MOLECULAR BASIS OF PATHOLOGY AND THERAPY IN NEUROLOGICAL DISORDERS

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Guest Editor:
Barbara Lukomska

MOLECULAR BASIS OF PATHOLOGY AND THERAPY IN NEUROLOGICAL DISORDERS

The 10th International Symposium

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Co-organizers:
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The Committee on Neurobiology of the Polish Academy of Sciences
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PROGRAMME

Thursday, November 25th, 2010

9:15	Opening of the Symposium

Session I

CELLULAR AND MOLECULAR MECHANISMS OF MEMORY DISORDERS

Chairs:	**Elzbieta Salinska (Warsaw, Poland)** **Radmila Mileusnic (Milton Keynes, UK)**
9:30 – 10:00	**Radmila Mileusnic (Milton Keynes)** What can the chicks tell us about the mechanisms of memory and protection from Alzheimer's disease?
10:00 – 10:30	**Tomasz Gabryelewicz (Warsaw)** From mild cognitive impairments to dementia
10:30 – 11:00	**Piotr Popik (Cracow)** Pharmacological modulation of memory
11:30 – 11:45	**Grzegorz Czapski (Warsaw)** Expression of Poly(ADP-ribose) Polymerases in hippocampus during systemic inflammatory response. The role of PARP-1 protein interactions in cognition
11:45 – 12:00	**Aleksandra Dys (Gdansk)** Acetyl-CoA the key point for cholinergic degeneration
12:00 – 12:15	Presentation of Becton Dickinson **Marek Michałowski** BD Pathway as an excellent tool for neurosciences
12:15 – 14:15	*Poster session*

Session II

NEUROPEPTIDES – ENDOGENOUS FUNCTIONS AND PROSPECTIVE CLINICAL APPLICATIONS

Chairs: **Andrzej Lipkowski (Warsaw, Poland)**
Istvan Krizbai (Szeged, Hungary)

14:15 – 14:45 **Istvan Krizbai (Szeged)**
In vitro models of the blood-brain barrier

14:45 – 15:15 **Sergiusz Markowicz (Warsaw)**
Peptide-based cancer vaccines: adjuvant vaccination with melanoma peptide-pulsed dendritic cells (DCs) in stage III melanoma patients

15:15 – 15:45 **Chaim G. Pick (Tel Aviv)**
Mild traumatic brain injury (mTBI) mouse model

15:45 – 16:15 **Anna Lesniak (Warsaw)**
Opioid peptides in peripheral pain control

16:45 – 17:00 **Michał Korostynski (Cracow)**
Gene expression profiling of psychotropic drugs effects in the brain

17:00 – 17:15 **Piotr Wojciechowski (Warsaw)**
μ-opioid receptors' share in cardiorespiratory response to systemic injection of the analgesic chimeric peptide in anaesthetized rats

Friday, November 26th, 2010

Session III

DIFFERENT TYPES OF SOMATIC STEM CELLS;
IMPLICATIONS IN NEUROLOGICAL DISEASE THERAPY

Chairs: **Barbara Lukomska (Warsaw, Poland)**
Eldad Melamed (Tel Aviv, Israel)

9.30 – 10.00 **Ewa Zuba-Surma (Cracow)**
„Key to Immortality" – the potential role of adult pluripotent stem cells (VSELs) in tissue and organ regeneration

10.00 – 10.30 **Leonora Buzanska (Warsaw)**
Biomimetic modulation of cord blood derived stem cells for neural fate control

10.30 – 11.00 **Piotr Rieske (Lodz)**
"Neural" differentiation of mesenchymal stem cells

11.30 – 12.00 **Eldad Melamed (Tel Aviv)**
Transplantation of human MSC in experimental neurodegenerative diseases

12:00 – 12:15 **Luiza Wojcik-Stanaszek (Warsaw)**
Potential role of MMP-2 and -9 in ischemia-stimulated neurogenesis in the gerbil hippocampus

12:15 – 12:30 **Mirosław Janowski (Warsaw)**
MRI-monitored cord blood-derived cell transplantation to the ventricular system of child with global cerebral ischemia - a case report

12:30 – 14:30 *Poster session*

Session I

CELLULAR AND MOLECULAR MECHANISMS OF MEMORY DISORDERS

SI-L1
What can the chicks tell us about the mechanisms of memory and protection from Alzheimer's disease?

Mileusnic R.
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One of the earliest cellular alterations in Alzheimer's disease is an intracellular accumulation of amyloid-β (Aβ), generated by altered processing of the amyloid precursor protein (APP) by β- and γ-secretases. However, APP also produces other fragments, including the neurotrophic and neuroprotective secreted form of APPα (sAPPα), generated by α-secretase activity. The sAPP contributes to normal memory function and its role as a key contributor to synaptic plasticity is supported by experimental findings showing that exogenous administration of sAPPα or small peptide fragments derived from its amino acid sequence enhance memory performance in mice, rats and chicks.
Although birds and mammals diverged about 270 million years ago, the chick APP gene sequence and enzymatic machinery for processing APP is almost identical to that of humans. The chick is thus a useful natural model in which to study the cell biology and developmental function of APP and a potential "assay system" for drugs that regulate APP processing. Using a one-trial passive avoidance task in day-old chicks, we reported that the amnestic effect of pre-training injections of anti-APP antibody or APP-antisense could be prevented by intracerebral injection of the pentapeptide RERMS, homologous to APP322-328, part of the growth-promoting E2 domain of sAPP. Subsequently, we have shown that this effect is due to the palindromic tripeptide sequence RER. Injected intracerebrally around the time of training, RER not only protects against the amnestic effects of downregulating or functionally blocking APP, but also against the amnestic effect of the Aβ1-42. To both cast light on the mode of action of the peptide, and to increase its efficacy as a potential memory enhancer, we have examined the behavioural responses in chick to the optically isomeric D- or diasteromeric (D/L) forms of the peptide as arginine-rich peptides easily cross cell membranes and bind to membrane-associated and cytoplasmic proteoglycans. One of them, acetylated- rER (Ac-rER, where the lower case indicates the D-isomeric form of the amino acid), is rapidly transported across the blood-brain-barrier, protects against Aβ-induced memory loss and enhances retention when injected peripherally up to 12 hr prior to training. Therefore, we propose that RER-related peptides may form the basis for a potential therapeutic agent in the early stages of Alzheimer's disease.

