MOLECULAR BASIS OF PATHOLOGY AND THERAPY IN NEUROLOGICAL DISORDERS

The 8th Polish Neurochemical Conference

Medical Research Centre
Polish Academy of Sciences
Warsaw
November 17–18, 2006

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PROGRAMME

Friday, November 17th, 2006

9.15 Opening of the Conference

SESSION I

MOLECULAR, METABOLIC AND STRUCTURAL ASPECTS OF BRAIN DISFUNCTIONS

Chairs: **Irena Nalepa (Krakow, Poland)**
Jan Albrecht (Warsaw, Poland)

9.30–10.00 **Leszek Kaczmarek** (Warsaw)
ICER isoforms and neuronal apoptosis in cortical *in vitro* culture

10.00–10.30 **Irena Nalepa** (Krakow)
Cellular and molecular aspects of depression

10.30–11.00 **Dieter Rausch** (Vienna)
Neuroprotective effect of ginsengocydes

11.15–11.45 **Paweł Grieb** (Warszawa) (Warsaw)
Failing brain glucose metabolism: Pathophysiological significance and potential therapies

11.45–12.00 **Malgorzata Beresewicz** (Warsaw)
Balance between JNK and MAP kinase cascades activation in hippocampus in norm and in ischemic pathology

12.00–12.15 **Michal Wegrzynowicz** (Warsaw)
Ammonia stimulates glutathione accumulation in the rat cerebral cortex *in vivo* and in cortical astrocytes in culture

12.15–12.30 **Anna Ronowska** (Gdansk)
Cholinergic cytotoxicity of zinc

12.30–12.45 **Ryszard Pluta** (Warsaw)
Ischemic breakdown of the blood-brain barrier in white matter responsible for final brain neurodegeneration and dementia

12.45–13.00 **Jacek Losy** (Poznan)
CXCL1 (GRO-alpha) and CXCL6 (Granulocyte Chemotactic Protein-) chemokines- are associated with stroke severity and short term stroke outcome

13.00–13:15 Presentation of the new products of PROSPECTA Co.

13.15–14.15 *Poster session*

SESSION II

NEUROPROTECTION STUDIES IN EXPERIMENTAL MODELS

Chairs:	**Joanna Strosznajder (Warsaw, Poland)** **Andrzej Szutowicz (Gdansk, Poland)**
14.15–14.45	**Julita Czarkowska-Bauch** (Warsaw) Does improvement of motor abilities after locomotor training of spinalized rats involve activation of neurotrophin system?
14.45–15.15	**Władysław Lason** (Krakow) Effects of neurosteroids on apoptotic processes in neuronal cells *in vitro*
15.15–15.45	**Robert Rejdak** (Lublin) New aspects of retinal degeneration: Mechanisms and potential neuroprotective strategies
15.45–16.15	**Anna Członkowska** (Warsaw) Stroke: Impact of experiments into clinic
16.30–16.45	Presentation of the newest products of APPLIED BIOSYSTEM Co.
16.45–17.00	**Andrzej Szutowicz** (Gdansk) Acetyl-CoA-dependent alterations in susceptibility of cholinergic neurons to neurotoxic signals
17.00–17.15	**Iwona Kurkowska-Jastrzebska** (Warsaw) Up-regulated glial TrkA may mediate neuroprotective effects of NGF-releasing, anti-MBP CD4 T cells administered into trimethyltin intoxicated rats
17.15–17.30	**Malgorzata Chalimoniuk** (Warsaw) Nitric oxide enhances expression and activity of cytosolic phospholipase A2 in PC12 cells with the different amyloid beta load
17.30–17.45	**Marzena Lazarczyk** (Warsaw) Antiproliferative properties of opioid-tachykinin hybride peptides on human glioblastoma cell line T98G

Saturday, November 18th, 2006

SESSION III

NEURAL STEM CELLS

Chairs:	**Krystyna Domanska-Janik (Warsaw, Poland)** **Nico Forraz (Newcastle upon Tyne, UK)**
9.30–10.00	**Colin McGuckin** (Newcastle upon Tyne,UK) Embryonic-like stem cells from umbilical cord blood
10.00–10.30	**Krystyna Domanska-Janik** (Warsaw, Poland) Neural commitment of cord blood stem cells (HUCB-NSC/NP): Identity and utility
10.30–11.00	**Thorsten Trapp** (Dusseldorf, Germany) Migration of somatic stem cells to neuronal injury

11.15–11.45	**Jukka Jolkkonen** (Kuopio, Finland) Stem cells and functional recovery in experimental stroke rats
11.45–12.15	**Mariusz Z. Ratajczak** (Louisville, USA) Stem cell plasticity explained by developmental deposition or embryonic/primordial germ cells in various organs
12.15–12.30	**Inga Markiewicz** (Warsaw, Poland) Defined cellular environment commands HUCB-NSCs fate determination
12.30–12.45	**Marcin Jurga** (Warsaw, Poland) Serum free culture of 3D scaffold-based aggregates of human neural stem cells derived from cord blood
12.45–13.00	**Ryan Reca** (Louisville, USA) Evidence that functional neural tissue-committed stem cells (NTCSC) reside in the human bone marrow and are mobilized into peripheral blood after stroke
13.00–13.15	**Piotr Rieske** (Lodz, Poland) Inventing combination instead of reductionistic model, to control the process of GFAP positive, human neural progenitors differentiation
13.15–14.15	*Poster session*

SESSION IV

NEURODEGENERATIVE DISEASES – CLINICAL STUDIES

Chairs:	**Urszula Fiszer (Warsaw, Poland)** **Piotr Janik (Warsaw, Poland)**
14.15–14.45	**Maria Barcikowska-Kotowicz** (Warsaw) Presymptomatic Alzheimer's disease as a task for diagnosis and treatment
14.45–15.15	**Urszula Fiszer** (Warsaw) Evidence of inflammatory reaction in pathogenesis of Parkinson's disease
15.15–15.45	**Piotr Janik** (Warsaw) Amyotrophic lateral sclerosis: how far away are we from the Charcot description?
16.00–16.30	**Malgorzata Siger** (Lodz) Magnetic resonance imaging in diagnosis of multiple sclerosis
16.30–16.45	**Jerzy Nowak** (Poznan) The possible participation of MSR virus in etiology of multiple sclerosis
16.45–17.00	**Grazyna Gromadzka** (Warsaw) The *APOE* epsilon4, gender, and stroke severity and one-year outcome
17.00–17.15	**Jolanta Florczak** (Poznan) Polymorphism of MTHFR, MTR and MTHFD1 as related to the oxidative DNA damage, and the level of thiols in Alzheimer's and Parkinson's diseases
17.15–17.30	**Andrzej Gomula** (Warsaw) The role of the hormone replacement therapy in curing Parkinson's disease and other diseases caused by hormonal deficit
17:30	Closing Remarks

Session I

MOLECULAR, METABOLIC AND STRUCTURAL ASPECTS OF BRAIN DISFUNCTIONS

S1-L2 Cellular and molecular aspects of depression

Nalepa I.
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Mood disorders (major depressive disorders and bipolar disorder) are among the most prevalent forms of mental illness. Biological hypotheses of depressive disorders stated that deficits of monoaminergic transmission in brain play an important role in their etiology and clinical studies over the past 50 years have attempted to uncover the specific defects in noradrenergic and serotonergic neurotransmitter systems by using a variety of biochemical strategies. Recent evidences indicate that an impairment of neuroplasticity may underlie the pathophysiology of depressive disorders. Neuroimaging and postmortem studies show that these disorders are associated with regional reductions in central nervous system volume and in the numbers and/or sizes of glia and neurons in discrete brain areas. Multiple abnormalities of regional cerebral blood flow and glucose metabolism in limbic and prefrontal cortical structures are observed as well. New hypotheses on the mechanism of action of antidepressants take into consideration their effects on neural plasticity and on remodeling of neuronal circuits. It is proposed that drugs of various classes have common antidepressant effects after chronic use because they may regulate transcription of the same set of downstream genes. Recent data show that antidepressant drugs, in addition to their primarily pharmacological action on the availability of neurotransmitters, exert major effects on signal pathways that regulate neuroplasticity and this way they promote restoring of neuronal functions.

S1-L4 Failing brain glucose metabolism: Pathophysiological significance and potential therapies

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Glucose is usually considered as a crucial, non-replaceable 'fuel' for brain energy metabolism. This 'classic' view seems to be compatible with immediate deleterious effects of acute hypoglycemia on brain function. However, many basic features of brain glucose metabolism remain uncertain. For example, it has not been conclusively determined whether in the brain glucose is metabolised uniformly by various cell types, or it is preferentially metabolised by astroglial cells. On the other hand, it has been shown that during fasting or strenous exercise human brain energy metabolism switches to ketone bodies (in particular to beta-hydroxybutyrate, beta-HB) as an alternative 'fuel' used by energy metabolism instead of glucose. In these situations brain glucose uptake can be reduced by 60% without any adverse effects on brain function. Compared to glucose, beta-HB are more efficient source of ATP and do not acidify brain tissues. There is some evidence that ketogenic diet may be useful in seizure control, whereas beta-HB which proved neuroprotective in experimental acute brain hypoxia/ischemia is under development as a new 'metabolic cytoprotective' drug. Not surprisingly chronic neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's (PD) disease, are characterized by profound decrease in brain glucose uptake, but several lines of evidence suggest that failure of brain glucose uptake may rather belong to causes of these neurodegenerations. An emerging concept of treatment of these diseases through the use of ketogenic diet or new pharmacologically acceptable forms of beta-HB will be presented.

SI-O1 Balance between JNK and MAP kinase cascades activation in hippocampus in norm and in ischemic pathology

Beresewicz M., Kowalczyk J.E., Zablocka B.
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Transient cerebral ischemia leads to a selective, delayed damage of neurons in vulnerable (CA1), but not in resistant (CA2-3, and gyrus dentus) region of hippocampus. The mechanism of different susceptibility is not well understood therefore we have concentrated on the role of the postsynaptic density proteins in that phenomenon. We have studied relationship between proteins implicated in the regulation of MAPK (PSD95, SynGAP, CaMKII) and JNK cascades (PSD95 and kalirin). Our data indicate that in control brains the activity of MAPK and JNK is higher in CA2-3/DG than in CA1 parts. These indicate that plasticity of ischemia-resistant part of hippocampus is greater then the vulnerable one. After ischemia we have found the decrease in the amount of kalirin-7 in CA2-3/DG, parallel with the substantial decrease in the amount of P-JNK unlike the double quantity of kalirin-7 in CA1, what can promote subsequent activation of JNK. Our data shows early postischemic attenuation of the MAPK/JNK activity, what probably reflects the adaptation response. Although many individual components of MAPKs and JNKs pathways have been changed by short ischemia, the vulnerability of CA1 to ischemia can be explained rather by their intrinsic weaker activity than by the prominent rearrangement of PSD95-connected first steps of these cascades. Additionally, opposite changes in the amount of kalirin-7 in CA1 and CA2-3/DG suggest that there is an "launch phase" leading to neuronal death in CA1 and full recovery in CA2-3/DG.
Supported by MSHE 2P04A 024 28.

SI-O2 Ammonia stimulates glutathione accumulation in the rat cerebral cortex *in vivo* and in cortical astrocytes in culture

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Ammonia neurotoxicity is associated with oxidative stress and astroglia-derived glutathione is a major antioxidant in the CNS. An earlier in vitro study indicated that cerebral cortical astrocytes in culture (cca) respond to ammonia treatment with increased glutathione accumulation (Murthy et al. (2000) Neurochem Int 37: 255), indicat-

ing a cell protective reaction. Here we show that in vivo infusion of ammonia to the prefrontal cortex (final concentration in the extracellular space – 5 mM) leads to increased accumulation of glutathione in the microdialysates, which adds credence to the in vitro observations. In our hands, increased intracellular glutathione content in ammonia-treated cca was associated with a gradual decrease of the GSH/GSSG ratio, and correlated with upregulation of cystine uptake, which occurred almost exclusively *via* the sodium-dependent XAG- system. The results suggest that ammonia upregulates a set of reactions in astrocytes rendering more glutathione for transfer to the extracellular space. This glutathione may become available for neurons, the cells where its content was found reduced upon ammonia treatment *in vitro* (Klejman et al. (2005) Neurochem Int 47: 51).

SI-O3 Cholinergic cytotoxicity of zinc

Ronowska A., Gul-Hinc S., Bielarczyk H., Jankowska-Kulawy A., Szutowicz A.
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Zinc is a trace element essential for brain cells. However, its excess in the brain was claimed to contribute to neurotoxic patomechanisms in Alzheimer's disease. The aim of this work was to find out whether cholinotoxic effects of Zn may be caused by impairment of acetyl-CoA metabolism. Zn in concentrations up to 0.1 mM caused no injury to SN56 cholinergic neuroblastoma cells. Further increase of Zn levels to 0.2 mM caused dose-dependent increase of nonviable cell fraction up to 89%. These changes correlated with Zn concentration-dependent inhibition of pyruvate dehydrogenase activity (r=0.95, P=0.002). Toxic, 0.15 mM Zn decreased aconitase, choline acetyltransferase activity, cytoplasmic acetyl-CoA and whole cell ATP levels by 55, 33, 35 and 62% respectively but caused no change in mitochondrial acetyl-CoA. No alterations in hexokinase, lactic dehydrogenase, succinate dehydrogenase, ATP-citrate lyase and acetylcholinesterase activities were found in these conditions. Lipoamide and lipoic acid reversed negative effects of Zn on cholinergic cells. Presented data indicate that cytotoxic and cholinosuppressive effects of Zn on cholinergic cells are caused by specific inhibition of pyruvate dehydrogenase and aconitase activities yielding decrease of acetyl-CoA synthesis and utilization in mitochondria as well as attenuation of its transport to cytoplasmic compartment. Such alterations of acetyl-CoA metabolism could increase mortality and suppress cholinergic neurons transmitter functions in encephalopathic brains.
Supported by MSHE grants 2P05A 110 30 and 2P05A 26 26.

SI-O4 Ischemic breakdown of the blood-brain barrier in white matter responsible for final brain neurodegeneration and dementia

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Pathology of white matter (WM) that is observed in ischemic brain indicates that similar changes contribute to Alzheimer's disease etiology. These changes have been seen in the subcortical and periventricular areas. Periventricular WM injuries in Alzheimer's disease and human brain ischemia referred to as leukoaraiosis and are responsible for function, memory and cognition. Recently it is not clear whether the blood-brain barrier (BBB) in WM after ischemia is altered in long-lived animals. We take into consideration investigation of BBB changes and amyloid precursor protein (APP) staining in perivascular space. Using rats, BBB changes, staining of APP around microvessels and platelets behavior were examined in ischemic WM with 1-year survival. White matter demonstrated chronic BBB changes. Toxic parts of APP deposits were associated with the microvessels. Additionally our study revealed platelet aggregates in- and outside microcirculation. APP deposits and platelet pathology correlated very well with BBB permeability. In summary progressing damage of the ischemic white matter may be caused not only by a degeneration of axons of neuronal cells destroyed during ischemia and also by changes in ischemic BBB vessels with deposition of toxic parts of APP (retrograde neuronal death). Chronic leaky BBB and platelets in the perivascular space with toxic parts of APP accumulation may be involved in the gradual maturation of neuropathological processes in ischemic white matter leading over a lifetime to severe neurodegeneration and progressive dementia.

SI-O5 CXCL1 (GRO-alpha) and CXCL6 (Granulocyte Chemotactic Protein-2) chemokines-are associated with stroke severity and short term stroke outcome

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Objective: Acute cerebral ischaemia induces local inflammatory reaction including expression of chemokines, which precedes relevant leukocyte infiltration contributing to tissue injury. The objective of the study was to test hypothesis that CXCL1 and CXCL6 chemokines,potent neutrophil chemoattractants, play a role in inflammation during early phase of ischaemic stroke. Methods: The CXCL1 and CXCL6 levels in the CSF and serum obtained during 24 h from 23 ischaemic stroke patients aged 72.2+/-10.8 years have been measured by ELISA. CSF and blood samples from 15 tension headache patients served as a control group. The neurological stroke severity was estimated with Scandinavian Stroke Scale (SSS) within 24 h of stroke (SSS-1) and two weeks later (SSS-2). Results: CXCL1 and CXCL6 levels were significantly elevated in the CSF of patients with ischaemic stroke in comparison with controls. Serum levels of studied chemokines did not differ from control values. CSF CXCL1 and CXCL6 levels correlated significantly with the neurological stroke severity within 24 hours and after twoo weeks from the onset of stroke. Conclusion: Our results suggest that CXCL1 and CXCL6 chemokines may play a role in the inflammatory reaction during early phase of ischaemic stroke and CSF CXCL1 and CXCL6 levels are associated with stroke severity and have predictive value for short term stroke outcome.

