriological studies of skin, tissue fluid, lymph and lymph nodes in patients with filarial lymphedema. *Am J Tropic Med Hyg* 1997, 57, 7-15.

Olszewski WL, Jaskłowska-Englisz M, Interewicz B: Hepatocyte transplantation – granulocytes recognize surface of isolated autologous hepatocytes as non-self and destroy them. *Transplant Proc* 1997, 29, 1113-1115.

Olszewski WL, Łukomska B, Durlik M, Namysłowski A, Laszuk D, Cybulska E: Nonspecific rapid elimination of transplanted allogeneic bone marrow cells. *Transplant Proc* 1997, 29, 2073-2074.

Olszewski WL, Łukomska B, Gawron W: *In vitro* liver tissue-lymphocytes adherence assay: a method for studies of lymphocyte interactions with liver cells in normal and pathological conditions. *Central-Europ J Immunol* 1997, 22, 286-291.

Szczęsny G: Angiogenesis and wound healing: I – the process of angiogenesis. *Medycyna Sportowa* 1997, 13, nr 76, 27-31 (in Polish).

Szczęsny G, Olszewski WL: Influence of heparin and non-steroid anti-inflammatory drugs on lymphatic drainage in tissues. *Medycyna Sportowa* 1997, 13, nr 66, 6-7 (in Polish).

Winnock M, Łukomska B, Saric J, Olszewski WL, Balabaud C: Characterization of the proliferative response to mitogens by liver-associated lymphocytes. In: *Cells of the hepatic sinusoid*, vol 6. Eds: E. Wisse, D.L. Knook, C. Balabaud. Kupffer Cell Foundation, 1997, pp. 463-465.

NEUROPEPTIDE LABORATORY

Head: Assoc. Professor Andrzej Lipkowski
5 Pawińskiego St., 02-106 Warsaw
Telephones: 668 53 88, 608 64 45
E-mail: lipkowski@cmdik.pan.pl

Research team

A. Lipkowski, P. Jakubowski, D. Kosson, B. Kwiatkowska-Patzer, I. Maszczyńska, A. Minich, A. Misicka-Kęsik, B. Sagan

STRUCTURE-ACTIVITY STUDIES OF NEW OPIOID ANALOGUES

This year's studies were part of several years of studies on the structure-activity relationship of opioid peptides and development of a new analgesics. Previous studies resulted in development of biphalin, opioid peptide analogue in which two tetrapeptide opioid pharmacophores are connected with hydrazide bridge. This year, the study concluded that only part of the biphalin is necessary for expression of full properties of the parent compounds. This observation created possibility of designing a new series of analogues in which one part of biphalin could be replaced with ligands selective to another, non opioid receptors. The example of such hybrides is AA501 which combines opioid and substance P selective ligands.

Cooperating units

New England Medical Center, Boston, USA (D.B. Carr).
Massachusetts General Hospital, Boston, USA (S.K. Szyfelbein).
University of Arizona, USA (V.J. Hruby).
Department of Anesthesiology, Medical School, Warsaw, Poland (J. Śliwowska, D. Julkowski).

PEPTIDES WITH β-STRUCTURE

Study of analogues of SPKK motive interacting with AT rich DNA has been conducted. The DNA-binding and molecular modelling of synthetic analogues resulted in conclusions that SPKK interacts with DNA in beta

conformation, and that hydroxyl group of serine is not necessary for inter-action.

Cooperating unit

Biochemical Department of Warsaw University, Warsaw, Poland (A. Jerzmanowski).

PEPTIDES ANALOGUES WITH AMPHIPHILIC-AMINO ACIDS

Many peptides and proteins interact with membrane proteins in an amphiphilic way. Stabilisation of such amphiphilic shape of flexible peptide chains through local incorporation of amphiphilic amino acids is a basic idea of this project. During the first year of the study, analogues of enkephalin with α-hydroxymethyl-tyrosine and hydroxymethyl-phenylalanine have been synthesised.

Cooperating unit

Department of Organic Chemistry, Technological University, Łódź, Poland (A. Olma).