SI-L2
From mild cognitive impairment to dementia

Gabryelewicz T.
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The significance of cognitive decline in the elderly has been widely discussed in the literature. In particular, complaints of memory impairment are common in the elderly and were found in 80% of various community samples. Different approaches have been proposed to describe this decline and its relationship with the development of dementia. Unfortunately, the difficulty in disentangling "normal", purely age-associated cognitive changes from changes related to early degenerative or vascular diseases has resulted in a lack of agreement on terminology and specific diagnostic criteria. Although there are a number of different definitions of mild cognitive deficits, the general concept is of a subjective memory impairment, and/or another domain in the context of cognitive impairment relative to age-matched controls and yet showing no loss of function and no dementia. Mild cognitive impairment (MCI) is a heterogeneous group with a variety of clinical outcomes. Most of the subjects will convert to dementia, but some MCI may never progress to any significant extent or even improve. The field of MCI research is currently focusing on identifying the risk factors of disease progression for the purpose of early therapeutic intervention, which may in turn delay or even prevent the onset of dementia. Individuals with a MCI are at an increased risk of developing dementia ranging from 3% to 15% per year. The prevalence of MCI varies from 15 to 30% of the population. Most of the studies demonstrate the age-dependent increase of MCI prevalence.

SI-L3
Pharmacological modulation of memory

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Efforts to enhance human potential - including cognitive performance - are probably as old as the humankind. Magic potions to produce superb strength, potency and wisdom can be found in the history of all cultures. Today, the medications sold by prescription as well as preparations available over-the-counter and advertised as "smart drugs" appear more and more desirable and thus, accessible. The re-birth of "memory pills" increased also the curiosity of their potential users regarding the mechanism of action, and efficacy of these products in enhancing mental capacity. While it appears unlikely that "Viagra for the brain" for cognitively-unimpaired subjects will become readily available, recent advances in psychopharmacology seem to offer pro-cognitive treatments for the disorders, in which cognitive impairments were not identified as the major health problem (schizophrenia and major depression). This lecture

will briefly summarize the knowledge on learning and memory mechanisms, discuss compounds enhancing learning and memory processes, and show the effectiveness of some old medications and novel compounds in an animal model of cognitive inflexibility.
Supported by the grant ProKog (UDA-POIG.01.03.01-12-063/09-00) co-financed by EU "Research and development of new technologies" within the Innovative Economy Program.

SI-O1
Expression of poly(ADP-ribose) polymerases in hippocampus during systemic inflammatory response. The role of PARP-1 protein interactions in cognition

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Poly(ADP-ribosyl)ation is covalent modification of proteins responsible for the alterations of their function. This process is catalyzed by poly(ADP-ribose)polymerases (PARP) family, consisted of 18 isoforms. Among target proteins, there are many DNA-related proteins and PARP-1 itself. In the brain, PARP-1 is responsible for more than 90% of poly(ADP-ribosyl)ation. PARP-1 plays a significant role in regulation of several transcription factors including NF-kB and p53. Our previous data indicated an important impact of PARP-1 in brain ischemia and in systemic inflammatory response (SIR).
In this study we have analyzed the expression of PARP family genes in hippocampus of mice subjected to lipopolysaccharide (LPS)-evoked SIR. Moreover, the effect of SIR on PARP-1/PAR protein interaction and on memory function was evaluated.
Mice C57BL6 were injected i.p. with LPS (1 mg/kg b.w.) alone or together with PARP inhibitors 3-aminobenzamide (30 mg/kg b.w.). The studies were carried out by using immunochemistry, microarray, real-time RT-PCR, and behavioral analysis.
Our data indicated the small effect of SIR on PARP-1 gene expression level, however, expression of genes for PARP-3, -9, -12 and -14 was significantly increased 12 h after LPS administration. The level of PAR in hippocampus was elevated during SIR indicating activation of protein poly(ADP-ribosyl)ation. Moreover, further analysis of LPS-affected genes indicated that among 83 proteins known for their direct interaction with PARP-1, genes for 21 are present in SIR-related interactome, along with several genes for transcription factors and proteins involved in signal transduction.
The enhancement of gene expression in hippocampus for several members of PARP family during SIR may be responsible for the alteration of PARPs function, higher level of PAR formation and for the modification of PARP/PAR protein interaction, transcription, cell signaling and memory. Our data indicated that SIR significantly decreases object recognition but has small effect on spatial memory. PARP-1 inhibitor protects against SIR induced molecular alteration in hippocampus and against SIR-evoked cognition impairment. Moreover, PARP-1 inhibitors significantly enhanced spatial memory in LPS treated mice. Our results indicate that inhibition of PARP-1 is a promising protective strategy during overactivation of inflammatory reaction. The role of other PARP family members in SIR is a target of our future investigation.

SI-O2
Acetyl-CoA the key point for cholinergic degeneration

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Inhibition of brain energy metabolism in demented subjects correlates with impairment their cognitive functions and loss of cholinergic neuron markers found in post mortem studies. However, mechanisms of preferential loss of brain cholinergic neurons in Alzheimer's disease and other encephalopathies, remain unknown. We demonstrate that neuronal acetyl-CoA metabolism may be a primary target for neurodegenerative insults. Several putative encephalopathy-inducing pathogens, such as aluminum, amyloid-beta, zinc, NO excess, interleukin 1b, hypoglycemia and thiamine deficit were found to decrease viability and transmitter functions of cholinergic neuronal cells in cultures as well as in whole brain models of neurodegeneration. They caused inhibition of pyruvate dehydrogenase activity that correlated directly with respective alterations of acetyl-CoA level in neuronal mitochondria and inversely with rate of cell death. Moreover, these pathogens caused greater suppression mitochondrial acetyl-CoA and viability of differentiated than nondifferentiated cholinergic neuronal cells. Decreased availability of intramitochondrial acetyl-CoA apparently suppressed its transport to cytoplasm. In consequence, these neurotoxins decreased acetyl-CoA level in the cytoplasmic compartment. It resulted in a prominent decrease in ACh content and its quantal release in differentiated cells. In nondifferentiated cells neurotoxic effects were much smaller or negligible. Significant direct correlations were found between cytoplasmic acetyl-CoA levels and different parameters of cholinergic metabolism. Neurotoxic signals were less harmful for resting microglial and astroglial than for neuronal cells. Several compounds, known to improve pyruvate and acetyl-CoA metabolism, such as lipoamide, acetyl-L-carnitine, flavonoids, prevented neurotoxic activities through the maintenance proper level of acetyl-CoA in the mitochondrial compartment. They also, stabilized transmitter functions, when added simultaneously with neurotoxic compounds. However, delay in neuroprotectant application, abolished its beneficial effects on cell survival. It might be due to irreversible inhibition of aconitase and isocitrate dehydrogenase by some neurotoxins. Presented data indicate that in encephalopathic brains, cholinergic neurons viability and their transmitter functions are affected by alterations of two functionally independent pools of intramitochondrial and cytoplasmic acetyl-CoA, respectively.
Supported by MNiSW projects NN401233333, 401029937 and GUMed fund St-57.