SI-P1 Involvement of FAK-coupled signaling in delayed neuronal damage in gerbil hippocampus after transient forebrain ischemia

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Focal adhesion kinase (FAK) is a non-receptor type of protein tyrosine kinase thought to play a major role in transducing extracellular matrix (ECM)-derived survival signals into cells. The function of FAK is tightly linked to its autophosphorylation at Tyr-397 and then recruitment of signaling molecules such as Src family kimases and adaptor protein p130Cas. Thus, modulation of FAK phosphorylation may affect several intracellular signaling pathways and may participate in a variety of pathological settings including brain damage arising from cerebral ischemia. We found that forebrain ischemia followed by reperfusion reduced the total amount of FAK as well as its phosphorylation at Tyr -397 to about 50% of the control. Concomitantly, a decreased association of FAK with Src kinase has been observed. The temporal profile of FAK suppression in the vulnerable CA1 region of hippocampus was matched by elevations of gelatinases activity, the key enzymes responsible for the proteolytic modification of extracellular matrix in the brain. These results are indicative of an involvement of ECM-FAK signaling pathway in ischemia-induced delayed neuronal degeneration.
Sponsored by MSHE grant 2P05A 09928.

SI-P2 PKC delta and beta translocation to mitochondria correlates with opposite susceptibility of hippocampal regions to ischemic insult

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Recent findings support the idea that mitochondrial integrity plays an important role in the propagation of ischemic signal and is crucial for the fate of cells. After cerebral ischemia signals connected with the initiation of apoptosis seem to integrate on mitochondrial membranes. PKC isoforms are implicated in the regulation of mitochondrial membranes integrity but their precise function is not fully understood. We examined the association of PKC isoforms with mitochondria and followed postischemic changes in their amount in mitochondria isolated from ischemia-vulnerable (CA1) and ischemia-resistant (CA2/3, DG) hippocampal regions in gerbil model of transient brain ischemia. Postischemic, biphasic translocation of PKC isoforms (α, β, γ, δ, μ) to mitochondria was observed. After 30–60 min of reperfusion the level of PKC proteins was elevated transiently up to 5 times of the control and again 72–96 h postischemia. Interestingly, PKC δ was translocated to mitochondria only in CA1 region, while significant elevation of PKC β was found in ischemia-resistant area. Additionally, in this region, the amount of PKC δ was slightly reduced. One of the targets of PKC δ is mitochondrial phospholipids scramblase 3 (PLS3), the enzyme responsible for cardiolipin (CL) translocation between two lipid compartments. Our data suggest that PKC δ translocation to mitochondria might regulate activity of PLS3 and therefore mitochondrial structure and respiration, and CL transport in apoptosis.
Supported by MSHE 2P04A 024 28.

SI-P3 Dynamics of post-stroke reorganization of cerebral cortex registered by metabolic mapping

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In most cases of stroke spontaneous recovery of impaired function can be observed. We mapped with 2-deoxyglucose changes in activation of cerebral cortex after focal infarct localized in the face representation of somatosensory cortex of rats. Stroke was induced by noninvasive photothrombotic technique and was centered on the cortical representation of vibrissae, the barrel cortex. At 1, 7, 30 and 60 days after stroke the activation of the brain, evoked by stimulation of vibrissae that projected to the barrel cortex damaged by the stroke, was mapped with 14C-2deoxyglucose (2DG) autoradiography. 2DG labeling was measured in the barrel field, auditory cortex, SII, representation of front paw and of rostral vibrissae of the snout. We observed a two-phase reaction to a local infarct. In the beginning, general activity decrease in the damaged hemisphere appeared and the contralesional hemisphere showed increased activation - significant increase of 2DG uptake was observed in all examined somatosensory representations at 1, 7 and 28 days after stroke. Later on the intact hemisphere activation returned to control values and the lesioned hemisphere showed considerable remapping of vibrissal input. Our results show a rapid remodeling of the activity pattern, with changes in the intact hemisphere preceding redistribution of activation in the lesioned one. At longer post-lesion times, activations of SII, rostral vibrissae and front paw region in damaged hemisphere were stronger, while those in the intact hemisphere became weaker, suggesting functional recovery of the lesioned hemisphere.

SI-P4 Homocysteine neurotoxicity: Involvement of calcium and mitochondrial alterations

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Our previous studies demonstrated that homocysteine (Hcy) neurotoxicity is mediated by both, NMDA receptors and group I metabotropic glutamate receptors. In the present work we investigated the role in Hcy neurotoxicity of calcium imbalance and mitochondrial dysfunction, two factors which have been implicated in the conventional mechanisms of excitotoxicity induced by glutamate (Glu). Primary cultures of rat cerebellar granule cells were incubated for 30 min in the presence of 1 mM Glu or 25 mM D,L-Hcy. The propidium iodide and Hoechst 33258 staining after 24 h demonstrated that at these concentrations both amino acids induce comparable neurodegeneration and chromatin condensation. Cyclosporin A (CsA), but not FK506 partially prevented Glu- and Hcy-induced neurotoxicity. Under these conditions Hcy induced negligible uptake of 45Ca, a weak stimulation of [3H]inositol phosphate release and insubstantial increase in intracellular calcium level, evaluated with fluo-3 fluorescent probe. Both, Hcy and Glu induced release of cytochrome c visualized using immunocytochemical method and confocal microscopy. Comparing to Glu, the

effects of Hcy were slightly less expressed and less sensitive to CsA, while FK506 did not modify mitochondrial alterations induced by both excitotoxins. These results point to involvement of mitochondrial alterations in acute Hcy and Glu neurotoxicity, whereas calcium imbalance seems to play a marginal role in Hcy-induced neurotoxicity.
Supported by grant MSHE 2.P05A.121.26.

SI-P5 Behavioral activity of MPEP in rats with experimental hypoxia

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Hypoxia disturbs glutaminergic transmission. The exact role of group I metabotropic glutamate receptors in causing posthypoxic injury is not yet clear. We investigated the influence of MPEP, a selective antagonist of metabotropic glutamate receptor subtype 5, on certain behaviors in control groups of rats and in rats after short-term hypoxia. The effect of MPEP, administered intravenously at dose of 1 mg/kg, was assessed using behavioral tests: the open field test, the passive avoidance response and the water maze.MPEP significantly enhanced locomotor and exploratory activity, impaired acquisition in the passive avoidance situation and learning in the water maze test in the last session. Hypoxia significantly inhibited motility of rats and profoundly impaired acquisition process in the passive avoidance test and spatial learning in the water maze. MPEP in hypoxia-treated groups of rats inhibited locomotor and exploratory activity vs MPEP-treated control group. Hypoxia did not changed the effects of MPEP on learning in the passive avoidance situation and in the water maze test. Summary, MPEP used in rats subjected to the short-term hypoxia, did not change acquisition process of learning. Hypoxia inhibited MPEP-induced enhancement of locomotor activity.
This work was supported by grant No 3-10585 from the State Committee for Scientific Research, Warsaw, Poland.

SI-P6 Vesicular glutamate transporters VGLUT1 and VGLUT2 in development of mouse barrel cortex

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Three vesicular glutamate transporters (VGLUT1-3) have been identified from neuronal tissue. VGLUT1 and VGLUT2 are the predominant transporters and they define the "glutamatergic" phenotype. They are implicated in development and plasticity of the nervous system. It has been shown that expression of VGLUT1 was affected after transient ischemia, in schizophrenia and unilateral hypoxic epilepsy model. In this study, using immunocytochemistry and western blot analysis, we analyzed postnatal developmental expression of VGLUT1 and VGLUT2 and their colocalization in the mouse barrel cortex. This particular part of neocortex is used as a model system to examine developmental and adult plasticity. We found that each transporter follow different developmental temporal pattern in the barrel cortex and their colocalization also changes from birth to adulthood. Both transporters are present in the neuropil, but VGLUT1 is localized uniformly within barrel cortex, whereas VGLUT2 can be found mainly in the barrel hollows. Early in postnatal life VGLUT2 dominates, then there is about 3-week period of more or less equal expression of the two transporters and in adult animals again VGLUT2 domination can be observed. In the septa during the examined period, invariably dominates VGLUT1 immunoreactivity. These roughly complementary distribution in the barrel cortex suggests synapse-specific differences in vesicular glutamate uptake, presumably related to differential synaptic functions and changes in their proportion can be potentially translated into susceptibility to different neurological diseases.

SI-P7 Dilation of the middle cerebral artery exposed to low extracellular sodium depends on the integrity of the endothelium

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Hyponatremia is defined as an electrolyte disorder occurring when serum sodium level falls below 135 mmol/l. It is frequently observed after brain trauma or subarachnoid bleeding and is typically associated with increased plasma level of vasopressin (AVP). Although hyponatremia has been reported to impair the recovery of neurosurgical patients its effect on brain vasculature is practically unknown. Therefore, present study was carried out to determine the effect of low sodium and increased AVP concentration in the extracellular fluid (ECF) on the calibre of the middle cerebral artery (MCA) of the rat. The experiments were performed on the isolated MCA mounted in the arteriograph chamber placed on an inverted microscope equipped with videocamera. Changes in the diameter of MCA were observed on the monitor and analyzed with a help of a customer made software. Replacement of the ECF 140 mM Na+ solution with 120 mM one resulted in the dilation of MCA by 17% (from 143 ± 10 μm to 167 ± 10 μm, $P<0.01$). Dilation was abolished when endothelial cells were mechanically removed or NO was inhibited. Addition of AVP to the ECF during normonatremia prior to the administration of low sodium buffer did not affect the diameter of MCA. However, when low sodium buffer was given together with AVP, constriction of MCA by about 16% ($P<0.02$) instead of dilation was observed. These results demonstrate that hyponatremia dilates cerebral blood vessels through endothelium- and NO-dependent mechanism. Constriction in response to low ECF sodium in the presence of AVP may explain the adverse vascular effect of AVP-associated hyponatremia observed *in vivo*.

SI-P8 Baclofen, LY367385 and MPEP influence MMP-2 and MMP-9 activity in rat's hippocampus after hypoxia

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The deregulation of balance between the glutamatergic and GABAergic systems is probably the most important factor of learning and memory deficits induced by hypoxia. The hippocampus which is important for learning and memory processes, is also very sensitive to hypoxia. Increased level of pro-MMP-2 and pro-MMP-9 in the hippocampus after ischemia suggested that substances, which inhibit activity of MMP, may reduce ischemia-induced deficit. We have shown that baclofen, an agonist of GABAB receptors, may interact with ligands of metabotropic glutamate receptors (mGluRs) and reduced hypoxia-induced memory deficit (Car et al. 2001, 2006). In the present study we focused on the measurement of MMP-2 and MMP-9 after joint administration of baclofen and antagonists of metabotropic glutamate receptors (mGluRs) group I (AIDA, LY367385, MPEP) in hippocampus of rats after short-term hypoxia (2% O2, 98% N2, 4 min). Gel zymography showed elevations in the MMP-2 and MMP-9 activity (pro and active form) after administration of baclofen with LY367385 or MPEP only, in the hippocampus of rats which undergone hypoxia. Concluding, the obtained results suggest important role of cooperation receptors of GABAB and mGluR1/5 in reconfiguration of extracellular matrix in hippocampus after hypoxia.
This work was supported by the grant No 3-10588 L from the State Committee from Scientific Research, Warszawa, Poland.

SI-P9 AIDA, LY367385 and MPEP influence MMP-2 and MMP-9 activity in rat's hippocampus after hypoxia

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Hypoxia impairs learning and memory and several studies demonstrated that glutamatergic excitotoxicity are probably involved in such neurocognitive deficits. Experiments shown that some ligands of metabotropic glutamate receptors (mGluRs) influenced hypoxia-induced impairment of memory (Car et al. 2004, 2006, Nadlewska et al. 2002, 2003) and antagonists of group I mGluRs elevated activity of MMP-2 and MMP-9 in hippocampus (Wiśniewska et al. 2006). We also obtained that hypoxia induced activity of MMP-9 in rat's hippocampus (Car et al. 2006). The objective of this study was to determine whether hippocampal matrix metalloproteinases (MMP-2 and MMP-9) are involved in cognitive activity of antagonists of group I mGlu (AIDA, MPEP and LY367385) in rats which undergone short-term hypoxia (2% O2, 98% N2, 4 min). Activity of MMPs was determined by gelatin zymography in homogenates from rat's hippocampus. Zymograms showed that MMP-2 and MMP-9 activity (pro and active forms) were elevated after administration of all tested antagonists. In summary, we suggested the role of the antagonist of group I mGluRs in reconfiguration of extracellular matrix in hippocampus of rats after hypoxia produced by MMP-9 and MMP-2.
This work was supported by the grant No 3-10588 L from the State Committee from Scientific Research, Warszawa, Poland.

SI-P10 Matrix metalloproteinases after cerebral photothrombotic stroke in rat

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Matrix metalloproteinases (MMPs) are involved in many pathological processes including brain injury. Their expression and activity are strongly activated after ischemia. Focal photothrombotic stroke is an animal model used to investigate processes occurring in the ischemic brain. However, the activation of MMPs in this particular experimental model has not been examined yet. In this study we focused on MMP-2 and MMP-9 because of their capacity to degrade the extracellular matrix components of the basement membrane that leads to breakdown of the blood-brain-barrier. Unilateral photothrombotic stroke, induced by rose Bengal technique, was located in the primary somatosensory cortex. MMP-2 and MMP-9 activity and expression were studied at various time points after stroke (1, 4, and 24 hours, 4 and 7 days). Using in situ zymography combined with immunofluorescence for neuronal marker NeuN, 1 hour after stroke we detected weak gelatinolytic activity within the cells, located in lesion core. Four and 24 hours after the lesion, MMPs activity was visible also in the neuronal nuclei and colocalized with NeuN. At later time points nuclear localization of MMPs activity was no longer visible. Four and 7 days post stroke the vascular activation was identified in the rim of infarct cavity. There was no activation of MMPs in contralateral side at any investigated time point. We confirmed these results by SDS-Page zymography using crude and nuclear tissue extracts.
Supported by statutory fund from the Nencki Institute.

SI-P11 Role of acetyl-CoA in cytotoxic and cytoprotective effects in cholinergic SN56 cells

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Depletion of acetyl-CoA may cause preferential loss of cholinergic neurons in encephalopathic brains. Therefore improvement of acetyl-CoA metabolism could alleviate cholinergic deficits. The aim of this study was to investigate whether cholinotropic and cholinoprotective activities of lipoic acid could be mediated by changes in acetyl-CoA levels in subcellular compartments of cholinergic cells. Amyloid-beta and sodium nitroprusside caused dose-dependent decrease of pyruvate dehydrogenase (PDH), choline acetyltransferase (ChAT) activities, ATP and acetyl-CoA content in mitochondria and cytoplasm as well as increased nonviable fraction of differentiated cholinergic cells. Lipoic acid reversed toxin-induced depression of ChAT and PDH activities, as well as acetyl-CoA content in mitochondrial and cytoplasmic compartments and improved cells survival. Significant negative correlations were found between PDH and ChAT activities, acetyl-CoA levels and cell mortality (r=-0.83–0.88, P=0.004–0.011) in neurotoxic and neuroprotective conditions employed here. The level of cytoplasmic acetyl-CoA positively correlated with mitochondrial

acetyl-CoA (r=0.91, P=0.002) whereas ChAT activity followed shifts in cytoplasmic acetyl-CoA (r=0.75, P=0.034). These data support the hypothesis that alterations in mitochondrial acetyl-CoA levels affect ability of cholinergic neurons to survive under neurotoxic conditions. On the other hand, cytoplasmic acetyl-CoA would regulate their transmitter functions.
Work was supported by MSHE grants 2P05A 110 30 and 2P05A 26 26.

SI-P12 The distribution of primary nitric oxide synthase- and parvalbumin-immunoreactive afferents in the dorsal funiculus of the lumbosacral spinal cord in the dog

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The present study is concerned with the occurrence and compartmentalization of primary afferent neuronal nitric oxide synthase (bNOS) and parvalbumin (PV) immunoreactive (IR) fibers in the dorsal funiculus of the lumbosacral spinal cord. The dorsal root bNOS-IR and PV-IR afferents in all lumbosacral segments segregate shortly before entering the dorsal root entry zone (DREZ) in such a way that large-diameter bNOS-IR + PV-IR axons enter the medial bundle of the dorsal root, while small-diameter bNOS-IR axons but not PV-IR participate in the formation of the lateral bundle which upon entering the DREZ tends to proceed in the ventrolateral direction and approach the dorsolateral border of the dorsal horn. Our study documents that the medial bundle of the dog is a large anatomical entity containing NOS and PV, the latter being a highly reliable, although not exclusive marker for fast-conducting Ia proprioceptive muscle afferents originating from muscle spindles. The labelling of bNOS-IR is quite apparent in the lateral bundle and in loosely-dispersed large axons of medial bundle. PV-IR is strictly limited to large-diameter axons of the medial bundle, while lateral bundle is lacking in any PV-IR.
Supported by VEGA Grant 2/5134/25 from SAS and NIH grants NS 32794, 40386 to M.M.