Publications

Goudas LC, Carr DB, Maszczyńska I, Marchand JE, Wurm WH, Greenblatt DJ, Kream RM: Differential effect of central versus parenteral administration of morphine sulphate on regional concentration of reduced glutathione in rat brain. *Pharmacology* 1997, 54, 92-97.

Misicka A, Lipkowski AW, Horvath R, Davis P, Porreca F, Yamamura HI, Hruby VJ: Structure-activity relationship of biphalin. The synthesis and biological activities of new analogues with modifications in positions 3 and 4. *Life Sci* 1997, 60, 1263-1269.

Przewłocka B, Łobuz D, Mika J, Lipkowski A, van Luijtelaar G, Coenen A, Lasoń W: Protective effects of TRH and its analogues in chemical and genetic models of seizures. *Pol J Pharmacol* 1997, 49, 373-378.

Romanowski M, Zhu X, Rawaswami V, Misicka A, Lipkowski AW, Hruby VJ, O'Brien D: Interaction of highly potent dimeric enkephalin analog, biphalin, with model membranes. *Biochim Biophys Acta* 1997, 1329, 245-258.

DEPARTMENT OF ENDOCRINOLOGY

Head: Professor Janusz Nauman
1a Banacha St., 01-201 Warsaw
Telephone: 659 75 62
Fax: 659 75 62
E-mail: janu@amwaw.edu.pl

T CELL INTERACTIONS WITH EXTRACELLULAR MATRIX PROTEINS IN THE AUTOIMMUNE THYROID DISEASE

Research team

J. Nauman, T. Bednarczuk, J. Kiljański

Pathogenesis of the thyroid-associated ophthalmopathy (TAO) is poorly understood. Although TAO is considered to be an autoimmune disorder, the nature and localisation of the primary antigen(s) being the target of the autoimmune reaction, predominating type of the autoimmune response and natural history of the disorder remain unknown. There is good evidence for the central role of T cells in TAO. The focus of our investigations is on the putative interactions of T cells with extracellular matrix (ECM) proteins as a potential key step in the pathogenesis of the thyroid-associated ophthalmopathy and a possible target of therapeutic intervention.

The results obtained so far indicate that peripheral blood mononuclear cells from patients with autoimmune thyroid disease complicated by active overt ophthalmopathy are stimulated towards proliferation by collagen I and that this stimulation may be antigen-dependent but not mediated *via* integrins. On the other hand, ECM proteins may be strong co-stimulators of T cells infiltrating orbital tissues. These results may reflect abnormalities of T cell – ECM proteins interactions playing an important role in pathogenesis of TAO.

Cooperating units

The Thyroid-Eye Disease Research Centre (where some of experiments were carried out by Dr. Tomasz Bednarczuk), the Allegheny Singer Research Institute, Medical College of Pennsylvania, Pittsburgh, PA, USA.

Department of Endocrinology, Medical School, Warsaw, Poland.
Department of Immunology, Transplantation Institute, Medical School, Warsaw, Poland.

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

Supported by the State Committee for Scientific Research: grant # 4 P05B 004 13

Research team

Z. Bartoszewicz, K. Konca, D. Krzeska

Sugar moiety of glycopeptide hormones is essential for their: presentation, regulation of the biological activity and conformational structure. Although prolactin (PRL) has traditionally been regarded as a nonglycoprotein pituitary hormone, it was recently discovered that a portion of PRL molecules is glycosylated in most species. The chemical structure of oligosaccharides and its monosaccharide composition in glycosylated prolactin (GPRL) has not been well characterized. However, there are several evidences that PRL sugar moiety may play an important role in the biosynthesis, secretion, plasma clearance, biological activity and immunoreactivity of the hormone. Therefore, using modern techniques we purified GPRL from pig pituitary and from human amniotic fluid. Our preliminary experiments involving lectin binding and glycosidase treatment revealed that both complex and high mannose type of oligosaccharide are present on GPRL isolated from pig pituitary. The monosaccharides, as characterized by a high-pH anion exchange chromatography system on Dionex, showed a composition typical for N-linked oligosaccharides, including: mannose, galactose, N-acetylglucosamine, N-acetylneuraminic acid and fucose.