SI-P1

Disturbances in carbohydrate metabolism in dementia

Wehr H., Bednarska-Makaruk M., Rodo M., Bochynska A., Graban A., Lojkowska W., Socha-Czechyra W., Ryglewicz D.
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Several authors observed a diminished insulin sensitivity in dementia. It was proposed that dementia could be treated as a kind of diabetes type 2 expressed mainly in the brain.
In insulin resistance states insulin levels in blood serum are usually elevated.
In this work a group of 140 demented patients was investigated: 63 of them were diagnosed as probable Alzheimer disease (AD), 33 as dementia of vascular origin (VaD) and 44 as mixed dementia (MD) and compared with 59 individuals without dementia. Dementia was diagnosed according to DSM-IV and the kind of dementia by NINCDS-ADRDA criteria. Fasting glucose and glucose after glucose load level was determined by enzymatic method. Fasting insulin concentration was assayed using ELISA using DRG kits. Homeostasis Model Assessment (HOMA-IR) - the most frequently used index of insulin resistance was calculated.
Statistically significant higher glucose levels after glucose load (impaired glucose tolerance IGT) were stated in the group of dementia as compared with controls. Elevated HOMA-IR values were more frequently observed in patients with dementia as compared with controls borderline significantly in the VaD and MD groups.
The results indicate abnormal glucose metabolism expressed as IGT as well as probable glucose resistance in dementia.

SI-P2

Paraoxonase1 gene p.q192r polymorphism and insulin resistance in dementia

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[1]Department of Genetics and [2]First Department of Neurology Institute of Psychiatry and Neurology; [3]Warsaw University of Life Sciences, Warsaw, Poland

The association of insulin resistance and dementia was observed by several authors. The activity of paraoxonase1 (PON1), the enzyme playing a protective role against oxidative stress, was decreased in demented patients.
The aim of this study was to investigate relationship of PON1 Q192R polymorphism and insulin resistance.
From demented patients (59 with probable AD, 24 with VaD and 38 with MD) and 51 controls DNA was isolated and Q and R isoforms were identified basing on Humbert et al.method (1993) with minor modifications. In the same subjects the following determinations were performed: serum fasting glucose, glucose 2 hours after 75g glucose load and fasting insulin using DRG ELISA kits. HOMA-IR index was calculated..
In the patients with dementia 47.9% of QQ , 43% of QR and 9.1% of RR genotypes were identified and in the controls respectively 54.9, 35.3 and 9.9 ones .
Statistically significant associations of the R allele carriers with insulin level and with HOMA-IR index were stated. Borderline significant association with glucose after glucose load level (impaired glucose tolerance-IGT) were observed.
The results show the asssociation of Q192R PON1 polymorphism with insulin resistance. However due to the small sample size the results should be considered as preliminary.

SI-P3

Inhibition of mGluR5 but not mGluR1 disrupts memory retrieval in the one-trial passive avoidance task in one-day old chicks

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It is known, that group I metabotropic glutamate receptors (mGluRs) are involved in memory consolidation and reconsolidation. The Ca2+ signal derived from IP3 receptors (IP3R) stimulation via mGluRs activation, initiates protein synthesis that is necessary for complete memory consolidation, whereas it is suggested that during retrieval and reconsolidation of memory other mGluR1/5 triggered mechanisms may be involved. Recently mGluRs have received considerable attention as a potential drugs target for many neurological disorders, but their influence on learning and memory is still unclear. With the increasing number of people suffering memory dysfunction of different origin, unravelling of the mGluRs role in memory processes may be very important.
The aim of this study was to detect differences in the role for mGluR1 and mGluR5 in memory retrieval at different times after initial training and remainder of the task.
One-day old chicks were trained to avoid pecking metal bead covered with bitter-tasting substance methylanthranilate. Two experimental groups were given a reminder by presenting similar metal bead 2 or 24 h after initial training. Bilateral injections of mGluR1 antagonist LY367385 (7.5 nmol/hemisphere), mGluR5 antagonist MPEP (20 nmol/hemisphere) or IP3R antagonist 2-APB (2.5 nmol/hemisphere) were made directly into the IMM region of chick brain. Injections were made immediately after initial training or reminder, and at 2, 3 and 24 hours later.
Our results demonstrate, that in the one-trial passive avoidance task in chicks, injection of the mGluR1 and mGluR5 antagonists into chick brain region IMM shortly after training resulted in permanent amnesia. Injection of MPEP at other times (2, 3 and 24 h after initial training) resulted in transient amnesia observed 1-2 h after injection and lasting up to 4 hours. The same effect was observed for 2-APB. Blocking mGluR1 and mGluR5 immediately after reminder resulted in similar transient amnesia, same as blocking IP3R. Injections of MPEP or 2-APB at later times after reminder also resulted in

transient amnesia, however the effect was weaker when reminder was given 24 h after training. mGluR1 antagonist applied later than in a short time after training or reminder had no effect on memory recall.
Presented data suggest that at least in the chick model, activation of mGluR1 and mGluR5 is necessary for complete memory consolidation and reconsolidation, whereas mGluR5 are additionally involved in retrieval processes which is dependent on Ca2+ release from IP3 activated intracellular calcium stores.

SI-P4
Cell cycle malfunctions in lymphocytes from sporadic Alzheimer's disease patients

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Alzheimer's disease (AD) constitutes one of the leading causes of disability with enormous socioeconomic costs. Most of AD cases occur sporadically and have unknown etiology (SAD). The Abeta peptide deposition in neuritic plaques is one of AD major hallmarks. Nevertheless, the role of Abeta as a primary driver in AD progression arouses controversy. Mounting evidence suggests that neurodegeneration results from the cell cycle reentry occurring in AD neurons. Recent data indicate that some of the cell cycle changes can be also observed in peripheral cells. Thus, our aim was to investigate whether any cell cycle abnormalities occur in transcriptome or proteome of lymphocytes from SAD patients. This study was performed in immortalized lymphocytes from 18 SAD patients and 26 healthy age-matched individuals. PCR arrays experiments showed that 43% of the 90 investigated cell cycle genes were down-regulated in SAD, whereas 4% were up-regulated comparing to control lymphocytes. Since most significant changes referred to the genes engaged in G1/S control, we assessed the levels of key proteins involved in G1/S transition with immunobloting. The most striking difference occurred in p21 protein level, which was significantly elevated for SAD in respect to controls. Furthermore, we measured distribution of cells in G1, S and G2/M cell cycle phases using flow cytometry. Our results showed increased % of cells in G1 phase with adequate decrease in % of cells in S phase for SAD lymphocytes. However, estimation of proliferation rate for SAD and control lymphocytes revealed no significant differences. We therefore used the following methods to assess the lengths of G1 and S phases: flow cytometry analysis of PI-labeled cells after nocodazole treatment and BrdU pulse chase labeling. SAD lymphocytes indicated significant prolongation of G1 phase and simultaneous shortening of S phase. In addition, treating the cells with gamma-secretase inhibitor L685,458 did not affect the observed cell cycle dysregulations, which highlighted that the impairments of cell cycle in SAD lymphocytes are not linked to gamma-secretase activity.
Taken together, this study emphasizes that disturbances in the cell cycle control are common for AD neurons as well as SAD lymphocytes. Thus, human lymphocytes could be used in further studies on AD pathogenesis and diagnostics.