SI-P13 The participation of nitric oxide synthase on nociceptive and proprioceptive afferentation

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The aim of the present study was to examine the effect of nitric oxide synthase (NOS) inhibitors on changes NOS pools in the spinal cord of rabbits after unilateral sciatic nerve transection and survival for 2 weeks. The rabbits were treated with NNLA, an inhibitor of neuronal isoform of NOS (nNOS) in a dose 20 mg/b.w. for 12 days, and with an inhibitor of inducible isoform of NOS (iNOS), aminoguanidine (AG) in a dose 100 mg/b.w. for 4 and 12 days. Our attention was focussed on the dorsal part of L4–L6 segments, receiving sensory inputs from the sciatic nerve through sensitive neurons localized in respective dorsal root ganglions and on motor neurons of the sciatic nerve, originating in the ventral horn of L4–L6 segments. Sciatic nerve transection increased the expression of nNOS in the dorsal part of the spinal cord on ipsilateral side. The treatment of animals with NNLA effectively reduced nNOS in both, the dorsal horn and the dorsal column of lower lumbar segments. Immunocytochemical analysis disclosed the up-regulation of iNOS-IR staining after peripheral axotomy in a-motoneurons. The changes in iNOS expression were not corrected by AG treatment for 4 days. However, the expression of iNOS in a-motoneurons had decreased significantly, when the animals were treated with AG for 12 days.
Supported by the VEGA Grant 2/5134/25 and PAS/SAS project 17/24-26.

SI-P14 Myelin glycoproteins as a target of Pb in the rodent model of prolonged exposure

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Oligodendrocytes, the myelin forming cells in the central nervous system, are vulnerable to Pb toxicity what may result in hypo- or demyelination but the exact mechanism of its action are not clear. Thus, it was of interest to discern whether prolonged exposure to Pb in drinking water in environmentally occurring doses, influences the level of myelin glycoproteins MAG and MOG in adult rat brains. The electron microscopic studies have shown structural abnormalities in axonal myelin sheath, which were paralleled by changes in myelin membrane fluidity measured by spectrofluorometry and electron paramagnetic resonance (EPR) techniques with a fatty acid spin label. In Pb-treated rats enhanced membrane fluidity was observed, as indicated by both anisotropy of the membrane and the order parameter. Myelin-associated glycoprotein (MAG) is a membrane component expressed preferentially on the innermost myelin wrap, whereas myelin oligodendrocyte glycoprotein (MOG) is an integral membrane protein expressed both in oligodendrocytes and outermost myelin lamellae. The expression of both myelin glycoproteins was found to be decreased in lead-exposed rats to a similar degree (80–85% of control values). These results suggest that Pb intoxication leads to disturbances in myelin sheaths. The lower expression of glycoproteins may be almost one of the reasons leading to the changes in integrity of the myelin membrane.

SI-P15 Inflammatory features of glial reaction in immature lead-exposed rat brain

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It is known that young organisms are particularly susceptible to lead (Pb) toxicity. Central nervous system is one of the principal sites of its toxic action and glial cells accumulate and store this metal. Activation of glial cells is a well-known phenomenon observed in many pathological conditions and connected with overexpression of several proteins, like glial fibrillary acidic protein (GFAP) and S-100b. The latter is known to be involved in progression of glial-mediated pro-inflammatory reaction in which ATP is suspected to be one of the factors influencing the release of cytokines via activation of the purinergic receptors. The effect of lead was examined in immature rat brains towards the potential proinflammatory reaction of glial cells. In experiments 15-day-old rats were injected with lead acetate (15 mg/kg, i.p.) for two weeks. After exposure to lead we observed increased level of GFAP and S-100b in all examined parts of brain. The release of proinflammatory cytokines: IL-1b, TNF-a in hippocampus and IL-6 in forebrain of Pb treated rats was noticed. In parallel with these changes we observed increased expression of purinergic receptor P2X7 in hippocampus of Pb treated rats. The results suggest that under lead toxicity conditions in immature rat brain glial activation is induced with coexisting inflammatory response.
The study was partially supported by grant 2PO5F01326 from the Ministry of Scientific Research and Information Technology.

SI-P16 Changes of the expression of particulate guanylate cyclases and their role in the cerebral cortical slices of hyperammonemic rats

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Recent in vitro evidence suggested that ammonia affects the expression of particulate guanylate cyclase (pGC) in astrocytes, which may contribute to changes in cGMP generation in the brain during hyperammonemia (HA). (Konopacka et al. 2006). Here we measured the effect of chronic hyperammonemia (a model of ammonia-supplemented diet) on: (i) expression of pGC-A and pGC-B mRNA in the cerebral cortex using real-time PCR technique (ii) cGMP generation in the cerebral cortical slices stimulated with the respective ligands: atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP), respectively. HA decreased the expression of pGC-B mRNA but not pGC-A mRNA in the cerebral cortex. pGC-B mRNA expression was not altered in the cerebellum or hippocampus of these rats. In control slices, stimulation of cGMP accumulation by CNP but not by ANP was greatly inhibited with an glial metabolism inhibitor, fluoroacetate (FA), indicating that a proportion of pGC-B is located in astrocytes. Hyperammonemia had a tendency to increase the CNP-dependent cGMP accumulation. There was no striking difference in the stimulatory effects of ANP on cGMP accumulation between slices from control and hyperammonemic rats. The results indicate that in chronic hyperammonemia, an FA-vulnerable population of cerebral cortical astrocytes may be the cells in which pGC-B-mediated synthesis of cGMP undergoes compensatory activation.
Supported by MSHE grant no 2PO5A06426.

SI-P17 Ammonia inhibits particulate guanylate cyclase-dependent cGMP formation and calcium transport in a rat brain endothelial cell line

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Ammonia is a neurotoxin involved in hyperammonemia-related neurological disorders. In the CNS cells, neurotoxic effects of ammonia are thought to be mediated by activation of the NMDA receptor/NO/cGMP pathway involving the soluble form of guanylate cyclase (sGC). An alternative pathway for cGMP formation involves activation of particulate guanylate cyclase (pGC) stimulated by natriuretic peptides (ANP, BNP, CNP). Recently it was proven that ammonia alters pGC mediated cGMP formation in cultured astrocytes (Konopacka et al. 2006, Neurochem Int 48: 553). This study investigated, to our knowledge for the first time, the effect of ammonia on pGC activity and expression in endothelial cells forming the blood brain barrier. A rat brain endothelial cell line (RBE-4) was employed which is characterized by abundance of pGC but absence of sGC. Cells were subjected to 1 h treatment with 1 mM or 5 mM ammonia. Ammonia produced a substantial decrease of cGMP synthesis, and depressed CNP-dependent calcium accumulation in these cells. A transiently decreased expression of mRNA coding for pGC-A and pGC-B was also noted which may be functionally reflected after cessation of ammonia exposure. A decline in pGC-mediated synthesis of cGMP may contribute to disturbances of hemodynamics of the cerebral blood vessels during hyperammonemia. No change of either parameter was anymore observed following a 24 h treatment with ammonia, indicating adaptation to the exposure.
Supported by a State Committee for Scientific Research grant 2P05A 06426 (to M.Z).

SI-P18 alpha-Synuclein and its neurotoxic fragment induce apoptotic pathway in PC 12 cells with different amyloid beta load

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alpha-Synuclein (ASN) and its neurotoxic fragment NAC (non-amyloid beta component of Alzheimer's disease amyloid) are suggested to play a crucial role in amyloid beta aggregation and neurodegeneration. However, the precise mechanism of ASN action remains unclear. In the present study, we investigated the role of ASN and NAC in cells viability. Investigations were performed on PC12 control cells and PC12 cells transfected with human amyloid precursor protein (APPwt) or bearing the Swedish double mutation (APPsw). Immunochemical, spectrophotometrical and spectrofluorometrical methods were used in this study. Our results showed that non-aggregated ASN and NAC induced PC12 cells death in concentration dependent manner by 50% and 70%, respectively at 10 microM. Analysis with Hoechst 33342, indicated that ASN and NAC activated apoptotic signaling. ASN induced caspase-3 activation by 36% and decreased the level of poly(ADP-ribose) polymerase (PARP) immunoreactivity. Inhibitor of caspase-3 (Z-

DEVD-FMK, 100 microM) and mitochondrial permeability transition pore blocker (cyclosporine A, 2 microM) partially prevented ASN-evoked cell death. These findings indicate that ASN and its degradation product NAC induce apoptotic pathway in PC 12 cell lines and the significance of their effects depends on the molecular events activated by intracellular amyloid beta accumulated in APP transfected cells.
Supported by MSHE grant 2PO5A4129.

SI-P19 alpha-Synuclein stimulates nitric oxide synthase (NOS) by activation of NMDA receptor

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alpha-Synuclein (ASN) is a small (14 kDa), abundant, intrinsically disordered presynaptic protein, whose aggregation is believed to be important in neurodegenerative diseases. The central domain of the protein, the non-amyloid component (NAC), is probably responsible for ASN aggregation and toxicity. The aim of our study was to determine the effect of ASN and NAC on nitric oxide synthase (NOS) activity. The studies were carried out using radiochemical and immunochemical methods. Here we report that ASN is liberated from synaptoneurosomes into extracellular space during oxidative stress evoked by FeCl2 /ascorbate (25 microM/250 microM), H2O2 (500 microM) and NO donor sodium nitroprusside (SNP, 1 mM). Extracellular ASN and NAC (10 microM) stimulated NOS activity in rat cortical slices by about 20% and 50%, respectively. These toxic proteins affected constitutive isoforms of NOS (nNOS and eNOS) without effect on inducible protein (iNOS). N-methyl-D-aspartic acid (NMDA, 100 microM) enhanced NOS activity by about 100%. Inhibition of NMDA receptor by MK-801 and APV prevented NOS activation evoked by NAC. Synuclein and NAC peptide induced [45]Ca2+ influx into cortical synaptoneurosomes by 42% and 56%, respectively. Our findings indicate that ASN and NAC stimulated NOS activity through inducing Ca2+ influx by NMDA receptor.
Supported by MSHE grant 2PO5A4129.

SI-P20 The role of cyclooxygenases and lipooxygenases in mechanism of amyloid beta toxicity

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Amyloid beta (Abeta) deposition in brain is a hallmark of Alzheimer's disease (AD). Mutations in Abeta precursor protein (APP) are associated with early-onset of familiar AD. Our previous data demonstrated that cells with the Swedish double mutation in APP (APPsw) characterize high level of Abeta and nitric oxide (NO). Moreover our data indicate that NO play important role in release of arachidonic acid (AA), the substrat for cyclooxygenases (COX) and lipooxygenases (LOX). The aim of this study was to examine the role of COXs and LOXs in Abeta toxicity. The relationship between NO and AA metabolism was investigated using NO donor, sodium nitropruside (SNP). We used control PC12 cells and two PC12 cell lines transfected with human amyloid beta precursor protein (APPwt) or bearing the Swedish double mutation APPsw. Our results showed relationship between Abeta/NO intracellular concentration and enhancement of cPLA2 and oxidative stress level. The increased translocation of NFkappaB subunit, p65, into nucleus in APPsw was significantly higher comparing to the other cell types. These events were partly prevented by inhibitors of NO synthases, COXs and LOXs. Cell death induced by SNP was also significantly higher in APPsw cells comparing to the other cell types and was reduced exclusively by COXs inhibitors. Our results indicate that COXs and LOXs may contribute to oxidative stress and play important role in mechanism of Aß toxicity.
Supported by grant PBZ-MIN-001/PO5/16 from Ministry of Science and Higher Education.

SI-P21 Effect of cocaine sensitization on the expression of Galpha(q/11) proteins in rat amygdala

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Cocaine, the blocker of monoamine transporters, is known to induce behavioural sensitization and neuroadaptive changes in reward brain system. G proteins are key components of cellular signalling that link receptors to effectors and G(q/11) family is coupled to phospholipase C pathway. The aim of the study was to assess expression of mRNA and protein of Galpha(q) and Galpha(11) in the amygdala of rats after induction of cocaine sensitization, the animal model of drug addiction. Wistar rats were treated with cocaine (COC, 10 mg/kg/i.p.) or saline (SAL) for 5days. On day 10 half of rats from either group received a challenge dose of COC and others received SAL. Locomotor activity (distance travel in cm) was enhanced (three-fold) by challenge dose of COC compared to the acute drug effect. Galpha(q) and Galpha(11)mRNA and protein level was measured by real time RT-PCR and Western blotting, 2 and 48 hours after the last injection. Two hours after acute COC, the Galpha(11) protein expression increased (~44%) compared to SAL control. COC challenge dose induced a significant but transient increase (~45%) in Galpha(q) protein. Interestingly, after 48 hours, there was a decrease (~42%) in Galpha(q) protein of rats challenged with SAL and this effect was in opposition to the changes of Galpha(q) mRNA that was significantly increased (~56%) in this group of animals. Our results suggest that cocaine withdrawal induces changes in the Galpha(q) turnover which can be normalized by a subsequent dose of the drug.
Supported by MSHE grant 0674/P05/2005/29.

SI-P22 Selective decrease of SN1(SNAT3) mRNA expression in cerebral cortical astrocytes grown in an acidic medium

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A previous study revealed that two astroglia-derived tumor cell lines (C6; rat and T98G; human) respond to a 4 h incubation in

an acidified medium (pH 6.5) ("acid incubation", AI) with a significant decrease of expression of mRNA coding for the N system glutamine (Gln) transporter, SN1(SNAT3) (Sidoryk et al. 2006, Neurochem Int 48: 547–552). Here we show that a similar decrease occurs in rat cerebral cortical astrocytes subjected to AI under identical conditions. No change in the expression of mRNAs coding for other glutamine transporters: a system ASC (ASCT2) and a system A (ATA1(SNAT1)) transporter was noted. As judged from the incubation in the presence of a RNA polymerase II inhibitor (DRB), AI did not affect the stability of SN1(SNAT3) mRNA, indicating a true effect on RNA synthesis. AI did not affect the cell viability (MTT test), nor the overall or system N-mediated glutamine uptake, but at the same time led to a significant (adaptive?) increase of pHi in the cells (BCECF-AM fluorescence test) from pH 6.7 to pH 7.0. The results counter the view of a crucial role of SN1(SNAT3) in adapting the Gln transport and pH regulation in astrocytes exposed to acidic stress.

Supported by a grant from the Ministry of Science and Education (grant no 2 P05A 122 30).

SI-P23 The activities of AChE and BChE in rat brain are related to behaviour disturbances in the course of experimental breast cancer

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Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are significant in cognitive functions disturbances. Cognitive impairment is known to be associated with malignancy and in cases of breast cancer it is not clearly known if cognitive impairment observed results from neoplastic disease as such or if it is an effect paraneoplastic syndromes. The aim of this study was to evaluate the activity of AChE and BChE in brain regions in the course of experimental neoplastic disease in relation to behaviour. Material and methods: The frontal, temporal and occipital lobes, cerebellum and brainstem were used for analysis following 1 and 2 weeks after breast cancer transplantation in female Wistar rats. The activity of esterases were estimated spectrophotometrically in tissue homogenates. The behaviour tests were based on open-field, T-maze and elevated-plus maze and video-monitored. The software built-up by the authors used intuitive algorithms. Results: AChE activity was increased in cerebellum during first ($P<0.05$) and second week of the tumor growth ($P<0.05$) and after 2 weeks in brainstem ($P<0.05$). BChE was elevated after one week in brainstem ($P<0.05$), cerebellum ($P<0.05$), frontal ($P<0.05$) and temporal lobes ($P<0.05$). Abnormal motor behavior and spatial disorientation were evidenced. Conclusions: The stimulation of esterases involved in acethylocholine degradation was associated with motor activity impairment and spatial disorientation in the course of experimental breast cancer.

SI-P24 Functional analysis of doxycycline-inducible receptor for neurotrophin-3

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Neurotrophin-3 binds to and activates the receptor tyrosine kinase TrkC. The aim of our study was selection of appropriate doxycycline (DOX)-inducible promoter to express functional TrkC in PC12-Tet-On cells (rat neuroendocrine tumor cells with expression of reverse tetracycline transcriptional activator, rtTA). First, we studied the expression of luciferase reporter gene regulated by original TRE promoter (that contains direct repeats of 42 bp tetO sequences) or second generation TRE-tight promoter (that contains direct repeats of 36 bp tetO sequences), where tetO are DNA sequence elements that bind dimeric rtTA proteins. We found that DOX-induced expression of luciferase driven by TRE-tight promoter was much higher than expression driven by TRE promoter. We note that undesired leaky expression of luciferase in the absence of DOX was almost completely eliminated in experiments using tetracycline transcriptional silencer (tTS). Next, we confirmed that DOX-induced expression of TrkC in PC12 cells was able to increase transactivation activity of GAL4-Elk1 fusion protein in luciferase reporter gene assays. In conclusion, we suggest that cells with DOX-induced TrkC could be useful in the study of transcriptional regulation by TrkC-dependent signaling pathways.