Cooperating units

Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jablonna near Warsaw, Poland.
Department of Vertebrate Animal Physiology, Institute of Zoology, University of Warsaw, Warsaw, Poland.

ELASTICITY OF COMMON CAROTID ARTERIES IN PATIENTS WITH OVERT HYPERTHYROIDISM

Supported by the State Committee for Scientific Research: grant # 4 P05B 011 13

Research team

P. Bagiński, M. Czarkowski, L. Hilgertner

The study has been designed to check whether hyperthyroidism can change elastic properties of common carotid arteries. Preliminary results obtained on 40 patients are indicative of reduced elasticity of the common carotid artery in hyperthyroidism.

Publications

Bednarczuk T, Kennerdell JS, Wall JR: Thyroid-associated ophthalmopathy: etiology and pathogenesis. In: *Thyroid disease: endocrinology, surgery, nuclear medicine and radiotherapy*, 2nd edition. Ed. S.A. Falk. Lippincott-Raven Publishers, Philadelphia, 1997, pp. 341-357.

Krzeska D, Skórka B, Bartoszewicz Z: Hyperprolactinemia. *Endokrynol Pol* 1997, 480, 135-150 (in Polish).

THE LIBRARY

Head: Krystyna Marczakowska
5 Pawińskiego St., 02-106 Warsaw
Telephone: 658 46 77, 608 64 65
E-mail: library@cmdik.pan.pl

This year the library gained access to the Internet and in this way to all the major databases, which largely extended its routine services .

Present holdings:

Books, monographies and serial volumes: 10,502
Journals (number of titles): 164

Scope and subject profile: biochemistry, clinical sciences, endocrinology, immunology, neurosciences, pharmacology, physiology, surgery and transplantation.

PROMOTIONS

DOCTOR'S DEGREE

Maciej M. Łałowski
Composition of amyloid beta peptides and amyloid associated proteins in normal aging and Alzheimer's disease.

Mariusz Muzylak
Ultrastructural changes in the brain of rabbits treated with vincristine (VCR)

Marek Samochocki
Regulation of arachidonic acid release from glycerolipids of adult and aged brain cortex membranes and its contribution to the modulation of GABA-A receptor function

Barbara Zabłocka
Role of PKC in the induction of postischemic changes in the hippocampus

ORGANIZATION OF SYMPOSIA AND CONFERENCES

Symposium: "Response of lymphatic system to trauma, allograft and neoplasm", Warsaw, March 20, 1997.

Symposium: "Transplantation of hepatocytes and liver in metabolic disorders treatment", Warsaw, April 16, 1997.

3rd Polish Israeli Symposium "Peptides and proteins, from basic chemistry to medical application", Warsaw, May 18-25, 1997.
The idea of organizing Polish-Israeli bilateral symposia had been discussed for a very long time, but a concrete decision was made at the European Peptide Symposium in Interlaken. The major animators of such symposia were Prof. Zbigniew Grzonka (Poland), Prof. Mati Fridkin (Israel) and Prof. Chaim Gilon (Israel). The First Polish-Israeli Symposium on *Biology and Chemistry of Peptides* was organized by Prof. Grzonka in 1993 at the University of Gdańsk (Poland). The second symposium was organized two years later by Prof. Mati Fridkin at the Weizmann Institute of Science in Rehovot (Israel). Consequently, the third symposium was organized in Poland and was attended by 77 scientists from academia and industry, mainly from Israel and Poland. Nevertheless, also scientists from other countries, who have some scientific or sentimental connections with Poland and/or Israel, participated. During the symposium, 37 plenary lectures and oral communications were given by representatives of the scientific groups. In addition to the oral presentations, 22 posters were presented.

International Symposium: "Systemic and local immune response to infection, allotransplant and tumor", Warsaw–Pułtusk, September 15-16, 1997.

Polish-Japanese Symposium: "Antitumor and antibacterial reactions in human liver", Warsaw–Katowice, September 17-18, 1997.

Meeting on the sleep apnea syndrome, Warsaw, October 8, 1997.

Symposium: "Influence of surgical trauma on immunological system – attempt of postoperative immunological disorders improvement", Warsaw, October 9, 1997.