SI-P5
The role of cyclin-dependent kinase 5 in pathomechanism of Alzheimer's disease

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Alteration of Amyloid Precursor Protein (APP) processing, leading to overproduction of Amyloid beta (Ab), and hyperphosphorylation of Microtubule-Associated Protein (MAP) tau remain in the center of pathomechanism of Alzheimer's disease (AD). The recent data indicated that oligomeric form of Aβ is responsible for memory impairment in AD. Also deregulation of protein phosphorylation processes plays an important role in AD pathology. Cyclin-Dependent Kinase 5 (CDK5) is responsible for aberrant phosphorylation of both MAP tau and APP. Moreover, CDK5 may also phosphorylate other proteins potentially involved in AD pathogenesis, i.a. Glycogen Synthase Kinase-3b (GSK-3b), NMDAR, p53.
The aim of the present study was to analyze the participation of CDK5 in cell death processes occurring in PC12 cells overexpressing APP. As a model, we used cells transfected with human wild-type APP (APPwt) and human APP with Swedish mutation (APPsw). Real-time PCR and Western blotting were used for analysis of expression and phosphorylation of CDK5, CDK5R1, CDK5R2, GSK-3β. Cytotoxicity was evaluated by MTT and LDH tests. To determine the role of CDK5 in AD patients, the association of human CDK5 gene with AD risk was analyzed.
Our data demonstrated enhanced cell death and cell cycle disturbances in PC12 cells transfected with APP gene, comparing to control PC12 cells. Real-time PCR analysis indicated increased level of mRNA for CDK5 gene in APPsw cells. Significantly decreased phosphorylation of CDK5 on Tyr15 was observed in APPwt and APPsw cells, what can be responsible for lowering of Cdk5 activity. Moreover, Cdk5-dependent phosphorylation of GSK-3β on Ser9 was also decreased, what can be responsible for GSK-3β activation, hyperphosphorylation of MAP tau and alteration of cell function.
Our genetic analysis indicated that there is no association between polymorphism of *CDK5* gene and the risk of AD in investigated Polish population (178 healthy controls, 71 EOAD

and 204 LOAD cases). Significant differences in serum level of cholesterol, LDL, vitamin B12 and homocysteine between AD patients and healthy controls were found, but there was no association of tested CDK5 genotypes with biochemical parameters.
These results indicated the important role of CDK5 - GSK-3β interplay in pathomechanism of AD.
This study was supported by MSHE Grant N N401 014635.

SI-P6

Role of lipoxygenases and poly(ADP-ribose) polymerase in amyloid beta cytotoxicity

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** Both authors have contributed equally to this study*

The roles of 12/15-lipoxygenase(s) (LOX), poly(ADP-ribose) polymerase (PARP-1) activity and mitochondrial apoptosis inducing factor (AIF) protein in the molecular processes evoked by amyloid β (Aβ) toxicity were investigated in PC12 cells that express either wild-type (APPwt) or double Swedish mutation (APPsw) forms of human Aβ precursor protein. Different levels of Aβ secretion characterize these cells. The results demonstrated a relationship between the Aβ levels and LOX protein expression and activity. High Aβ concentration in APPsw cells correlated with a significant increase in free radicals and LOX activation, which leads to translocation of p65/NF-κB into the nucleus. An increase in AIF expression in mitochondria was observed concurrently with inhibition of PARP-1 activity in the nuclear fraction of APPsw cells. AIF accumulation in mitochondria may be involved in adaptive/protective processes. However, inhibition of PARP-1 may be responsible for the disturbances in transcription and DNA repair as well as the degeneration of APP cells.
Under conditions of increased nitrosative stress, evoked by the nitric oxide donor, sodium nitroprusside (SNP, 0.5 mM), 70-80 % of all cells types died after 24 h, significantly more in APPsw cells. There was no further significant change in mitochondrial AIF level and PARP-1 activity compared to corresponding non-treated with SNP cells. Only one exception was observed in PC12 control, where SNP significantly inhibits PARP-1 activity. Moreover, SNP significantly activated gene expression for 12/15-LOX in all types of investigated cells. Inhibitors of all LOX isoforms and specific inhibitor of 12-LOX enhanced the survival of cells that were subjected to SNP. We conclude that the LOX pathways may play a role in Aβ toxicity and in nitrosative-stress-induced cell death and that inhibition of these pathways offers novel protective strategies.
Supported by MS&HE grant NN40113938 and MRC statutory theme No 7.

SI-P7

SNCA, PARK2 and LRRK-2 mutations in Polish patients with sporadic Parkinson's disease

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Parkinson's disease (PD) is one of the most common neurodegenerative disorders in Poland.
Although the genetic basis of familial PD is now well established, the majority of PD is sporadic and occurs without a clear mode of inheritance.
The etiology of sporadic PD remains unknown, but it is currently assumed that genetic susceptibilities may be involved.
The observation that mutations in α-synuclein (SNCA), parkin (PARK2)and leucine-rich repeat kinase 2 (LRRK2) genes are common in familial PD and increasing evidence supporting a direct role for PARK2 and LRRK-2 in sporadic both early- and late-onset disease make those genes a particularly compelling candidate for intensified investigation.
The aim of the study was analysis and identification of SNCA, PARK2 and LRRK-2 mutation in Polish patients with sporadic PD.
Peripheral blood was collected from 34 patients with sporadic PD clinical diagnosis (the average age 58 years), and 22 patients with the other neurological diseases (the average age 55 years) as well as from 25 healthy donors (the average age 60 years).
Genomic DNA was isolated using standard protocols. SNCA mutations analysis was performed to exclude one of the familial forms of PD. Restriction-enzyme digestion of polymerase-chain reaction (PCR) amplified genomic DNA fragment of SNCA exon 3 detected no G88C mutation. PCR-amplification of parkin exons 2 and 4 also detected no exon deletion. Moreover exon 41 of LRRK-2 gene as well as exons 4, 7 and 11 of PARK2 gene was screened using real-time PCR/HRM and exon sequencing.
None of the patients as well as control subjects tested had mutation of LRRK2 gene. These results are consistent with previous reports in the Polish population
Mutation in tested exons of PARK2 gene were identified in 20,6% patients with sporadic PD, 4,5% patients with the other neurological disorders and 4,0% control subjects. All detected mutations were heterozygous. One of the PD patients had two mutations in PARK2 gene (G1281A, G601A).
It can be concluded, that both G88C SNCA and G2019S LRRK-2 mutations as well as deletion of 2 and 4 exon of parkin gene are rare causes of PD in Poland. Moreover point mutation in PARK2 seems to be associated with sporadic PD in polish population. Thus, the results of this study suggest that screening for PARK2 mutations may be a component of genetic testing for sporadic PD.