This work was supported by grant PBZ-MIN-005/P04/2002.

SI-P25 Dominant negative form of the CREB subfamily of transcriptional activators inhibits TrkC-induced activation of the nur77 promoter

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The orphan nuclear receptor Nur77 is a member of the Nur family of transcription factors which also consists of Nurr1 and Nor-1. The nur77 gene is induced with immediate-early kinetics by various external stimuli in neuroendocrine tumor PC12 cells, including response to activation of receptor tyrosine kinases, elevation of cAMP and calcium influx. The promoter region of the nur77 gene contains four near AP-1 (NAP) sites TGCGTCA. Results of Yoon and Lau (1994, Mol Cell Biol 14: 7731) indicated that JunD, but not CREB, was responsible for induction of transcription of nur77 in response to stimulation of PC12 cells with nerve growh factor. However, our results indicate that transcriptional activation of the nur77 promoter in response to activation of the receptor tyrosine kinase TrkC is inhibited by A-CREB, a genetically engineered isoform of CREB that functions by heterodimerizing with endogenous CREB, CREM, ATF1 proteins and preventing their interaction with DNA. In conclusion, we suggest that activation of the nur77 promoter requires cooperation of both the CREB subfamily activator and the heterodimer containing JunD.

This work was supported by grant PBZ-MIN-005/P04/2002.

SI-P26 Activation of ERK1/2 and CREB by angiotensin II and IV in rat glial cells

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Angiotensin (Ang) peptides activate several signaling molecules, alter blood pressure, cell growth and memory. We evaluated the effect of angiotensin peptides on astroglial cell growth, apoptosis, oxidative stress, intracellular calcium and on expression and activation of two important signaling molecules in memory formation: a stimulus-induced transcription factor – the cAMP response element binding protein (CREB) and extracellular signal-regulated protein kinase 1/2 (Erk1/2). Ten days old cultures of rat glial cells were treated for 24 h with Ang II (10 μM), Ang IV (10 μM) or both peptides in the presence or absence of AT1 receptor antagonist-losartan (100 μM), AT2 receptor anantagonist-PD123319 (100 μM) and/or protease inhibitor-bestatin (100 μM). The treatment did not significantly affect cell proliferation, apoptosis and oxidative stress but intracellular calcium was increased almost 2-fold in cells treated with Ang II but not with Ang IV. This effect was partly abolished by losartan. Ang II increased CREB expression and to a lesser extent ERK1/2 and Thr202/Tyr204-phosphorylated ERK1/2 expression, while Ang IV significantly enhanced both ERK1/2 forms. These changes were partly abolished by losartan but not by PD123319 or bestatin indicating that the effect of Ang II on astroglial cells could be coupled to the up-regulation of CREB and ERK1/2 functionality, most probably *via* activation of AT1 receptors.

SI-P27 PSD95 in learning-induced plasticity of the adult cerebral cortex

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PSD95 is a scaffolding protein that links NMDA receptor subunits to cytoskeleton, adhesion molecules and intracellular transduction pathways. As a part of NMDA receptor complex PSD95 is implicated in synaptic plasticity. We examined PSD95 involvement in learning-induced plasticity of cerebral cortex of adult mice. In our learning model whiskers stimulation is paired with the aversive stimulus in a 3 days-long classical conditioning training. Functional representation of whiskers stimulated during the training enlarges and the change depends on NMDA receptor activation. PSD95 mRNA and protein level were examined in the cortex during and after the training. In subcellular fraction enriched in postsynaptic densities PSD95 protein level increased after the training by about 40% as analysed by Western blotting method. Twenty-four hours later PSD95 protein level returned to control concentration, although plastic change is still present. After the first training session, level of PSD95 was unchanged. Changes in PSD95 mRNA level were checked by *in situ* hybridisation method with antisense oligonucleotide as a probe. No changes in mRNA expression were found at any of the examined time points. In the P2 fraction PSD95 protein level was elevated 1 hour and 24 hours after the training and 24 hours but not 1 hour after 3 sessions of increased whiskers stimulation. The results suggest that PSD95 plays a role in a late phase, but not in induction or maintenance of plastic changes in adult cerebral cortex. Rise of PSD95 protein level in synapses after the training is linked with increased total level of PSD95, but not due to increase in its transcript level.

Session II
NEUROPROTECTION STUDIES IN EXPERIMENTAL MODELS

SII-L1 Does improvement of motor abilities after locomotor training of spinalized rats involve activation of neurotrophin system?

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Improvement of locomotion due to exercise has been well documented in the cats after complete spinal cord transection. The effect has been attributed to plasticity, suggestive of remodeling of spinal neuronal network. We hypothesized that neurotrophins (NT), important for neuronal plasticity, play crucial role in recovery processes activated by exercise. In intact rats, 4 weeks of moderate (1 km daily) walking led to activation of BDNF, NT-4 and their receptor TrkB FL in the lumbar spinal cord but did not modify other NT receptors (TrkB TK, p75, Trk C, TrkA) and ligands of TrkC (NT-3) and TrkA (NGF), pointing to BDNF/TrkB FL as the most responsive to exercise. We asked whether an improvement of locomotor ability after the training of spinalized rats, is accompanied by changes of BDNF in the lumbar spinal cord. Three groups of rats were used: intact, spinalized non-trained and trained. Six weeks after surgery an increase of BDNF immunoreactivity (IR) in neuropil and in neuronal perikarya of motor nuclei was found. Training did not change this effect. In contrast, the density of BDNF IR fibers in motor nuclei was lower in spinalized than in intact rats, and training caused an increase of BDNF IR fiber density. Higher density of BDNF positive fibers in motor nuclei speaks in favor of the hypothesis that locomotor training of spinalized animals might cause remodeling of the spinal locomotor network.
Supported by grants: MSII K056/P05/2003; statutory for the Nencki Institute.

SII-L2 Effects of neurosteroids on apoptotic processes in neuronal cells *in vitro*

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Neurosteroids are important modulators of the central nervous system function and may be involved in protecting neuronal cells against damaging agents. However, little is known about their interactions with apoptotic processes. To this end, in the present study we evaluated effects of some excitatory and inhibitory neu-

rosteroids on staurosporine-induced apoptosis in human neuroblastoma SH-SY5Y cells. Staurosporine (1 microM for 24 h) enhanced caspase-3 activity and significantly decreased mitochondrial membrane potential. Dehydroepiandrosterone (DHEA, 0.01–1 microM), dehydroepiandrosterone sulfate (DHEA-S, 0.1 and 1 microM) and pregnenolone (PREG, 0.1 and 1 microM) attenuated effects of staurosporine on both caspase-3 activity and the mitochondrial membrane potential. Pregnenolone sulfate (PREG-S) and allopregnanolone inhibited the staurosporine-induced alterations in both apoptotic parameters only at the lowest concentrations. Neuroprotective effects of neurosteroids were positively verified by Hoechst staining. Moreover, calcein assay showed that DHEA, DHEAS and PREG enhanced viability of staurosporine-treated cells and these effects were partially reversed by inhibitors of phospatidylinositol 3-kinase (PI3-K) and extracellular signal-regulated mitogen-activated protein kinase (ERK-MAPK). This study indicates that neurosteroids at physiological concentrations prevent SH-SY5Y cell damage related to activation of mitochondrial apoptotic pathway. Furthermore, neuroprotective effects of DHEA and DHEAS appear to depend on PI3-K and ERK/MAPK activation.

Support: Grant nr 2 P05A 079 27 from the Ministry of Science and Higher Education.

SII-L3 New aspects of retinal degeneration: Mechanisms and potential neuroprotective strategies

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Glaucoma is one of the most common forms of optic neuropathies and retinal degenerations. Preventing visual loss is being studied with neuroprotective therapies. Visual restoration requires a restoration of retinal ganglion cells (RGC) and their axons. Experimental studies have yielded a wealth of information related to the mechanism of RGC death following injury either to the myelinated ganglion cell axon or to the ganglion cell body. A battery of agents now exist that can blunt animal ganglion cell death irrespective of whether the insult was to the ganglion cell body or the myelinated axon. Whether this information can be applied for use in patients remains a matter of debate, and major obstacles need to be overcome before the laboratory studies may be applied clinically. Discussions on the etiopathogenesis of glaucoma center on elevated IOP and ocular disorders of vascular function. The mechanisms of axonal damage induced by ischemia are explained and the resultant possible neuroprotective effect mechanisms will be discussed (ischaemic preconditioning, endogenous neuroprotection, kynurenine pathway in the retina). Relevant questions and possible therapeutic approaches will be discussed. Finally, perspectives of neuroprotective treatment of glaucoma and other degenerative diseases will be presented.

SII-O1 Acetyl-CoA-dependent alterations in susceptibility of cholinergic neurons to neurotoxic signals

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Competition between energy and acetylcholine producing pathways yielding relative shortage of acetyl-CoA in cholinergic neurons may cause their susceptibility to neurodegenerative conditions. Highly differentiated SN56 cholinergic neuroblastoma cells demonstrated lower activity of pyruvate dehydrogenase (PDH), mitochondrial acetyl-CoA and higher activity of choline acetyltransferase (ChAT), acetylcholine content, cytoplasmic acetyl-CoA level, Ca accumulation and p75 receptor density than the nondifferentiated ones. Inverse correlation existed between acetyl-CoA content and cholinergic activities. Aluminium, NO excess, amyloid-beta, zinc, separately or in combination caused grater damage in differentiated than in non differentiated cells. Acetyl-L-carnitine reversed neurotoxin-evoked suppression of PDH, ChAT activities, mitochondrial acetyl-CoA in differentiated cholinergic cells through the restoration cytoplasmic acetyl-CoA, but failed to improve cell survival. Lipoic acid decreased mortality and elevated cholinergic markers in toxin treated cholinergic cells increasing their acetyl-CoA levels, PDH activity and antioxidative capacity. Significant correlations were found between parameters of acetylcholine, acetyl-CoA metabolism and cell mortality in these experimental conditions. Thus, in cholinergic neurons particular elements of pyruvate-acetyl-CoA-acetylcholine pathway form a functional unit which assures their uniform response to neurotoxic and neuroprotective conditions.

Supported by MSHE grants 2P05A 110 30 and 2P05A 26 26.

SII-O2 Up-regulated glial TrkA may mediate neuroprotective effects of NGF-releasing, anti-MBP CD4 T cells administered into trimethyltin intoxicated rats

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The cells from the immune system are capable of infiltration and localization to regions of CNS injury and also are able to produce and secrete neurotrophic factors. Evidence has been provided that T cells do produce BDNF and NGF. We investigated the influence of administration of autoimmune T cells on hippocampal neurodegeneration induced by trimethyltin (TMT). Lewis female rats were subjected to i.p. injections with 8 mg/kg of TMT alone, or followed the next day by i.v. injection of anti-MBP CD4+ GFP+ T cells (4 millions/animal). We have found that neurodegeneration in the CA4/CA3 pyramidal cell region (NeuN immunopositive and Nissl stained neurons counting) was significantly less in animals receiving TMT plus transferred T cells compared to those

receiving TMT alone. Since neuroprotection by neurotrophic factors produced by T cells could be afforded through their respective receptors, we investigated the occurrence and cellular localization of hippocampal TrkA, the high affinity receptor of a putative T cell released NGF. We have found that TMT intoxication not only up-regulated TrkA, localized both on immature and mature astroglia, but that this effect was further enhanced by administration of T cells. The data suggest that neuroprotective action of T cell released NGF could be exerted *via* the glial TrkA.

SII-O3 Nitric oxide enhances expression and activity of cytosolic phospholipase A2 in PC12 cells with the different amyloid beta load

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Cytosolic phospholipase A2 (cPLA2) preferentially liberated arachidonic acid (AA), which is known to be elevated in Alzheimer disease (AD). The aim of this study was to investigate the possible relationship between enhanced nitric oxide (NO) generation observed in AD and cPLA2 activity in PC12 cells with different amyloid beta (Aß) load. The PC12 control cells, PC12 cells bearing the Swedish double mutation (APPsw) and transfected with human APP (APPwt) were used. APPsw secreted 6 and 26 fold Aß compared to APPwt and control PC12 cells, respectively. The increase of NO synthase activity, cGMP and free radicals levels in APPsw and APPwt PC12 cell was observed. cPLA2 protein level and activity were higher in APPsw and APPwt PC12 cells comparing to control PC12 cells. Moreover, phosphorylated cPLA2 protein level was higher in APPsw and APPwt PC12 cells *versus* PC12 control cells. Incubation of [3H]AA prelabelled all investigated cell groups (APPwt, APPsw, PC12) with NO donor stimulated [3H]AA release. The higher NO induced AA release was observed in PC12 control cells. Inhibitors of cPLA2, NO synthase and gamma secretase reduced significantly AA release in all investigated cells. These results indicated that intracellular Aß peptides enhances expression and activity of cPLA2 by NO regulated pathway.
Supported by MSRIT grant PBZ-MIN-001/PO5/16.

SII-O4 Antiproliferative properties of opioid-tachykinin hybride peptides on human glioblastoma cell line T98G

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Tachykinins are endogenous neuropeptides synthesized in various human cells including neuronal and glial cells in the central nervous system. They act as neurotransmitters and/or neuromodulators and induce DNA synthesis leading to stimulation of cell division and proliferation. Substance P (SP) is the member of the large tachykinins family that triggers its biological responses via well-established tachykinin NK1 receptors. It has been documented that malignant brain tumours of glial origin demonstrate overexpression of the functional NK1 receptors. We investigated the effect of selected SP-antagonists, opioid (biphalin) and synthesized hybrides of both of these substances on the growth and proliferation of T98G human glioblastoma cells. Immunocytochemical analysis evidenced the inhibitory effect of opioid peptides, SP-antagonists and tachykinin-opioid hybrides on proliferation of glioma cells determined by Ki-67 proliferation marker. The results of this study suggest the potential inhibitory effect of NK1 receptor antagonists and opioid peptides in proliferation abilities and growth of neoplastic astroglial cells in malignant gliomas in vitro. The effect of reduction of neoplastic cells proliferation by application of SP-antagonist-opioid hybrides *in vitro* might have some implication in synergic anti-neoplastic and analgetic therapy in malignant glial brain tumours.

SII-P1 Diazepam and cyclosporin A: The promising neuroprotectants

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We have investigated molecular background accountable for the neuroprotective potential of cyclosporin A (CsA), diazepam and minocycline *in vitro* on organotypic rat hippocampal culture treated with 30 mM tert-butylhydroperoxide (TBH) or 100 mM NMDA and *in vivo* after transient gerbil brain ischemia. We have found that 5 mM diazepam, 30 uM minocycline or 0.25 uM CsA showed significant cell protection (>50%) *in vitro. In vivo,* postischemic treatment with CsA or diazepam resulted in more than 60% of alive CA1 neurons comparing with 15% of survivors in untreated, postischemic animals. We tested whether the protective effect of diazepam was solely due to the drug-induced hypothermia or additionally to the inhibition of mitochondria-induced apoptosis. Diazepam in normothermic conditions did not revealed significant protection (21.3 ± 1.6%). Importantly, hypothermia alone induced at the time of diazepam-evoked hypothermic effect, did not prevent neuronal cell loss after ischemia to the same extend as diazepam did (42.8 ± 9.2% and 72.4 ± 14.5%, respectively). Moreover, CsA and diazepam reduced the efflux of cytochrome c out of mitochondria in ischemia compromised CA1 neurons as well as in mitochondria treated *in vitro* with TBH. Our results confirm crucial role of mitochondrial pathway in postischemic neuronal apoptosis and suggest that neuroprotective action of diazepam consist of two independent components – reduction of body temperature and hypothermia-independent inhibition of mitochondrial apoptotic pathway.
Sponsored by MSHE grant 2P05A00927.

SII-P2 Behavioral evaluation of ischemic damage of CA1 hippocampal neurons: Effects of preconditioning

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In Mongolian gerbils global forebrain ischemia induces disruption of the nest building and hyperactivity immediately after the insult, which corresponds to the extent of damage of the hippocampal neurons developing 3 days later. Preconditioning by a brief episode of sublethal ischemia induces protection of CA1 hippocampal neurons against a lethal ischemic insult. We examined how preconditioning with 2-min ischemia affects disturbances in the nest building behavior and locomotor activity induced by the injurious 3-min ischemia. Morphological examination confirmed that preconditioning significantly reduces neuronal damage in CA1 evoked by injurious ischemia. Behavioral studies demonstrated that preconditioning reduced the locomotor hyperactivity and latency in the nest building after test ischemia, in comparison to sham or naive animals. Results indicate that nest building test and measurement of locomotor activity may be utilized for an early *in vivo* prediction of the extent of ischemic brain damage and tolerance induced by ischemic preconditioning.