International Symposium "Transplantation of cells and tissues-problems of immunosuppression", Warsaw, October 13, 1997

International Symposium "Experimental and clinical pathophysiology", Warsaw, October 13-16, 1997.

The symposium was organised to celebrate the 30th anniversary of the Institute.

Number of participants: 244, including plenary sessions speakers from Austria, Canada, Finland, France, Germany, Japan, Poland, Spain, Sweden, UK and USA.

Program:

1. Plenary sessions:
   - Cardiovascular Pathophysiology (chair: Sir John Vane, Krystyna Cedro-Ceremużyńska)
   - Molecular Genetics: Key to Research in Neuromuscular Diseases in 21 Century (chair: Lewis P. Rowland, Irena Hausmanowa-Petrusewicz)
   - Control of Energy Metabolism at Rest and During Exercise (chair: John E. Greenleaf, Krystyna Nazar)
   - Pathophysiology of Glia: Tools and Perspectives (chair: Jose Regino Perez-Polo, Jan Albrecht)
   - Brain Ischemia (chair: Tadeusz Wieloch, Jerzy Łazarewicz)
   - Molecular Mechanisms of Signal Transduction in Brain – Physiology and Pathology (chair: Lloyd A. Horrocks, Joanna B. Strosznajder)
   - Immune and Neuroendocrine Response to Transplant, Trauma and Infection (chair: Waldemar L. Olszewski, Wojciech A. Rowiński)
   - Excitotoxicity and Metal Neurotoxicity (chair: Jarda T. Wróblewski, Urszula Rafałowska)

– Neuronal and Myelin Pathology in the Central Nervous System (chair: Henry M. Wisniewski, Krystyna Domańska-Janik)
– Neurobiology of Respiration (chair: Yoshiyuki Honda, Mieczysław Pokorski)
– Intracranial Volume/Pressure Relations in Normal and Pathological Conditions (chair: Hans J. Reulen, Zbigniew Czernicki)
– Development of the Central Nervous System (chair: Krystyna Wiśniewski, Maria Dąmbska)

These 2-2.5h sessions included 5-6 presentations each, by invitation only.

2) Poster sessions (2 multidisciplinary sessions, 60 posters).

No abstracts were printed.

Program Committee:
Jan Albrecht
Krystyna Cedro-Ceremużyńska
Zbigniew Czernicki
Krystyna Domańska-Janik
Irena Hausmanowa-Petrusewicz
Hanna Kaciuba-Uściłko
Jerzy Łazarewicz
Mirosław J. Mossakowski
Waldemar Olszewski
Mieczysław Pokorski
Urszula Rafałowska
Joanna B. Strosznajder

Organizing Committee:
Jan Albrecht
Maria Barcikowska-Litwin
Krystyna Cedro-Ceremużyńska
Bronisław Głód
Paweł Grieb
Wojciech Hilgier
Halina Hoffman
Jerzy Jurkiewicz
Eugeniusz Kaczmarczyk
Andrzej Lipkowski
Jerzy W. Łazarewicz
Danuta Maślińska
Mieczysław Pokorski
Marek Samochocki
Joanna B. Strosznajder
Halina Weinrauder
Ewa Wójcik-Ziółkowska
Teresa Zalewska

Sponsors:
Bank PeKaO SA, Bio-Rad Laboratories Polska, Glaxo-Wellcome Polska, Hewlett-Packard Polska, InLab BTH, KEBO-Lab, Pfizer Corporation, Press S.c., Servier Polska, Shimadzu Polska, Shoeller Pharma.

Symposium: "Experimental and clinical pathophysiology". Session: Immune and neuroendocrine response to transplant, trauma and infection, Warsaw, October 15, 1997.

4th International Symposium on intracranial hypertension and cerebral ischemia in clinical practice, Warsaw/Pułtusk, October 17-18, 1997

Symposium: "Inflammations of skin and vessels in extremities", Warsaw, November 26, 1997.

Neurochemical Conference: "Molecular mechanisms of neurotransmission and signal transduction in physiology and pathology of CNS", Warsaw, December 12, 1997.