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SI-P8

Phrenic and hypoglossal activity in animal model of Parkinson's disease

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Besides extrapyramidal motor disorders, disturbances in breathing form an important part of the Parkinson's disease (PD) syndrome. Respiratory disturbances include alternations in respiratory pattern, pulmonary ventilation and a dysfunction of upper-airway muscles. We investigated whether the animal model of Parkinson's disease created by unilateral injection of 6-hydroxydopamine (6-OHDA) into the striatum induced changes in neural respiratory activity to the diaphragm and upper-airways muscles and the respiratory response to hypoxia. The respiratory effects and response to intermittent hypoxia were studied in animals after unilateral infusion of 24 µg 6-OHDA (4 µg/1µl) or 6 µl of vehiculum into the striatum. Two weeks following the infusion the rats were anesthetized, subjected to bilateral midcervical vagotomy, paralyzed and artificially ventilated. Relation between activity of the phrenic and hypoglossal nerves was analyzed during normoxic ventilation and during acute intermittent hypoxia, composed of five episodes of 11% hypoxia introduced every 3 minutes, in the control and 6-OHDA lesioned animals. Amplitude, frequency of integrated phrenic and hypoglossal nerve activity and minute phrenic and hypoglossal activity were calculated. The results showed that unilateral nigrostriatal dopaminergic destruction in the 6-OHDA model of Parkinson's disease did not evoke an alternation of the respiratory pattern of the phrenic and hypoglossal activity in anesthetized, normoxic rats. The difference between the control and 6-OHDA lesioned animals appeared when respiratory drive increased due to hypoxia. While the magnitude of the phrenic response to each episode of hypoxia was almost unchanged in the lesioned vs. control animals, the hypoglossal hypoxic response attained significantly higher levels in 6-OHDA lesioned rats. The biphasic response to hypoxia was maintained, however, a decline of the hypoglossal hypoxic response was more emphasized in the PD model. Changes in the respiratory frequency due to hypoxia were comparable in both groups of rats. In conclusions this study reveals that central dopamine depletion elicited by unilateral infusion of 6-OHDA into the nigrostriatal region develops a modification of the respiratory hypoxic response and intensifies an asymmetry of the phrenic and hypoglossal nerve activity. Uneven hypoxic drive to the diaphragm and the muscles of upper respiratory tract is a probable cause of this modification and might be a source of respiratory disturbances in the Parkinson's disease.

SI-P9

Homocysteine, cysteine and gluthatione in epileptic patients treated with antiepileptic drugs

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Today, epilepsy is regarded as the most common neurological disease.

AEDs pharmacotherapy, used to treat patients with epilepsy affect plasma concentrations of sulfur amino acids: Hcy, Cys and GSH. These are the main intracellular thiols, whose anabolic and catabolic pathways are closely linked. Through transsulfuration of Hcy arises Cys, who further participates in the synthesis of GSH. It has been shown that the increase of plasmatic Hcy in patients with epilepsy taking AEDs can be affected by a number of factors including diet, type of pharmaceutical preparations taken, the duration of treatment, and genetic factors.

The study group consisted of 63 patients with idiopathic epilepsy, 28 women and 35 men, aged from 18 to 65. Among patients with epilepsy 55 people, 24 women and 31 men, aged from 18 to 65 were treated with various AEDs, and 8 patients, 4 women and 4 men, aged between 18 and 65 were before the inclusion the anticonvulsant therapy. A preliminary analysis in our studies was conducted on 38 people from the control group, 28 women and 10 men, aged from 22 to 67, without symptoms of dementia or any other neurological disorders.

The concentration to sulfur amino acids (Hcy, Cys, GSH) in plasma has been identified by HPLC (high performance liquid chromatography) with electrochemical detection. The analysis was performed in Thermo Hypersil BDS C18 column
(250x4, 6x5 µm) using phosphate buffer with 12.7% acetonitrile as mobile phase for the determination of Hcy and GSH, and phosphate buffer with 10% acetonitrile as buffer mobile phase for the determination of Cys.

Studies show that AEDs pharmacotherapy in patients with epilepsy leads to
a significant increase in Hcy- treated in polytherapy, especially in the application of VPA and the long-term treatment. In addition, in patients with epilepsy treated with VPA in monotherapy, plasma Cys concentration is significantly reduced. Moreover, it was observed that long-term AEDs treatment and mild HHcy (Hcy>16 µmol/l) in patients with epilepsy leads to a significant increase in GSH concentration.

It can be assumed that patients with epilepsy using AEDs are exposed to high oxidative stress, which is an index for the observed higher concentrations of GSH, the main intracellular antioxidant. It also seems that only the combined supplementation of vitamins B and FA in patients with epilepsy treated with AEDs may contribute to the effective regulation of Hcy- the risk factor for vascular diseases.

SI-P10

Metformin reduces the production of toxic molecules and affects the profile of cytokines release in LPS-stimulated rat primary microglial cultures

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The results of recent studies suggest that metformin, in addition to its application for treating of type 2 diabetes, may also have therapeutic potential for treating neuroinflammatory diseases where reactive microglia play an essential role. However, the molecular mechanisms of action by which metformin exerts its anti-inflammatory effects remain largely unknown. Activation of AMP-activated protein kinase (AMPK) constitutes the best-known mechanism of metformin action. It is also known that some of metformin biological responses are not limited to activation of AMPK but mediated by AMPK-independent mechanisms.
In this study we attempt to evaluate the effects of metformin on non-stimulated and LPS-activated rat primary microglial cell cultures. Our results support the conclusion that AMPK activated by metformin is involved in the regulating the release of TNF-alpha at the early phase of secretion. Furthermore, we found that the effects of metformin on the release of TNF-alpha at the later phases of secretion, IL-1beta, IL-6, IL-10 and TGF-beta1 as well as on the expression of arginase I, iNOS, NF-kappaB p65 and PGC-1-alpha are not AMPK-dependent because the pretreatment of LPS-activated microglia with compound C (a pharmacological inhibitor of AMPK) did not reverse the effect of metformin. Considering the described properties of metformin, we suppose that the shift of microglia towards "alternative activation" may form the basis of the drug's beneficial effects observed in animal models of neurological disorders.