SII-P3 Effect of methylnicotinamide on MMPs activity after neonatal cerebral hypoxia-ischemia

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Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that degrade the extracellular matrix and carry out key functions during development and after injury. The aberrant excessive activity of MMPs, especially MMP-9, contributes directly to neuron apoptosis and brain damage after cerebral ischemia. Therefore it is logical to anticipate that the inhibition of MMPs leads to the reduction of neuronal death. Recent experimental data show that 1-methylnicotinamide (MNA) reduces infarct size induced by neonatal cerebral hypoxia-ischemia (H-I). Using the same H-I model we studied the possible contribution of MNA to MMP-9 and MMP-2 activity. Seven-day-old rats were subjected to left common carotid artery ligation and hypoxia (7.3% O_2 in N_2 for 75 minutes). The activity of MMPs was determined by zymography. Our results show that H-I resulted in significant elevation of MMP-2 and MMP-9 activity in the H-I forebrain. Injection of MNA resulted in a marked decrease of MMP9 activity at 48 h after H-I insult. Contrary, the activity of MMP-2 gradually increased during the investigated time of recovery, reaching maximal value at 14 days. These results indicate the different roles of MMPs in HI brain. It seems that the inhibition of MMP-9 in brain of the animals treated with MNA may contribute to the protection of neuronal cells after HI whereas the rise in activity of MMP-2 in the later stages after injury might enable the migration of precursor cells to replenish lost neurons.
Supported by MSHE grant 101/T09/2003/11.

SII-P4 MTEP, a selective mGluR5 antagonist, reduces excitotoxic neurodegeneration in rat hippocampus

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Extensive research into glutamate receptors in the central nervous system has shown an important role of metabotropic glutamate receptors (mGluR) as potential targets for neuroprotective drugs. In the majority of studies on neuroprotection, potentially protective compounds were tested before, simultaneously or shortly after damage. Such procedures are markedly different from the situation faced in the clinic. Therefore in the present study we tried to find out whether the potent and highly selective mGluR5 antagonist 3-[(2-methyl-1, 3-thiazol-4-yl)ethynyl]-pyridine (MTEP) had neuroprotective action even when administered several hours after an excitotoxic injury. Neuronal damage was induced by unilateral injection of kainic acid (KA; 2.5 nmol/1 µl) into the CA1 region of the hippocampus in male Wistar rats. MTEP (1, 5 or 10 nmol/1 µl) was administered into the CA1 30 min before, or 30 min, 1, 3 or 6 hours after KA. Seven days later, brains were taken out and analyzed histologically to estimate the total number of neurons in the pyramidal layer of the CA of the dorsal hippocampus using a stereological method. It was found that KA induced extensive neurodegeneration (50% loss) of CA neurons. MTEP given 30 min before, or 30 min to 6 h after KA significantly attenuated the neuronal damage. The obtained results indicate that MTEP can prevent excitotoxic neuronal injury *in vivo*. Since the neuroprotection by MTEP was still evident even 6 h after KA administration, its potential therapeutic role may be suggested.

SII-P5 Neuroprotective potential of methylnicotinamide versus nicotinamide *in vivo* and *in vitro*

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Recent papers demonstrated the neuroprotective potential of nicotinamide (NAM) applied in high doses. However, its metabolite 1-methylnicotinamide (MNA) may be neurotoxic. In this study we compared neurotoxicity and neuroprotective potential of NAM and MNA *in vivo* and *in vitro*. In 7-day-old rats brain hypoxia/ischemia (HI) was induced by unilateral carotid occlusion followed by 75 min exposure to hypoxia. The excitotoxic lesion in rat pups was induced by the intrastriatal NMDA injections. The drugs were administered i.p. 30 min after the insult. Two weeks after HI or 24 h after NMDA injection brain damage was evaluated. We noticed a significant dose dependent neuroprotection in both *in vivo* models by NAM (at 250 and 500 mg/kg) and by MNA (at 30 and 60 mg/kg). Injection of both substances did not change weights and volumes of the control hemispheres. In the in vitro neurotoxicity tests NAM and MNA were administered for 24 h to primary cultures of rat cerebellar granule cells. NAM and MNA administered in the concentration of about 10-2 M significantly reduced neurotoxicity induced by 24 h exposure to homocysteine. In addition MNA was neuroprotective in high micromolar range.

Under similar conditions both substances did not interfere with glutamate neurotoxicity. These results confirmed neuroprotective potential of NAM *in vivo* and *in vitro*. Moreover they demonstrated that in contradistinction to previous speculations MNA is practically devoid of neurotoxicity and by itself induces neuroprotection.
Supported by MSHE grant - 101/T09/2003/11.

SII-P6 Upregulation of IGF-1B (MGF) in neonatal stroke

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Perinatal hypoxia-ischemia occurs in approximately 1 in 4000 to 1 in 10000 newborns. There is a number of reports showing protective effect of IGF-1 in models of hypoxia-ischemia (H–I). MGF (IGF-1Ec), an alternatively spliced variant of IGF-1 translating into an isoform with a unique C-terminus, was found to be expressed in the ischaemia-resistant hippocampal neurons *in vivo* in a gerbil model of transient adult brain ischaemia, suggesting that the endogenous MGF might have an important neuroprotective function. Therefore, we have investigated whether MGF splicing occurs during normal brain development and if it is expressed in response to neonatal H-I. Unilateral carotid occlusion with 8% hypoxia has been used to study mRNA content using semi-quantitative RT-PCR of MGF in the time course of reperfusion after global ischemia in the newborn rats. Both IGF-1 and specific MGF splicing occurred during normal postnatal brain development. However, MGF expression was transient and evident only up to day 11–12 and in the adults there was no significant MGF mRNA presence in the brain. H-I insult produced prolonged expression of MGF but not IGF-1 in the damaged brain. The finding that MGF is expressed at specific stages in development as well as in response to H-I insult not only suggests that this particular splice variant may play a significant developmental role but also indicates that MGF constitute an important endogenous neuroprotective mechanisms activated both in the postnatal and adult brain.

SII-P7 Induction of areactivity of rat lymphocytes stimulated by myelin antigens

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Recently, it has been proposed to apply method of oral tolerance to the treatment of autoimmune diseases. The aim of our study was to use hydrolizate of pig spinal cord proteins (mixture of neuroantigens) to induce oral tolerance in animal model of sclerosis multiplex (SM) – experimental allergic encephalomyelitis (EAE). The female Lewis rats were fed with pig spinal cord hydrolizate in high dose for one week before immunization, which was induced by injection of guinea pig spinal cord homogenate. As a control were used animals with EAE only, hydrolizate only, albumine fed and intact ones. Clinical course was observed and graded in five steps scale. On the top of clinical symptoms (13th day post immunization) the rats were sacrificed and the spleens were removed. Spleen cells were used for culture. Proliferation of them was measured by [3H]thymidyne incorporation and expressed in cpm (average of triplicate samples). After 7 days of cells culture the inhibition of proliferation was observed in hydrolizate fed animals in comparison to control ones. The result shows that hydrolizate of pig spinal cord proteins has a modulatory effect on the immune reaction particularly on the orally induced antigen specific modulation of autoimmune response.

SII-P8 cGMP-dependent protein kinase is involved in MPP+-induced cytosolic cPLA2 activation in dopaminergic neuronal cell line PC12

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Previous studies have shown that nitric oxide (NO)/cGMP pathway is up-regulated in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced Parkinson disease (PD) in mice. Since NO is known to be involved in cPLA2 regulation, we investigated if cGMP/PKG signaling participate in MPP+-induced cPLA2 activation and cells death. Our results indicated that MPP+ caused time-dependent enhancement in arachidonic acid (AA) release into medium from [3H]AA prelabeled PC12 cells. Moreover, AA release was significantly decreased (60%) in time dependent manner (1–24 h) by inhibitor of cPLA2 (ACOCF3). Ca2+-independent PLA2 inhibitor (BEL), decreased by 30% MPP+-induced AA release 24 h after treatment of PC12 cells. The enhancement of AA release was accompanied by a significant increase of total and phosphorylated cPLA2 protein level. PKG inhibitor (KT5823) decreased significantly cPLA2 activity and also total and phosphorylated cPLA2 protein level in MPP+ treated PC12 cells. The same effect was observed in the presence of other protein kinase inhibitors: PKC, (GF109203X) and ERK1/2 (U0126). We also observed dose-and time-dependent decreased viability of MPP+ treated PC12 cells. The cPLA2 and PKG inhibitors also prevented against MPP+-induced enhancement of free radicals production and PC12 cell death. These results indicate that cGMP/PKG signaling pathway up-regulated by MPP+ in PC12 cells is involved in activation of cPLA2.
Supported by the statutory theme 13 of Medical Research Centre.

SII-P9 17β-estradiol application after MPTP intoxication prevents the depletion of tyrosine hydroxylase in striatum of aged male mice

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The neuroprotective action of estrogen (Es) against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been shown in various reports on both female and male mice, however only when given chronically prior to MPTP insult. In this study, we tested the chronic effects of estrogen (17b-estradiol, 0.25 mg per pellet, 21-d release) in male mice (12 months old) to function as a neuroprotectant when administered prior to (Experiment 1) or after (Experiment 2) MPTP treatment. Tyrosine hydroxylase (TH) concentrations in the striatum were measured by Western blot methods at 1, 7, and 21 (Exp.1) and 7 and 21 (Exp.2) day post MPTP intoxication to assess the neuroprotective action of Es on nigrostriatal system. MPTP treatment reduced striatal TH within 1–21 days following intoxication. We indicated that Es exerted a neuroprotective effect upon nigrostriatal system when administered at 7 days prior MPTP intoxication, consistent with previous reports. Surprisingly, we also observed that Es protected the striatum from MPTP insult when Es administered at 3rd day post MPTP injection. The implantation of Es pellets after intoxication attenuated the MPTP-induced loss of striatal TH at 7 and 21 time-points. Future research will define more clearly the molecular mechanisms by that estrogen elicits its protective influence on nigrostriatal system also when given after MPTP insult.

SII-P10 Neuroactive steroids regulate plasma membrane calcium pump in PC12 cell lines

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Neuroactive steroids have been shown to modulate the excitability of neuronal membrane. Their concentration in some brain regions appears to be higher than in the plasma, defining the potentially active role in neurons. Recent evidence showed that neuroactive steroids may also serve as scavengers for free radicals. The neuroprotection was detected in the aged or injured brain with the disrupted calcium homeostasis. Plasma membrane calcium pump (PMCA) is an integral part of Ca2+ regulatory system, and plays a prominent role in restoring Ca2+ concentration to a basal level. PMCA is very sensitive to inhibition by reactive oxygen and nitrogen species; hence it may be a sensitive target for oxidative stress in the age-associated neurodegenerative states. The existence of multiple PMCA isoforms differs between tissues, and functional capacities of Ca2+-ATPase decreased with age due to oxidative modification of the protein molecules. The aim of our study was to determine if and how the non-genomic steroids action depends on PMCA isoforms composition in the membranes. We analyzed the 45Ca2+ uptake in the membranes of differentiated PC12 cell lines with suppressed neuron-specific isoforms PMCA2 and 3, in the presence of increased concentration (from 1 nM to 1 μM) of DHEA, DHEAS, PREG and PREGS. Our results indicate that neuroactive hormones can use non-genomic mechanisms to control the intracellular calcium concentration regulated by PMCA, and the effects depend on both, the membrane PMCA composition and the steroids structure.
Supported by grants 2 P05A 03529, 503-6806-2 and 502-16-197

SII-P11 GSTs in PC12 cell lines with suppressed PMCA isoforms

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GSTs are a family of enzymes that play important role in protecting cells against toxins and ROS and represent a major neuroprotective mechanism. Intracellular Ca2+ is essential for excitability, synaptic plasticity and neurite outgrowth, but its overload leads to the cell death. PMCA, encoded by 4 genes, is the most sensitive enzyme in decreasing Ca2+ level. PMCA1 and 4 are ubiquitous, but PMCA2 and 3 are characteristic for neuronal cells. To elucidate the role of PMCAs under stress conditions, we constructed stable PC12 lines with suppressed expression of PMCA2, PMCA3 or both isoforms. The modified profile of PMCAs generated changes in morphology of PC12 to a pseudo-neuronal phenotype. The resting and tapsigargin-stimulated levels of Ca2+ were increased in the lines without PMCA2, and increased number of apoptotic cells was detected. We observed differences at cellular GSH concentration and at the ratio of oxidized to the total GSH content. To characterize the relationship between modified PMCA pattern and GSTs in our PC12 lines, we evaluated the mRNA level and the specific activities of GSTA4 and MGST1. We observed differences in the mRNAs expression as well as in the activity of both GSTs in all cellular fractions of the transfected cell lines. This could suggest that suppression of both neuron-specific PMCA isoforms can alter the pattern of cell's GSTs activity as a putative adaptive mechanism against Ca2+ induced stress.
Supported by grants 2 P05A 03529, PBZ-MIN-012/P04/2004, 503-6806-2 and 502-16-197

SII-P12 The effect of TRH and its analogs on hydrogene peroxide-induced toxicity in differentiated human neuroblastoma SH-SY5Y cells

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Thyrotropin releasing hormone (pGlutamyl-Histidyl-Prolinamide, TRH) is known to exert neuroprotective effects *in vitro* and *in vivo*, however, its potential utility is limited due to its rapid metabolism. The aim of present study was to evaluate effects of stable TRH analogues on hydrogen peroxide-induced toxicity in retinoic acid-differentiated human neuroblastoma SH-SY5Y cells. Exposure of SH-SY5Y cells to 0.5 mM hydrogen peroxide resulted in significant increase of LDH (lactate dehydrogenase) efflux. Furthermore, a decrease in cell viability was verified by MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl-tetrazolium bromide) assay. The obtained data showed that TRH and its analogs RGH-2202 (L-6-keto-piperidine-2-carbonyl-L-leucyl-L-prolinamide) and Z-TRH (N-(carbobenzyloxy)-pGlutamyl-Histidyl-Prolinamide; AWL-4102) (0.01–50 mM, 24–72 h) decreased hydrogen peroxide-induced cell damage in concentration and time-dependent manner. Among the

three studied compounds, the most effective was Z-TRH. These data point to potential efficacy of new stable TRH analogues in reducing oxidative stress-related cell damage.
This study was supported by grant no. 2 PO5A 155 30 from Ministry of Science and Higher Education.

SII-P13 The effect of antidepressant drugs on glucocorticoid receptor-mediated gene transcription in the presence of low and high concentration of corticosterone

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Many data have indicated that an increased level or action of glucocorticoids is involved in the pathogenesis of major depression. Antidepressant drugs are known to inhibit some effects exerted by glucocorticoids or stress not only by lowering glucocorticoid level but also by inhibiting glucocorticoid action on gene transcription. The aim of these studies was to compare antidepressants effect of low (physiological) and high (stress) corticosterone concentration on glucocorticoid receptor-mediated gene transcription. Mouse fibroblast cells (L929), stably transfected with a mouse mammary tumor virus (MMTV) promoter linked with chloramphenicol acetyltransferase (CAT) reporter gene (LMCAT cells) were cultured with antidepressant drugs and next corticosterone at concentrations of 50 nM or 1 uM to stimulate reporter gene transcription. CAT activity was determined in cell lysates using [14C]-chloramphenicol and n-butyryl coenzyme A. It has been found that imipramine, desipramine, fluoxetine and tianeptine strongly and reboxetine weakly, inhibited CAT gene transcription induced by both corticosterone concentrations. Venlafaxine inhibited GR function only in the presence of high concentration of corticosterone. The obtained results indicated that independently of the degree of GR stimulation antidepressant drugs inhibited corticosterone-induced gene transcription. The inhibitory action of antidepressant drugs on GR function exerted by high corticosterone level seems to be beneficial, but the attenuation of action produced by basal glucocorticoid level may be disadvantageous.

SII-P14 An involvement of phoshatidylinositol 3-kinase in anti-apoptotic action of 1,25-dihydroxyvitamin D3 and its low-calcemic analogues in SH-SY5Y cell line

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The active form of vitamin D3 and some of its related compounds prevent neuronal damage, however, their effects on apoptotic processes in neuronal cells have not been studied in detail. In the present study, we investigated the effects of 1,25-dihydroxyvitamin D3 and its low-calcemic analogues, PRI-2191, PRI-1890 and PRI-1901 on staurosporine-evoked apoptosis in human neuroblastoma SH-SY5Y cells. Staurosporine (1 microM, 24 h) enhanced the caspase-3 activity, decreased mitochondrial membrane potential and increased the number of apoptotic cells as visualized by Hoechst staining. The results showed that 1,25-dihydroxyvitamin D3 and PRI-2191 (5–500 nM), attenuated the staurosporine-induced caspase-3 activity, whereas PRI-1890 and PRI-1901 were less active. Moreover, 1,25-dihydroxyvitamin D3 (50, 500 nM) and PRI-2191 (500 but not 50 nM) reversed the staurosporine-induced decrease in mitochondrial membrane potential. The neuroprotective effects of the secosteroids were positively verified by Hoechst and calcein staining. Further experiment revealed that a selective inhibitor of phosphatidylinositol 3-kinase (PI3-K), wortmannin, antagonized the effect of 1,25-dihydroxyvitamin D3 and PRI-2191 on staurosporine-induced apoptosis. These data indicate that 1,25-dihydroxyvitamin D3 and its analogues inhibited mitochondrial pathway of apoptosis in SH-SY5Y cells and that activation of PI3-K/Akt signaling pathway appears to play an essential role in the antiapoptotic action of secosteroids.
Supported by Grant No. 3 P05A 025 25 from the Ministry of Sciences and Higher Education.