SI-P11

Neuroprotective effects of 1MeTIQ in vitro involve induced tolerance to excitotoxicity

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1,2,3,4,-Tetrahydroisochinolines (TIQ) are endogenous substances present in brain in low concentrations. Several TIQ derivatives are neurotoxic producing a Parkinson's syndrome. In turn 1-methyl-1-,2,3,4,-tetrahydroisochinoline (1MeTIQ) was found to reduce neuronal death in various models of neurotoxicity. Our previous results revealed that 1MeTIQ reduces excitotoxicity in the primary culture of rat cerebellar granule cells, inhibits glutamate evoked 45Ca accumulation in neurons and suppresses [3H]MK-801 binding to rat cortical membranes. Thus we hypothesized that this compound may be attributed to NMDA receptor antagonists. To verify this supposition, in the present study we compared the neuroprotective potential of 1MeTIQ with the established uncompetitive NMDA receptor antagonists MK-801 and memantine. The primary cultures of rat cerebellar granule neurons were briefly exposed to glutamate, and the substances tested were either co-applied with the excitotoxin or we tested their ability to induce tolerance to glutamate by pre- or post-conditioning. Consequently, 100, 250 or 500 μM 1MeTIQ, 0.5 μM MK-801 or 5 μM memantine were applied for 30 min either together with glutamate, 24 or 48 h before (pre-conditioning), or 0.5 h, 1 h and 3 h after (post-conditioning) exposure to 100 μM or 250 μM glutamate. Our results demonstrated that MK-801, memantine and 1MeTIQ induce an almost complete neuroprotection when co-applied with glutamate. Similar effects of 1MeTIQ and of the established NMDA receptor antagonists were observed in the pre-treatment experiments, even with 48 h lag between application of tested substances and the excitotoxic challenge. In the post-treatment experiments, MK-801 and memantine as well as 500 μM 1MeTIQ applied up to 3 h after brief exposition to glutamate significantly decreased the excitotoxic lesion, while 1MeTIQ in lower concentrations was ineffective. Thus, we demonstrated that 1MeTIQ acts as a weak NMDA receptor antagonist. The new finding of this study is that 1MeTIQ like MK-801 or memantine is neuroprotective when administered before and after exposure to glutamate. We suggest that 1MeTIQ induces long-lasting tolerance of cultured neurons to the excitotoxic insults, and consequently that the mechanism of neuroprotective effects of 1MeTIQ observed under various experimental conditions may be partially attributed to tolerance developing after pre- or post-treatment of neurons with this substance.
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SI-P12

Distinct cardio-respiratory response patterns elicited by neuropeptide Y and neuropeptide Y 13-36

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Neuropeptide Y (NPY) and its receptors have been involved in many physiological functions such as: regulation of cardiovascular system, anxiety, circadian rhythm, pain processing, inflammation, and among others, regulation of breathing. Microinjections of NPY to the dorsal medulla oblongata evoked respiratory and cardiovascular depression (Barraco et al., Brain Res. Bull., 1990; Dunbar et al., Am. J. Physiol., 1992). There is also evidence that respiratory failure or severe dyspnoea in humans was related to a high content of NPY in the infundibular nucleus (Corder et al., Neuroendocrinol., 1990).

The objective of this study was to determine and compare the effects of systemic administration of neuropeptide Y and neuropeptide Y 13-36 (NPY 13-36) on the pattern of breathing and cardiovascular function and to evaluate the contribution of vagal input and the role of NPYY1 and/or Y2 receptors in these responses.
Anaesthetized, spontaneously breathing rats were used. Tidal volume was measured at tracheostomy. The timing components of the breathing pattern, arterial blood pressure and heart rate were recorded. Intravenous injection of 100µg/kg^{-1} of NPY before and after midcervical vagotomy induced immediate slowing down of the respiratory rate and decreased tidal volume. Depressed ventilation was accompanied by a significant hypertension and bradycardia. Blockade of NPYY1 receptors with an intravenous dose of 5 mg/kg of BMS 193885, significantly reduced post-NPY cardio-respiratory effects.
NPY 13-36, an agonist of NPYY2 receptors, at a dose of 10 mg/kg provoked completely different respiratory response consisting of increased tidal volume, short-lived acceleration of the respiratory rhythm resulting in hyperventilation. Increased blood pressure but no effect on heart rate were observed. Section of the lung vagi abrogated the increase in respiratory rate thus reducing an enhanced ventilation. The rise in blood pressure was diminished.
This study shows that intravenous injection of neuropeptide Y by acting on NPYY1 receptors outside of the lung vagi depresses ventilation by decreasing tidal volume and respiratory rate. Hypertension and bradycardia occur also besides this pathway and might result from the activation of peripheral vascular or heart Y1 receptors. Yet, NPY 13-36 acting through NPYY2 receptors stimulates ventilation augmenting the tidal component of the breathing pattern independent of the vagal pathway. This latter mediates the respiratory timing and hypertensive responses to NPY 13-36.

SI-P13
Complex mechanisms of tetrabromobisphenol A neurotoxicity in primary cultures of rat cerebellar granule cells