SII-P15 Involvement of adenosine receptors in L-DOPA-induced oxidative stress in rat striatum

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Presently, it is widely believed that nigral neuronal death is caused by oxidative stress generated by enzymatic oxidation of the endogenous neurotransmitter DA and its exogenous precursor, L-DOPA, which is used as a main therapy of Parkinson's disease. DA and L-DOPA toxicity is mediated through formation of reactive oxygen species: superoxide, hydroxyl radical and semiquinones. In our study, we investigated the role of adenosine A1 and A2A receptor ligands in free radicals generation and a possible mechanism of their neuroprotection in L-DOPA-induced oxidative stress in rat striatum. P-hydroxybenzoic acid (pBA, 2.5 mM) was infused through microdialysis probes for free radicals measurement in dialysates from rat striatum. The reaction product of pBA with hydroxyl radical, 3,4-dihydroxybenzoic acid (3,4-DHBA) was assayed with HPLC-ED. Direct intrastriatal infusion of L-DOPA (50 uM) markedly increased the dialysate level of DA and 3,4-DHBA. It is shown, that the adenosine A1 agonist N6-cyclopentyladenosine (CPA), a non-selective A1/A2A receptor agonist 2-chloroadenosine (2-CADO) and selective A2A receptor agonist CGS 21680 (25–100 uM) decrease the level of 3,4-DHBA in dialysates from rat striatum. Similar effect on 3,4-DHBA level was produced by caffeine, a non-selective A1/A2A adenosine receptor antagonist. Our study suggests that a decrease of hydroksyl radical generation by adenosine receptor ligands results from attenuation of L-DOPA- derived DA level in the striatum. Instead, caffeine effect may be linked to scavenging activity of this methylxanthine or to its MAO-B blocking properties.

SII-P16 The protective effect of CDP-choline on neuronal changes in a model of slow glutamate excitotoxicity *in vitro*

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The defective neuronal and/or glial glutamate transport is thought to contribute in progressive neuronal loss in several neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). The organotypic tissue culture model of chronic glutamate excitotoxicity is particularly useful for the study of motoneurons (MNs) loss. Our previous ultrastructural studies performed on this model documented a subset of different modes of neuronal cell death. The aim of this ultrastructural study was to determine the potential neuroprotective effect of CDP-choline on neuronal changes in glutamate excitotoxic model *in vitro*. The study was performed on organotypic cultures of the rat lumbar spinal cord subjected to 100 μM DL-threo-b-hydroxyaspartate (THA) and 100 μM CDP-choline. At different time after experiment the cultures were processed for electron microscopy. Chronic THA exposition induced several distinct types of morphological changes such as necrosis, apoptosis and autophagocytosis. After the exposition to THA and CDP-choline both, necrotic and apoptotic injury of MNs occurred, whereas typical apoptotic changes were seen sporadically. The spinal cord cultures exposed to CDP-choline alone exhibited well-preserved MNs and astroglial cells. This results evidenced neuroprotective effect of CDP-choline against neuronal apoptotic changes in a model of chronic excitotoxicity *in vitro*.

SII-P17 Effects of doxorubicin on undifferentiated and retinoic acid (RA)-treated neuroblastoma SH-SY5Y cells

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Doxorubicin is a commonly drug used in anti-cancer therapy and in experimental studies is employed to induce cell death by activating extracellular pathway of apoptosis. In order to find out, whether toxic effects of doxorubicin depend on state of differentiation of cells, we evaluated effects of this agent on undifferentiated and retinoic acid (RA, 10 muM)-treated SH-SY5Y cells cultured in medium containing high (10%) and low (1%) serum level. Doxorubicin (0.1–10 muM) in dose- and time-dependent way decreased cell viability and increased cell death as estimated by MTT reduction and lactate dehydrogenase assays, respectively. The toxic effects of doxorubicin were more profound in undifferentiated cells in the presence of low serum level. Similar effects were observed after treatment of SH-SY5Y cells with staurosporine (0.5 muM), which is widely used as proapoptotic agent in in vitro studies, acting mainly by intracellular pathway of apoptosis. The obtained data indicate that susceptibility of human neuroblastoma SH-SY5Y cell line to toxic effects of both doxorubicin and staurosporine is attenuated in RA-differentiated cells. In this respect the caution must be taken when studied putative neuroprotective agents in models engaging neuroblastoma cell lines.
The study was supported by MSHE grant No. 1874/P01/2006/31.

SII-P18 The effect of PLC inhibitor U-73122 on angiotensin peptides-induced changes in protein tyrosine kinases activity in rat anterior pituitary

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The peptides from angiotensin family: Ang III, Ang IV, Ang 1-7 are involved in control of pituitary cell growth, proliferation and apoptosis and have inhibitory effect on tyrosine kinases (PTK's) activity. The physiological and pathological events caused by Ang peptides proceed via AT1 and AT2 receptors or by other specific receptors. Stimulation of AT1 receptor results in generation of inositol trisphoshate and diacylglycerol by phospholipase C (PLC). The aim of this study was to investigate the effect of PLC inhibitor U-73122 on angiotensins-induced changes in PTKs activity. Samples were pretreated with PLC inhibitor (10 μM) alone and then AngIII, AngIV and Ang1-7 were added in concentrations (10-9–10-11 M). Gamma[32P] - ATP was added to the mixture containing homogenate and artificial specific substrate for PTKs – poly Glu,Tyr 4:1. The activity of protein tyrosine kinase was defined as amount of pmoles of 32P connecting with poly GluTyr/mg of protein/min. The results showed that PLC inhibitor abolished the inhibitory effect of Ang III on tyrosine kinases activity. The little changes were observed in case of Ang IV and U-73122 treatment. PLC inhibitor had no effect on Ang 1-7-induced changes on PTKs activity. The results suggest that Ang III and Ang IV can modulate protein tyrosine kinases activity *via* mechanism dependent on PLC.
This work was supported by Medical University of Lodz Grants Nr 502 - 16 – 301, 503-686-2 and Polish Ministry of Education Grant no 059/P05/2004/27.

Session III
NEURAL STEM CELLS

SIII-O1 Defined cellular environment commands HUCB-NSCs fate determination

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Local environment of adult CNS dictate the fate choice of NSC. We have established human umbilical cord blood derived cell line with neural stem cell characteristic (HUCB-NSC). The aim of our study was to investigate the effect of cells isolated from neonatal rat brain on HUCB-NSC differentiation *in vitro*. Methods: HUCB-NSC ($5 \times 10^4/cm^2$) labeled with CMFDA were seeded on confluent

monolayer of primary astrocytes, neurones, oligodendrocytes and microglial cells from neonatal rat brain or endothelial cells (t-End line), co-cultured for 7 days and then stained for neural markers. Results: Rat astrocytes induce HUCB-NSC differentiation mostly into neurones (80% TUJ1+; 62% MAP-2+ cells). Similarly, close vicinity of oligodendrocytes promotes HUCB-NSC differentiation into neurons (88% TUJ1+ cells). In the presence of neuron-enriched cultures HUCB-NSC differentiate generally into oligodendrocytes (65% O4+ cells). Microglia and EC stimulate HUCB-NSC differentiation into neurons (41% and 36% TUJ1+ cells) as well as astrocytes (58% and 51% GFAP+ cells), respectively. Interestingly, the presence of EC maintains high number of HUCB-NSC to remain in undifferentiated state (23% Nestin+ cells) in comparison to the influence of astrocytes or microglial cells (0% and 4% Nestin+ cells, respectively). Conclusions: Distinct cell types isolated from neonatal rat brain influence the fate of HUCB-NSC *in vitro*. It seems that local cellular environment may also affect terminal differentiation of NSCs after their transplantation into the brain.
Supported by MSHE grants: 2 P05A17729; 2 P05/A05430.

SIII-O2 Serum free culture of 3D scaffold-based aggregates of human neural stem cells derived from cord blood

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Many neurological disorders are caused by a loss of nerve cells. However, cell replacement therapy up to now is insufficient to meet the restorative needs. New approach for such therapy requires the introduction of neural progenitors as well as more mature cells to constitute the proper microenvironment. The aim of the study was to establish the three dimensional scaffold-based aggregates (3D-SBA) of neural stem cells (NSC) in prospect of their further transplantation. Neural-like stem cell line derived from umbilical cord blood (HUCB-NSC) was cultured in defined serum-free media on human protein scaffolds. NSC present in 3D-SBA expressed pluripotent cell genes (Oct4, Sox2) as well as neural-progenitor genes (Nestin, GFAP). Such 3D constructs differentiated *in vitro* in the presence of dBcAMP and ECM proteins or have been transplanted on rat organotypic hippocampal cultures (HOC) or into adult rat brain underwent stroke by ouabain injection. After dBcAMP treatment HUCB-NSC 3D-SBA differentiated into mature neurons (GABARAP+, GluR1+) and astrocytes (S100beta+). Similar differentiation with host tissue has been noticed after transplantation on HOC. Preliminary observation revealed that after transplantation in rat brain many 3D-SBA-originate neuroblasts migrated towards the stroke area. We conclude that 3D-SBA of HUCB-NSC facilitate migration, differentiation and integration of transplanted cells into the brain where appropriate cellular composition of such 3D-SBA is necessary.
Supported by MSHE grants no: 2P05/A177/29, 2P05/A054/30 and 3TO/9B/03029.

SIII-O3 Evidence that functional neural tissue-committed stem cells (NTCSC) reside in the human bone marrow and are mobilized into peripheral blood after stroke

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The concept that bone marrow derived cells participate in neural regeneration remains controversial and the specific cell type(s) involved remains unknown. We recently reported that murine BM contains a population of CXCR4+ cells that express mRNA for various markers of tissue-committed stem cells (TCSC), including neural TCSC (Leukemia 2004, 18: 29–40). Here we show that these cells can be isolated from murine BM as a population of Sca-1+lin-CD45- cells expressing neural lineage markers and form neurospheres *in vitro* which in secondary cultures differentiate into neuronal and macroglia lineages. Our data demonstrate that neural TCSC (i) are very rare (ii) present in BM from young mice while being barely detectable in older mice (iii) are mobilized into peripheral blood during mobilization and (iv) are chemoattracted for potential brain regeneration in an SDF-1-CXCR4 dependent manner. In humans the corresponding population of cells is present among CXCR4+CD34+AC133+CD45- BMMNC. Our studies performed on 14 patients with stroke demonstrated in the peripheral blood an increase in the number of CXCR4+CD34+AC133+ cells expressing neural TCSC. Thus, we conclude that bone marrow is a potential source of CD45- neural TCSC for brain repair and we provide for the first time evidence that neural TCSC residing in bone marrow account for neural differentiation of BM-derived cells. These observations provide rationale for further studies aimed at optimizing therapeutic brain regeneration by BM-derived neural TCSC.

SIII-O4 Inventing combination instead of reductionistic model, to control the process of GFAP positive, human neural progenitors differentiation

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Instructive models of differentiation are most popular amongst neurologists. Surprisingly stochastic nature of differentiation is rarely considered by them. During our experiments GFAP+ neural progenitors (GFAP+NP) were differentiated towards neural cells, or into the fibroblastic cells depending on cell culture condition. It shows that environmental factors offer opportunity to change fate of presented here progenitors. Nevertheless, we were not able to instruct GFAP+NP to choose between neuronal or glial fate. However in accordance with stochastic models percentage of neuronal cells was increased by implanting quasi-cloning method to the differentiation protocol. Obtaining fibroblastic cells resembled in certain aspects epithelial to mesenchymal transition observed during neural crest derivatives formation. In terms of molecular mechanism, obtaining

the neuronal, astrocytic and fibroblastic cells seemed to be accomplished in accordance with model of silencing the superfluous genes expression. Our analyses suggest that reductionistic models of differentiation focusing on either only stochastic, or only environmental events should evolve to combination models, informing that prediction of stem cells fate requires assembling together stochastic, and environmental factors. It should help to design more constructive for cellular transplantologists differentiation protocols.
This research was supported by Temple University Philadelphia and MSHE Grant No. N401 046 31/1195.

SIII-P1 Close cell contacts stimulates generation and propagation of neural progenitors from human umbilical cord blood

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Cord blood is a new source of neural progenitors which could be used for cellular therapies in neurological disorders. The aim of the study was to examine the different initial culture conditions of HUCB to obtain cell population enriched in putative neural progenitors. Methods: CD34(-) fraction of HUCB-mononuclear cells (MNC) was cultured in two different cell density: high – HD (10^7/ml) and low – LD (10^6/ml) in DMEM/F12 medium with 30% FBS. Stem cell/early progenitor markers expression was studied in freshly isolated cells (DIV0) and after 24 h of culture by RT-PCR, FACS and immunochemical analysis. Results: Cells from HD culture (10^7/ml) formed floating aggregates while cells from LD culture (10^6/ml) remained as single cells. After 24 h in aggregation-promoting conditions the percentage of cells displaying high Hoechst 33342 efflux (SP fraction) increased in comparison to non-aggregating culture (1.5% vs. 0.2%). RT-PCR analysis of cells from HD revealed higher Oct-3/4 and Sox-2expression than cells from LD culture. Moreover, cells expressing pluripotency (Oct-3/4) and early proneural markers (Nestin, NF200), revealed the increase in aggregating cultures in comparison to non-aggregating conditions: Oct-3/4 (15.2% vs. 2.9%), Nestin (40% vs. 3.2%), NF200 (31.5% vs. 3.2%). Conclusions: The initial culture of CD34(-) HUCB-MNC in condition promoting cell aggregation and facilitating direct cell contacts can be decisive for proneural HUCB-NSCs commitment, selection and proliferation.
Supported by MSHE grant: 2 P05A17729

SIII-P2 Developmental response of human umbilical cord blood-derived neural-like cells to selected neurotoxins

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The aim of this work was to develop a new human cell-based alternative method for the assessment of developmental neurotoxicity (DNT). The influence of selected compounds on HUCB-NSC was evaluated at the different developmental stages (undifferentiated, spontaneously differentiating and lineage directed cells). Neural cell type-specific responses to toxic agents were measured in HUCB-NSC culture grown 14 days in standard media vs. that grown in the presence of cytokines and neuromorphogens enhancing their differentiation into neuronal, astrocytic and oligodendroglial-like cells (respectively CNTF, PDGF-BB + RA and T3 containing media). Cell viability was measured by MTT and LDVC assays and changes in expression of neural cell-type specific markers by immunodetection with antibodies against ß-tubulinIII and MAP-2 for neurons, GFAP and S100ß for astrocytes and GalC and O4 for oligodendrocytes. It was shown, that Methylmercury Chloride (MeHg) and L-Glutamate had significant influence on the survival, proliferation and differentiation of HUCB-NSC, while Paracetamol, Theophiline or D-glutamate had no significant effect. HUCB-NSC was more sensitive to MeHg at non-differentiated, early developmental stage than along its differentiation toward three neural lineages. In contrast, L-glutamate had not significant influence on the viability and expression of neural markers in non-differentiated cells, while at the later stages of development selectively affected differentiated neurones.
Supported by MSHE grant: 2 P05A17729

SIII-P3 Transplantation of neurospheres and organoids derived from human umbilical cord blood onto hippocampal organotypic slice culture

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Neural stem cells (NSCs) *in vivo* occur in specified regions of CNS called stem cells niches. Our recent results allows to postulate that neurospheres (N-HUCB) and organoids (O-HUCB) derived from human umbilical cord blood may be treated as the models of NSCs behavior in their niches. The aim of our study was to assess survival, migration and differentiation of grafted N-HUCB or O-HUCB cells on rat hippocampal organotypic slices (HOC). In further perspective such monitoring of interactions between host tissue and alien cells *in vitro* will be helpful for optimizing HUCB-NSC transplantation procedures in rat models of brain diseases. Methods: HOC was grown for the first week in DMEM with 25% horse serum supplement. After 5 days serum was gradually withdraw, and finally from day 8th the culture was maintained in serum free medium. Transplantation of N-HUCB was performed: on freshly isolated HOC or on slices cultured by 7 days in the medium alone. O-HUCB was transplanted exclusively on freshly isolated HOC. All grafts were placed in the three different regions of the hippocampus: CA, DG and enthorinal cortex. Results: We observed a strong activation of host neuron progenitors in tissue surrounding transplanted neurospheres. Moreover this activation was never observed in DG region suggesting stratification of these inductory signals. N-HUCB and O-HUCB transplanted on HOC revealed capability to differentiate toward neurons and integrate with rat hippocampal cytoarchitecture.
Supported by MSHE grant: 2 P05A17729.

SIII-P4 The influence of ECM proteins on clonal growth and

neural differentiation of Human Umbilical Cord Blood Neural Stem Cells (HUCB-NSC)

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The goal of the study was to investigate the role of ECM proteins on clone formation, proliferation and differentiation of HUCB-NSC. Methods: HUCB-NSCs were plated on matrix protein-coated (laminin, fibronectin and poly-L-lysine) plates at clonal density (50 cells/cm^2) and cultured in DMEM/F12+ITS2%FBS. After 11 days HUCB-NSC clones were immunostained for neural (beta-tubulin III) and astroglial (S100beta) markers. Results: The number of clones was significantly higher when HUCB-NSCs were plated in the presence than in absence of matrix components (104 ± 21 vs. 39 ± 5). On laminin or poly-L-lysine-coated surfaces, HUCB-NSCs clones appeared earlier and have grown more rapidly as compared to fibronectin-coated or uncoated plastic surfaces. There was no significant difference in relative number of beta-tubulin III and S100beta - positive cells grown on different surfaces. Interestingly, smaller clones (up to 10 cells) comprised undifferentiated cells and astrocytes or neurons (exclusively), while larger clones (over 10 cells) gave rise to similar proportion of these two neural phenotypes. Conclusions: Laminin and poly-L-lysine increased HUCB-NSCs clonogenicity. Such clones revealed two phases of growth: early – when either astrocyte or neuronal commitment is favorable and late – when clone composition comprised both neural phenotypes. It seems that matrix signals can influence early clones growth/migration but not their differentiation commitment.
Supported by MSHE grants: 2 P05A17729; 2PO5A05430.

SIII-P5 Behaviour of neural stem cells in sequentially monitored culture

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The goal of the investigation is to design some tools to detect and describe a pattern of cell behaviour in the cell culture. The neural stem cells from the HUCB-NSC line, established in the Medical Research Center Polish Academy of Sciences, were used to obtain quantitative data and knowledge about cell growth in culture. This knowledge will be used on both biological and mathematical levels. The monitoring of cell culture growth was based on sequences of images of the neural stem cell culture, acquired every 15 or 20 min. After up to 40 hours of observation the cells in samples were fixed and stained for visualise cells' nuclei (Hoechst), neurons (Tubulin III) and astrocytes (S100B). Some of immanent features of the observed phenomenon were extracted in semiautomatic or manual procedures using image processing and analysis methods. Several types of events were depicted, e.g., cell division and cell death, changes in cell shape and position, formation of a clone and cell fusion. Then the lineage trees were constructed for cells under division in the observed area. Based on these trees the mean time between two consecutive cell divisions was measured as 25.5 ± 5 hours. Daughter cells after division, in which two neurons were detected, appeared to be similar one to another in shape more than daughter cells after the division, in which one neuron and one cell of another type were detected.

SIII-P6 New model of experimental stroke in rats: Cellular networking in response to injury

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One of the most promising new treatments for neurological disorders could be replacement of injured cells by neural stem cells (NSC) transplantation. *The aim* of the study was to analyze the changes in rat brain following intrastriatal ouabaine (OUA) injection, considered as a model of stroke. Methods: The unilateral damage of striatum by stereotactic OUA injection (1 ul/50 nmol) was performed in Wistar rats immunosuppressed with CsA. Thereafter, at day 2, 3, 7, and 30th, rat brains were analyzed histologically and immunohistochemically. Results: Activation of ED1 (mikroglia/macrophages), GFAP (astrocytes), vWF (endothelial cells) and MMPs (matrix metalloproteinases) was found after injury in ipsilateral hemispheres of all rats. The highest number of ED1(+) cells were noticed 2–3 days after OUA injection then decreased with time of observation. In contrast, activated GFAP(+) expressing astrocytes accumulated at the border of lesion up to 30 days. Qualitative changes in vascular structure (vWF) suggesting active angiogenic process were observed in striatum and ipsilateral cortex regardless on the time of observation (2–30 days). The activity of MMPs in tissue surrounding lesion rose continuously during whole observation period. In conclusion: OUA- induced focal brain damage causes acute inflamatory macrophage/microglia infiltration, reactive astrocytosis leading to post-stroke scar formation, vast angiogenic response in tissue surrounding core of infarct with associated remodeling of ECM by activated matrix proteases.
Supported by MSHE grant: 2PO5A05430.

SIII-P7 Xenotransplantation of human umbilical cord blood neural stem cells (HUCB-NSC) into immunosuppressed rat brain

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Recently, in our laboratory human umbilical cord blood neural stem cell line (HUCB-NSC) was established. The aim of the study was to analyze the survival, migration and differentiation of HUCB-NSC transplanted into intact rat brain. Methods: HUCB-NSC (2×10^4/20 ul) labeled with Hoechst or transfected with GFP gene was stereotactically transplanted (tx) into intact brain of CsA immunosuppressed adult Wistar rats. Cell detection was performed 24 h, 48 h, 72 h, and 7 days after tx using Abs anti-GFP, NuMa, HLA-class I, GFAP, NF200. Additionally ED1 staining was used

for macrophages and microglia. Results: Analysis of rat brain revealed viable HUCB-NSC (Hoechst+ or GFP+) cells migrating from tx site and dispersed through the host brain tissue 1, 2, 3, and 7 days after grafting. Immunohistochemical studies confirmed that these cells were of human origin (HLA class I+ or NuMa+). Concomitantly, host macrophage/microglia (ED1+) infiltration was noticed around HUCB-NSC injection site. The density of inflammatory cells picked up at the 2nd day and declines with longer observation time. HUCB-NSC which migrated out from the injection site were negative for neuronal (NF-200) and astrocyte (GFAP) phenotype markers. Conclusions: HUCB-NSC transplanted into the striatum of adult rats stimulate inflammatory reaction associated with host cell recruitment and release mediators. The question arises to which extend this process is detrimental for grafted cells and how to support cell survival.
Supported by MSHE grant No 2PO5A05430.

SIII-P8 Human umbilical cord blood derived neural progenitor (HUCB-NP) intra-arterial infusion improves sensorimotor deficits in adult rats with ouabain induced brain lesion

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We developed experimental model of long-lasting motor deficits using ouabain induced lateral striatum focal injury of rat brain. In this model, considered as a stroke model, rats presented significant deficits in performance of walking beam task – characteristic impairments of limbs contralateral to injured hemisphere. Lesion extension in caudal direction correlates with performance of walking beam. The goal of the study was to analyze the functional efficacy of HUCB-NP in experimental stroke recovery. Methods: For experimental model of stroke 1 ul of 5 mM ouabain was injected into right striatum (coordinates: A 0.5; L 3.8; V 4.7) of Wistar rats. To study the effect of HUCB-NP on rat functional recovery 8 × 106 HUCB-NP was infused into right internal carotid artery 48 h after brain damage. Behavioral studies were performed to test the functional recuperations. Results: HUCB-NP treated rats presented improved performance of walking beam task at 15 and 30 days after surgery in comparison to non-treated. Infusion of HUCB NP improved also open field exploratory behavior which was affected by brain injury. Apomorphine induced rotations as well as turning tendency in the open field did not show clear differences between HUCB-NP treated and non-treated rats. It is concluded that HUCB NPs therapy may induce enhancement of functional recovery from selected deficits following focal brain damage.
Supported by MSHE grant 2P05A05430.

SIII-P9 The role of PrPc protein in neural differentiation of GFAP-positive NSCs into neuronal and glial line

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One of PrPc putative functions is its involvement in cell differentiation. To investigate a possible role of PrPcin *in vitro* differentiation of GFAP-positive neural stem cells (NSCs) into neuronal and glial cells, we examined the expression pattern of *PRNP* gene in relation to differentiation at mRNA (RT-PCR) and protein level (immunocytochemistry). The expression of *PRNP* gene was detectable in undifferentiated subset of cells and in the final stage of differentiation. Semiquantitative analysis showed slight increase of *PRNP* mRNA level in differentiated subset of cells. Immunocytochemistry assay allowed to detect the PrPc protein in all examined stages of NSCs differentiation both in neuronal and glial lineage. Comparison of cellular prion protein expression between both lineages revealed a slight increase of PrPc level in the majority of MAP2-positive neuronal cells and no significant changes of this protein level in most of GFAP-positive astrocytic cells. However, the examined NSCs exhibited largish heterogeneity of PrPc level in the final steps of differentiation. Our findings suggest that PrPc participates mainly in the differentiation of neuronal lineage, however, the presence of PrPc in glial cells may imply PrPc involvement in differentiation of astrocytic cells as well.
The study was supported by MSHE grants No 2P05A1329; No N401 046 31/1195.

Session IV
NEURODEGENERATIVE DISEASES – CLINICAL STUDIES

SIV-L3 Amyotrophic lateral sclerosis: How far away are we from the Charcot description?

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In 1869 Jean-Martin Charcot described the clinical and neuropathologic features of a disorder with selective degeneration of the upper and lower motor neurons, which Charcot termed amyotrophic lateral slerosis (ALS). ALS is a neurodegenerative disease with malignant clinical characteristics resulting from the death of motor neurons. At present, similarly to the Charcot time, ALS is a completely incurable disease leading to death on average within 3 years. Approximately 20% of patients can survive more than 5 years, and only about 10% of ALS patients can survive more than 10 years. Most cases of ALS are sporadic and about 10% of all cases are inherited, the so-called familial ALS (FALS. Approximately 20% of FALS cases are caused by missense mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1). Since 1993 some 135 mutations have been found in the SOD1 gene with different modes of inheritance. Three other genes: Alsin, Senataxin, and VABP are also known to cause different and very rare forms of FALS. A further 7 genetic loci have been identified but the genes remain unknown. The molecular basis of sporadic (idiopathic) ALS is mostly unknown; only about 3% sporadic cases have mutations in SOD1. A wide range of molecular mechanisms responsible for neurodegeneration in ALS have been proposed, including glutamate excitotoxicity,

mitochondrial dysfunction, oxidative stress, protein aggregation, proteasomal dysfunction, axonal transport deficits, and abnormal growth factors signaling. The future treatment of ALS is likely to be based on new neuroprotection and neurorestoration strategies, including gene silencing methods (siRNA) and stem-cell therapy.

SIV-O1 The possible participation of MSR virus in etiology of multiple sclerosis

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Although the etiology of multiple sclerosis is still unknown, the potential role of viral pathogenic agent in MS development is strongly suggested. Among of viruses the multiple sclerosis-associated retrovirus (MSRV) is often taken into consideration. Aim of the study was to assess the copy number of MSRV pol and gag genes in MS patients compared to healthy individuals and persons with myasthenia. The material was peripheral blood lymphocytes from 60 patients with MS, 12 patients with myasthenia and 20 healthy persons. The FISH studies with labeled PCR products of pol and gag MSRV genes in nuclei, chromosomes and chromatin fibers were done. MSRV pol and gag sequences were found in both MS patients and controls. The copy number of MSRV pol sequence was significantly greater in MS patients (6–24 copies on nucleus) than in myasthenia (4–5 copies) and normal individuals (3–6 copies). MSRV pol sequence appeared on chromatin fibers as tandem repeats longer than in healthy persons and patients with myasthenia. MSRV pol sequences were present on 1, 2, 3, 4, 5, 7, 10, 14, 17, and X chromosomes. MSRV gag sequences were found in lower number of copies compared to pol and were in a range of 2–4 copies in both MS patients and controls. In conclusion, evident difference in MSRV pol copy number between MS patients and control suggests that MSRV pol may play some role in the etiology of multiple sclerosis.
Scientific work has been supported by Ministry of Scientific Research and Information Technology funds (Grant No 2 PO5A 139 28).

SIV-O2 The APOE epsilon4, gender, and stroke severity and one-year outcome

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Background and purpose: An important genetic factor associated with the risk, severity, and prognosis in neurodegenerative diseases and in acute brain injuries, is the apolipoprotein E gene (APOE) e4 allele. In some studies the APOE e4 effects were modified by gender. We aimed to evaluate the APOE e4 effects on ischemic stroke (IS) severity and on one-year outcomes in men and women. Patients and methods: 330 men and 336 women were studied. Neurological and functional condition of patients was assessed with the Scandinavian Stroke Scale, Barthel Scale, and Rankin Scale. Information on patients' survival status was also collected. APOE genotyping was performed by the PCR-RFLP method. Results: Men possessing the APOE e4 allele had more severe neurological impairment in the acute phase of the IS, and increased risk of death within 1 month (OR=3.00, P<0.00), 3 months (OR=2.25, P<0.05), and one year (OR=2.30, P<0.05) after the IS, compared to men not having this allele. Among women APOE e4 allele status was not related to stroke severity and outcome. Men non-carriers of APOE e4 had less severe neurological impairment and lower mortality than women. APOE e4-positive men had as severe strokes and as high 1-year mortality as female patients. Conclusion: APOE e4 a significant predictor of acute IS severity and 1 year mortality in men. APOE e4-negative men have less severe neurological deficit and higher mortality than women. When men possess the APOE e4, they have similar IS severity and mortality as female patients.

SIV-O3 Polymorphism of MTHFR, MTR and MTHFD1 as related to the oxidative DNA damage, and the level of thiols in Alzheimer's and Parkinson's diseases

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Alzheimer's (AD) and Parkinson's (PD) diseases are accompanied by oxidative DNA damage, reflected by augmented levels of 8-oxo-2'-deoxy-guanosine (8-oxo2dG). Oxidative stress is intensified by homocysteine (Hcy), which originates from methionine (Met) and may undergo remethylation, due to involvement of MTHFR, MTR and MTHFD1 or transsulfuration to cysteine (Cys). Studies aimed at determination of 8-oxo2dG, Hcy, Met, and Cys in AD and PD and in the controls, using HPLC/EC/UV, and estimation, by restriction analysis, frequency of following gene polymorphisms: MTHFR (C677T, A1298C, G1793A), MTHFD1 (G1958A) and MTR (A2756G). In the control the least frequent genotypes were: TT (C677T), CC (A1298C), AA (G1793A), AA (G1958A) and GG (A2756G) with a tendency for increased frequency in AD and PD. 8-oxo2dG were significantly increased only in AD harboring following genotypes: MTHFR, CC and CT (C677T), AA (A1298C), as well as GG (G1793A); MTR, AA (A2756G); and MTHFD1, GA (G1958A). In AD levels of Hcy were significantly increased only in the patients with: MTHFR, CC and CT (C677T), and MTHFD1, AA (G1958A) genotypes. In PD, levels of Hcy were significantly increased in the patients with MTHFR, CT, (C677T), AA and AC, (A1298C) and GG, (G1793A), as well as MTR, AG, (A2756G) genotypes. In PD metabolism of Hcy to Met and Cys seemed to be more disturbed and L-dopa treatment significantly increased Hcy level. Polymorphisms of MTHFR, MTHFD1, MTR may be linked to the pathogenesis of AD and PD.

SIV-O4 The role of the hormone replacement therapy in curing

Parkinson's disease and other diseases caused by hormonal deficit

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In the course of hormone replacement therapy in males with Parkinson's disease and concomitant androgen deficit it was noticed, that supplementation of pre-existing hormonal deficits resulted in a marked improvement in parkinsonian symptoms, even if original treatment regimen remained unchanged. Tremor of upper extremities and muscular rigidity resolved or decreased markedly. Some patients experienced improvement of legibility of their writing. Patients regained their former vigor. A change in their lifestyle was made possible due to the lack of limitations imposed by the disease – they could resume activities previously made impossible by the illness. The authors are aware of the fact that unequivocal assessment of improvement in Parkinson's disease is very difficult, as many of its symptoms can not be measured digitally. Therefore, they documented their observations in the form of a video film, which depicts changes occurring in men with Parkinson's disease undergoing hormonal replacement therapy. This work is a turning point in considering different ways of treating men with Parkinson's disease. At the same time, the work presents the effects of hormone replacement therapy on many other diseases, which were hitherto treated without hormone replacement therapy. An important influence of androgen therapy was demonstrated on type 2 diabetes, osteoporosis, depressive states, sexual disorders and prostate diseases. Research results presented in this work are based on the analysis of 800 men undergoing hormone replacement therapy.

SIV-P1 Polymorphism of the paraoxonase-1 (PON-1) gene promoter and dementia

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Paraoxonase-1 (PON-1) is an antioxidative plasma enzyme associated with HDL (high density lipoproteins). Its important role is inhibition of LDL (low density lipoproteins) oxidation. Oxidatively modified LDL play an essential role in atherosclerosis development. Oxidative stress is also an important factor in neurodegeneration. Polymorphism of PON-1 gene promoter exerts an influence on gene expression and enzyme activity. Polymorphism C-108T of PON-1 gene was investigated in patients with Alzheimer's disease where degenerative processes are the prevailing ones, with dementia of vascular origin, with mixed dementia and in a control group. For genotype identification the method of PCR-RFLP according to Brophy et al. (2001, Am J Hum Gen) was used. Preliminary results show a tendency to more frequent occurrence of the allele causing lower expression of the gene in the group with dementia of vascular origin. This could cause a less effective antioxidative defense in these patients.