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Tetrabromobisphenol A (TBBPA) is a brominated flame retardant considered as the environmental toxin affecting the brain. The exact molecular mechanisms of the TBBPA-induced neurotoxicity are unclear, however recent studies suggest a role of the NMDA receptor-mediated excitotoxicity and/or of calcium imbalance. To verify this hypothesis in the present study we examined relation between toxicity of TBBPA applied to cultured neurons at the micro molar concentration range and activation of the NMDA receptors as well as changes in the intracellular calcium homeostasis, oxidative stress and a decrease in the mitochondrial membrane potential. Experiments were performed using an in vitro model of the primary cultures of rat cerebellar granule cells at 7th day in vitro. To evaluate TBBPA neurotoxicity, the cells were exposed for 30 min to TBBPA, and neuronal viability was tested after 24 h with propidium iodide staining. Changes in calcium homeostasis were characterized by measuring 45Ca uptake and increases in fluorescence of calcium-sensitive probe fluo-3. Changes in the mitochondrial membrane potential and in free radical production were evaluated using the fluorescent probes rhodamine 123 and DCF, respectively. The results demonstrated that TPPBA in the concentration-dependent manner in the range of 25 - 100µM induced severe neurotoxicity. TBBPA in concentrations exceeding 5 µM triggered rise in the intracellular calcium level, which was sensitive to inhibitors of ryanodine receptors 2.5 µM bastadin 10 with 200 µM ryanodine, but not to 2ABP, which inhibits IP3 receptors. TBBPA in concentrations above 25µM activated 45Ca uptake, which was sensitive to uncompetitive NMDA receptor antagonist 0.5 µM MK-801. Moreover we observed accumulation of the reactive oxygen species and a drop in the mitochondrial membrane potential evoked by TBBPA applied at micro molar concentrations. The toxic effect of TPPBA in concentrations up to 10 - 15 µM was insensitive to antagonists of NMDA receptors and ryanodine receptors, MK-801 and bastadin 10 with ryanodine, respectively. This points to a role of the mechanism of TBBPA neurotoxicity other than excitotoxicity and calcium imbalance. Tentatively we identify it as the oxidative stress. Collectively, these data point to complexity of the mechanisms of toxic effects of TBBPA. Depending of TBBPA concentrations they comprise oxidative stress, the release of calcium from ryanodie sensitive stores in endoplasmic reticulum and activation of NMDA receptors.
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SI-P14
Agonists of mGluRs II/III attenuate the staurosporine-induced toxicity in human neuroblastoma SH-SY5Y cells

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Agonists of metabotropic glutamate receptors group II and III (mGluRs II/III) show neuroprotective effects in in vitro and in vivo models of excitotoxicity. However, their influence on neuronal apoptosis remains unknown. In this study the effect of agonists of mGluRs II/III on staurosporine (St)-evoked LDH release was estimated in undifferentiated (UN-) and retinoic acid (RA)-differentiated human neuroblastoma SH-SY5Y cells. It has been found that LY354740 (0.01-100 microM) and ACTP-I (0.01-100 microM), a nonspecific agonists of mGluRs group II and III, respectively when given alone had no effect on cell proliferation and cell viability. However, both of these compounds partially decreased the St-induced cell death in UN- and RA-SHSY5Y. The selective agonist of mGluR7, AMN082 in low concentrations (0.001-1 microM) had no effect on cell proliferation/viability and tended to attenuate the St-induced toxicity only in UN-SHSY5Y. On the other hand, AMN082

in higher concentrations (>10 microM) had the cell damaging effect in both UN- and RA- SHSY5Y cells. This study indicates that agonists of mGluRs II/III have potential to attenuate cell death evoked by staurosporine - a well recognized inducer of apoptosis.
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SI-P15
Zinc as early neurotoxic signal in cholinergic SN56 neuroblastoma cells

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Preferential loss of septal cholinergic neurons is a main cause of cognitive deficits in various encephalopathies. Zinc excess is one of multiple pathologic signals contributing to mechanisms of Alzheimer's and other neurodegenerative diseases. We suggest that zinc may be involved in early excitotoxic phase of neuronal injury. In homogenates of SN56 cholinergic neuroblastoma cells, Zn caused instant inhibition of pyruvate dehydrogenase (PDH), aconitase, isocitrate dehydrogenase (IDH) and ketoglutarate dehydrogenase (KDH) activities with Ki values equal to 0.08, 0.008, 0.005 and 0.005 mM, respectively. Short term, 30 minute exposition to Zn caused a concentration dependent increase in mortality of cAMP/retinoic acid differentiated SN56 cholinergic cells (DC) that was two times higher than that of differentiated ones (NC). Zn also decreased cytoplasmic acetyl-CoA as well as ACh content and inhibited its release. Exposition of DC and NC to increasing concentrations of Zn yielded concave up non saturable accumulation plots that reached level of 60 nmol/mg protein at 0,15 mM extracellular concentration of a cation. In these conditions no change in whole cell Ca level was observed. However the level of intramitochondrial Ca was decreased by 30%, at 100 % increase of cytoplasmic Ca. Significant, direct correlation between Zn accumulation and cytoplasmic Ca concentration ($r=0.97$, $p=0.028$) and the inverse one with mitochondrial Ca ($r=-0.96$, $p=0.028$) were found, respectively. On the other hand, 24 h cell exposition to 0,15 mM Zn increased its intracellular content from 1.4 to about 6 nmol/mg protein at simultaneous 40% decrease of whole cell Ca level. Zn caused no significant changes in the density of ZnT1 and ZnT4 transporter proteins in the cells. Presented data indicate the coexistence in SN56 cell plasma membranes low density - high-affinity and high density - low affinity Zn-transporting sites. Inhibition of mitochondrial Na-Ca exchanger by accumulated Zn might cause depletion of Ca in mitochondria. In addition chronic exposition to Zn apparently induced adaptative mechanisms eliminating excess of the metal from the cells. These changes may directly inhibit intramitochondrial acetyl-CoA synthesis and its transport to cytoplasmic compartment, yielding impairment of cell viability and suppression their transmitter functions.

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SI-P16
Biochemical changes in the brain of rats with different resistance to hypoxia exposed to cadmium toxicity

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Individual resistance to hypoxia is one of the cardinal features of the mammalian body. This resistance is closely related with different enzymatic activity system of metabolizing xenobiotics, in particular, the activity of cytochrome P450 and microsomal oxidation system of liver. Low and high resistance to hypoxia may be an important criterion in the individual approach to the pharmacotherapy of diseases associated with conditions of hypoxia, and can also be used to predict and prevent early and long-term complications of drug therapy. The goal of the study was to estimate the effect of cadmium (II) chloride intoxication, on the activity of some metabolic enzymes (alanine transaminase ALT, aspartate transaminase AST, lactate dehydrogenase LDH and succinate dehydrogenase SDH), and the levels of lipid and protein oxidation processes in the brain of male rats with different resistance to hypoxia. The results suggest that the activity of metabolic enzymes (AST, LDH, SDH) is higher in animals presenting low resistance to hypoxia in the control group, and thus can serve as a compensatory reserve mechanism under unfavorable environmental conditions. However, rats with high resistance to hypoxia display an increased tension of regulatory mechanisms and a decreased ability of antioxidant system, which results in the activation of lipid and protein oxidation precesses under cadmium intoxication. Conclude, the cadmium intoxication decreases the activity of ALT in rats with high resistance to hypoxia, whereas the activity of AST and LDH is higher in the brains of cadmium-exposed rats with low resistance to hypoxia.