SIV-P2 Prion protein gene (PRNP) codon 129 polymorphism in Polish centenarians

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Polymorphism at codon 129 (Met/Val) of the prion protein gene (PRNP) is a known risk factor for Creutzfeldt–Jakob disease. Recently, a few papers have been published showing a possible association of this polymorphism and the occurrence of Alzheimer's disease. Moreover, it has been also suggested that Val/Val homozygosity at this codon may influence cognitive abilities in the elderly. The authors investigated the distribution of codon 129 polymorphism in 155 Polish centenarians, compared to a group of 213 Polish patients with probable AD and 171 controls without dementia. The percentage of Val/Val and Met/Met genotypes in centenarians was similar to AD patients and higher than in the control subjects. The results suggest that PRNP gene may be linked not only to prion diseases but to other degenerative dementias and/or survival as well. However, further research is needed to confirm this hypothesis.
The study was partially supported by MSHE; grant no.2PO5B 08 430 and PBZ-KBN-022/PO5/1999.

SIV-P3 Neuronal acetylcholine receptor alpha-7 subunit gene (CHRNA7) is expressed in lymphocytes of healthy individuals but not in the ADNFLE patients

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Neuronal nicotinic acetylcholine receptors (nAChRs) are cationic ligand-gated channels, found in the peripheral and central nervous system (CNS). Mutations in nAChR alpha-4 subunit gene (CHRNA4) are responsible for autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). Amongst the nAChRs, the alpha-7 subunit displays relatively unique features: it is widely expressed both in CNS and in lymphocytes, and it has been implicated to participate both in synaptic transmission and its modulation. The aim of the study was to investigate the expression level of CHRNA4 and CHRNA7 coding for the alpha-4 or alpha-7 nAChR subunits in lymphocytes obtained from three ADNFLE patients and ten healthy individuals to look for a possible link between the cholinergic and the immune systems. Total RNA was isolated from 1mln mononuclear leukocytes (MNLs) by acid guanidium-phenol-chloroform method. RNA was converted into cDNA using the RT-PCR method. RQ-PCR was conducted in a Light Cycler system and target cDNA was quantified. Our results provide evidence that CHRNA7 is expressed in MNLs from all healthy individuals, but not in the ADNFLE patients. Dysfunctional nAChRs in the ADNFLE patients might impair cholinergic transmission and a possible link between the cholinergic and immune systems might be disturbed. As expected, no CHRNA4 expression was evidenced in MNLs from both healthy and ADNFLE individuals.

SIV-P4 Chemokine-induced *in vitro* chemotaxis of mononuclear leukocytes in multiple sclerosis

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Active multiple sclerosis (MS) is characterized by the presence of perivascular inflammatory foci localized in the central nervous system (CNS). Inflammatory cells forming those foci migrate from the blood to the CNS. Several studies confirmed that chemokines and their receptors play an important role in that process. The major goal of this study was to analyze the migratory activity of subpopulations of peripheral blood mononuclear cells (PBMC) from the blood of MS patients stimulated by chemokine CCL5/RANTES. Moreover the impact of MS treatment with methylprednisolone (active MS) and mitoxantrone (progressing MS) on CCL5-induced chemotactic activity of PBMC subpopulations was analyzed. Chemotactic activity of mononuclear leukocytes was measured *in vitro* in Neuroprobe MBA96 chemotaxis chamber using fluorimetric reader. We observed that in active MS before any treatment *in vitro* chemotactic activity of lymphocytes after stimulation with CCL5 was significantly increased. Spontaneous migration of lymphocytes was similar in all studied groups. Treatment of MS with methylprednisolone and mitoxantrone diminished this activity to the level observed in control groups of patients with other neurological diseases (OND) and healthy controls (HC). This effect was dose dependent. We did not observe any significant changes in spontaneous and stimulated by CCL5 migratory activity of monocytes from MS patients. Our results suggest that in active MS chemokine-induced migratory activity of lymphocytes is increased. This activity may be significantly diminished by treatment with methylprednisolone and mitoxantrone.

SIV-P5 Long time effect of high doses glucocorticosteroids on mRNA expression for IL-6 and IL-8 in multiple sclerosis patients treated during relapse

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Glucocorticosteroids (GS) therapy during multiple sclerosis (MS) relapse is well established, however exact mechanism of their action is not fully known. Intravenously given methylprednisolone (IVMP) is thought to restore blood-brain-barrier (BBB) integrity at least by several mechanisms, including changes of pro- and anti-inflammatory cytokines. IL-6 and IL-8 seem to play interesting role in terms of MS pathogenesis. The aim of our study was to evaluate the effect of two high doses (500 mg vs. 1000 mg) of IVMP on mRNA expression for IL-6 and IL-8 in peripheral blood of MS patients during relapse. EDTA blood samples were collected before treatment, after 7 days, 14 days and 3 months from starting therapy. Percentage of patients with detectable IL-6 mRNA expression changed significantly only in group treated with higher dose of IVMP. No significant changes of IL-8 mRNA expression were noted. Interesting differences were found according to gender. Only higher dose caused significant increase of female percentage with detectable gene expression for IL-6 (at day 14 P=0.01) which decreased at month 3 (P=0.02). This effect was not observed in female group treated with 500 mg. No gender differences in IL-8 expression have been noted. As IL-6 is thought to be one of the cytokine with possible protective effect, described changes could be of important meaning. Our findings suggest that higher dose is probably more efficient in restoring proper balance of some parameters of the immune system.

SIV-P6 Impact of cytokines on pathomechanism of diabetic and alcoholic neuropathies

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To elucidate the impact of immunological factors in development of the neuropathies the expression of some cytokines: tumor necrosis factor α (TNFα), monocyte chemotactic protein-1 (MCP-1) and growth-regulated peptide alpha (GROα - CXCL1) in serum was studied. Twenty-seven patients with type 2 diabetes, 33 with chronic alcohol abuse and 20 healthy controls were included into the study. The type of neuropathy (involvement of axon, myelin or both) was evaluated by electrophysiological methods. (EMG and nerve conduction velocity). The cytokine levels were determined by ELISA method. For statistical comparison the nonparametric Mann–Whitney test was used. The evaluated material was divided according to clinical duration of neuropathy and electrophysiological pattern. Expression of TNF? in both types of neuropathy did not differ from the control material. Expression of MCP-1 was insignificantly higher in patients with alcoholic neuropathy. The same was noted in the cases of the demyelinating form *versus* axonal diabetic neuropathy. Serum level of GRO-α was significantly higher in patients with alcoholic neuropathy and in cases with demyelinating form of diabetic neuropathy than that in control subjects. GRO-α is a potent neutrophil chemoattractant, playing important role in various primary and secondary inflammatory processes. The results suggest that GRO-α may contribute to the mechanisms of alcoholic neuropathy and of demyelinating form of diabetic neuropathy.

SIV-P7 Deficiency of folic acid and vitamin B12, dyslipidemia, hyperhomocysteinemia in patients with dementia

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Disturbances of homocysteine (Hcy) metabolism favour atherosclerosis development in both small and large brain vessels. This increases the risk of incidents of vascular origin like subcortical lacunar strokes (S-VaD) or polyinfarcts in strategic areas (PS-VaD). The aim of this work was to estimate the levels of Hcy, folic acid, vitamin B12 and lipids in both these forms of dementia of vascular origin (VaD). Material: The patients were divided into two subgroups: 46 patients with VDP and 20 with VDW. The control group consisted of 62 per-

sons without dementia. Diagnosis was based on DSM-IV, NINCDS and AIREN criteria and ischemic Hachinsky scale. Methods: Homocysteine was determined by ELISA method using Boehringer kits. Folic acid and vitamin B12 were estimated by chemiluminescent methods and lipids by enzymatic methods. Results: 1.Hyperhomocysteinemia was significantly more frequent in VaD patients as compared with the controls. No significant differences were stated between subcortical and polyinfarct dementia. 2.Folic acid and vitamin B12 deficiences were observed more frequently (close to sinificant) only in individuals with PS-VaD. 3.In PS-VaD individuals more frequently than in the controls low HDL (high density lipoprotein) cholesterol was observed. Conclusion: Hyperhomocysteinemia is a common symptom in dementia of vascular origin.

SIV-P8 The expression of Tumor Necrosis Factor-alpha in migraineurs' macrophages

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Migraine is classically not recognized as inflammatory disorder, however there are numerous studies revealing the effect of cytokines on headache. Tumor necrosis factor alpha (TNF) as well as interleukin-1 (IL-1), Il-6 AND Il-8 may promote hyperalgasia. The aim of this study was to examine the expression of tumor necrosis factor alpha in monocytes of migraine patients. Material and methods: Twenty migraine patients (aged 39 ± 11) (16 females, 4 males) were included in the study. Ten healthy subjects (7 females, 3 males) were used as controls. The migraine patients were examined neurologically and assessed according to QMV, HimQ, MIDAS and MIGSEV scales. The heparinized blood was used for monocytes separation. Following meglumini amidotrizoas (Uropolinum) - Ficoll centrifugation the monocytes were isolated with the use of magnetic labeling system (MACS, Miltenyi Biotec). Monocytes underwent further immunostaining with the use of anti-TNF antibodies (Bender System). The final colour obtained in result of peroxidase-DAB reaction was estimated using ImageJ software. Results: We have found decreased expression of tumor necrosis factor alpha in monocytes originating from migraine patients compared to controls ($P<0.001$). There were no statistically significant differences between patients with and without aura. Conclusion: The decreased expression of TNF in circulating monocytes is believed to be responsible for increased serum cytokine level.

SIV-P9 Expression of chemokines CCL19, CCL20, CCL21, and CCL22 in CNS and peripheral tissues during ChREAE

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Chemokines are cytokines with chemotactic properties which play an important role in development of experimental autoimmune encephalomyelitis (EAE), considered to be best available model of human multiple sclerosis (MS). These cytokines initiate migration and accumulation of inflammatory cells in the central nervous system (CNS). In our previous studies we have shown upregulation of several classical chemokines and their receptors during attacks of ChREAE in CNS and peripheral tissues. The major goal of present study was to analyze expression of some relatively recently described inflammatory and homeostatic chemokines in the CNS and peripheral tissues at different stages of ChREAE. Animals with ChREAE were sacrificed and several organs including spinal cord, brain, spleen and kidneys were obtained. Using quantitative Rnase Protection Assay (RPA) and MicroArrays assays we analyzed expression of several inflammatory and homeostatic chemokines including CCL19, CCL20, CCL21, and CCL22. Localization of chemokine expression was analyzed with immunohistochemistry. In brains we observed increased expression of chemokines CCL19, CCL20, CCL21, and CCL22 during relapses of the disease as compared to disease remission. Expression of these chemokines in control animals was significantly lower. Expression of CCL19 and CCL21 was localized in the vicinity of inflammatory foci within the CNS. Expression of chemokines in kidneys was similar in control animals and in mice with ChREAE. Conclusions: Our results suggest the complex pattern of chemokines expression not only in the CNS but also in peripheral tissues during different stages of ChREAE.

SIV-P10 Beta-cells specific antibodies in neurological autoimmune disorders

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The coexistance of organ-specific auto antibodies and neurological autoimmune disorders have been noticed in clinical practice. Anti-GAD (Glutamic Acid Decarboxylase) were found in 80% of patients with insulin-dependent diabetes mellitus (IDDM) and stiff-person syndrome or epilepsia partialis continua. Anti-insulinoma-associated protein-2 (IA-2) were detected in 37% of IDDM patients. The aim of this study was to analyze the incidence of anti-GAD and IA-2 antibodies in autoimmune neurological disorders. Material and methods: Fifty-one patients with neurological autoimmune disorders (36 females, 15 males) were examined. Among them 30 patients had multiple sclerosis, 18 – myasthenia gravis, 1 patient – coexisting multiple sclerosis and myasthenia gravis, 2 patients – Guillain–Barre syndrome. Diabetes mellitus was present in 9 SM cases (6 IDDM), 9 myasthenia gravis cases (5 IDDM); 1 patient with Guillain–Barre syndrome had non-insulin-dependent diabetes (NIDDM) as well. Results: Anti-GAD antibodies were positive in 5 (56%) patient with coexisting multiple sclerosis and diabetes, and 1 patient (11%) with myasthenia gravis and diabetes, no patient with Guillain Barre and diabetes had anti-GAD antibodies. Anti IA2 antibodies were positive and presented as high levels only in one patient with multiple sclerosis and diabetes. Conclusion: Anti-GAD and anti-IA2 antibodies were specific for coexistence of diabetes and multiple sclerosis and to a lower extend myasthenia gravis. No presence of anti-GAD or anti-IA2 antibodies was revealed in non-diabetic patients wit autoimmune neurological disorders.

SIV-P11 Serum S-100 protein and Neuron Specific Enolase in patients with onconeuronal antibodies

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Neurological paraneoplastic syndromes are remote effects of cancer believed to be immune-mediated. The pathomechanisms involved in those syndromes await elucidation. Increased permeability of blood-brain barrier (BBB) may form a milieu of factors promoting the development of this pathology. S-100 protein and Neuron Specific Enolase (NSE) are recognized as indicators of BBB damage. The aim of this study was to evaluate the levels of S-100 and NSE in seropositive patients with neurological paraneoplastic syndromes. Material and methods: Eighty-four patients with onconeuronal antibodies revealed by means of indirect immunofluorescence and Western blotting (EUROIMMUN tests) were included in the study. Serum S-100 protein was estimated using electrochemiluminiscence immunoassay (ROCHE) and NSE - using ELISA kit (BIOMEDA). Four groups of patients were studied: anti-Hu, anti-Yo, anti-Ri positive and those with unspecific onconeuronal antibodies. Results: Basing on indirect immunochemistry and Western blotting we have found 19 anti-Hu positive subjects, 25 anti-Yo positive, 20 anti-Ri positive and 20 with unspecific antibodies. Twenty-five percent of anti-Hu patients had S-100 levels over reference value, what was the case for 20% of anti-Ri, 8% of anti-Yo and 5% of patients with unspecified antibodies. Mentioned worthy is that all patients with onconeuronal antibodies had NSE levels over the reference value. Conclusion: The presence of onconeuronal antibodies is linked to higher levels of increased blood brain barrier permeability markers in patients' serum.

SIV-P12 Relationship between NOS expression and dopamine concentration in murine model of Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disorder of unknown aetiology. The involvement of nitric oxide synthase (NOS) producing NO in the etiopathogenesis of PD is quite well documented. Therefore we decided to examine changes in iNOS, nNOS, eNOS protein and gene expression as well as neurotransmitters levels in the striatum of mice in course of PD related neurodegeneration induced by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Protein expression was assayed by Western Blot method, mRNA expression by PCR and HPLC was used to the examination of the levels of selected neurotransmitters. Correlation analysis revealed a negative correlation between iNOS protein expression and DA concentration as well as a positive correlation between iNOS protein expression and DA turnover (HVA/DA) in studied groups of animals. Hypothesis about the potential role of nNOS in neurodegeneration evoked by MPTP intoxication was supported by existence negative correlation between DA concentration and expression of mRNA for nNOS. There were not observed any changes in expression of mRNA and protein for eNOS as the result of MPTP-dependent injury of nigrostriatal dopaminergic projections. In summary, our results lead to the conclusion that enzymatic modification of iNOS and nNOS are potential purpose for further investigation of new antiparkinsonian compounds.

SIV-P13 Temporal pattern of glutamate transporters and metabotropic glutamate receptors expression during the course of Experimental Allergic Encephalomyelitis in rats

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Recent studies have suggested that glutamate neurotoxicity is involved in the pathogenesis of MS. Most important in clearing extracellular glutamate is the system of astroglial glutamate transporters, dysfunction of which may lead to the elevation of potentially toxic glutamate and in consequence to excitotoxic neurodegeneration. The present studies were undertaken to search the temporal pattern of glutamate transporters (GLAST and GLT-1) expression in different symptomatic phases of EAE. During the course of EAE the body weight and neurological deficits were monitored daily, so as duration of disease phases and the lethality. Western blot analysis revealed the increased expression of both excitatory amino acids transporters in brain homogenates of diseased rats. There is evidence for a functional cross-talk between mGluR5 metabotropic glutamate receptors and glutamate transporters, raising the hypothesis that these receptors act as a sensor of extracellular glutamate and contribute to the acute regulation of glutamate clearance by astrocytes. Thus, the protein expression of mGluR5 was also examined and revealed increased immunoreactivity during the course of diseased EAE rats. The present data suggest that overexpression of both glutamate transporters and mGluR5 receptors proteins may reflect a compensatory mechanism against increased glutamate concentration.