SI-P17
The group III metabotropic glutamate receptor agonists attenuate neuronal cell death in primary cortical cultures exposed to oxygen-glucose deprivation

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Oxygen-glucose deprivation (OGD) induces excitotoxic cell death mediated primarily by excessive release of glutamate. A growing body of evidence suggests that metabotropic glutamate (mGlu) receptors can modulate glutamatergic transmission, so these receptors are regarded as potential targets for neuroprotective drugs. Group III mGluRs (mGlu4, mGlu6, mGlu7 and mGlu8) agonists are known to reduce glutamatergic neurotransmission by inhibiting

glutamate release. Therefore in the present study we tried to find out whether the agonist of group III mGluR (1S,3R,4S)-1-aminocyclopentane-1,3,4-tricarboxylic acid (ACPT-1) and the first selective allosteric mGlu7 receptor agonist, N,N'-bis (diphenylmethyl)-1,2-ethanediamine (AMN082) have neuroprotective potential in primary neuronal cortical cultures exposed to oxygen-glucose deprivation, as an in vitro ischemic injury paradigm. In order to evoke toxic effects cortical cultures were exposed to OGD for 1 - 5 h. ACPT-1, at concentrations of 1, 10, 100 and 200 μM, or AMN082, at concentrations of 0.01, 0.1, 0.5 and 1 μM, were applied in two ways: twice, just before the start of OGD and immediately after OGD or once, immediately after OGD. Neurotoxicity was measured by lactate dehydrogenase (LDH) efflux from the damaged cells into the culture media 24 h after the end of OGD. It was found that a double application of ACPT-1 or AMN082 significantly attenuated the LDH release by 20-30% and 30-43%, respectively. A particularly important finding is that AMN082, given once after the end of OGD also significantly decreased ischemic-induced LDH release by 30%. These data were confirmed by immunohistochemical staining for the presence of characteristic neuronal protein MAP-2. In conclusion, the above results indicate that group III mGlu receptor agonists may have neuroprotective potential and may play a potential therapeutic role in neurodegenerative disorders.

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SI-P18

COX-2/PGI2 pathway is involved in neuroprotection induced by N1-methylnicotinamide in hypoxia/ischemia of newborn rats

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1-Methylnicotinamide (MNA) is a key metabolite of nicotinamide, which is an important cofactor in many metabolic pathways with intrinsic cytoprotective properties. Recent studies demonstrated that MNA induces in the periphery the anti-inflammatory and vasoprotective effects mediated by the COX-2/ PGI2 signaling pathway. The role of this pathway in the protection against ischemic injury has been emphasized by the increased risk of myocardial infarction, stroke or susceptibility to excitotoxic insults caused by COX-2 inhibitors. This suggests that administration of MNA resulting in activation of COX-2/PGI2 might provide neuroprotection in brain ischemia. The aim of this study was to evaluate neuroprotective abilities of MNA in the model of brain hypoxia/ischemia (HI) in 7-day old rat pups, and to assess its effects on COX-2 activity and eicosanoid levels in the ischemic brain. The results demonstrated that i.p. administration of 62.5 mg/kg MNA 30 min after HI induced a significant neuroprotection and a marked increase in COX-2 activity 6 and 24 h after the insult. This effect was not accompanied by changes in the COX-2 protein level, as measured with Western blotting. The analysis of the level of eicosanoids: 6-keto-PGF1alpha-a which is a stabile metabolite of PGI2, PGE2, and TXB2 in brain of rats treated with MNA after HI demonstrated in the ipsilateral cortex a significant increase in 6-keto-PGF1alpha 30 min after the treatment, while PGE2 and TXB2 levels did not change. Pretreatment with rofecoxib (COX-2 inhibitor) or indomethacin (unselective COX inhibitor) decreased the level of 6-keto-PGF1alpha in the ipsilateral cortex to values observed in the untreated ischemic brain. To test the hypothesis that neuroprotection evoked by MNA in HI model depends on activation of prostacylin receptors, the selective antagonist of these receptors RO 324479 was applied. However, pretreatment of rat pups subjected to HI with RO 324479 (10 mg/kg i.p.) did not influence significantly the neuroprotective effect of MNA. Further studies are needed to assess the role of COX-2/PGI2 in the MNA-evoked neuroprotection.

SI-P19

Neuroprotective potential of 1-methyl-1,2,3,4,-tetrahydroisochinoline in brain ischemia: role of NMDA receptor antagonism

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Various endogenous teterahydroisochinoline derivatives present in the mammalian brain have been considered as neurotoxic substances. However 1-methyl-1,2,3,4-tetrahydroisochinoline (1MeTIQ) is known for its mild neuroprotective potential of the unclear mechanism. On the one hand 1MeTIQ exhibits anti-dopaminergic activity and reduces the neurotoxic effects of MPTP and rotenone, decreasing also the behavioral effects of MK-801 in rats in vivo. On the other hand the results of our previous study demonstrated that 1MeTIQ in vitro prevents glutamate-induced excitotoxicity in cultured neurons suggesting that this effect may be ascribed to its inhibitory effect on NMDA receptors. It is well known that the antagonists of NMDA receptors provide neuroprotection in brain ischemia, however the anti ischemic properties of 1MeTIQ were not tested previously. The aim of our present study was to verify in vitro putative antagonistic effects of 1MeTIQ on the NMDA receptors and to evaluate its neuroprotective potential in the animal models of brain ischemia. The receptor binding experiments using membranous fractions isolated from the rat brain cortex confirmed that 1MeTIQ in high micro molar concentrations inhibits in a concentration-dependent manner the specific binding of [3H] MK-801, while the binding of [3H]glutamate remains unaffected. The hypothesis that 1MeTIQ may be attributed to NMDA receptor antagonists acting as channel blockers was also supported by the results of experiments utilizing primary cultures of rat cerebellar granule cells submitted to acute NMDA and glutamate excitotoxicity. Under these conditions 1MeTIQ applied at high micro

molar concentrations provided a pronounced neuroprotection and significantly inhibited generation of the calcium signal. The in vivo ischemic experiments demonstrated that application of 1MeTIQ in the dose of 50 mg/kg 30 min before the insult in the model of global forebrain ischemia in Mongolian gerbils or its repeated application in the same dose after hypoxia-ischemia in the rat model of perinatal asphyxia provided significant neuroprotection. In the gerbils treated with 1MeTIQ we observed the morphological and behavioral symptoms of neuroprotection and the postischemic hypothermia characteristic for medication of brain ischemia with the NMDA receptor antagonists. Collectively these data offer new arguments confirming the hypothesis that 1MeTIQ acts as a weak uncompetitive antagonist of NMDA receptors, providing the neuroprotection under various excitotoxic and ischemic conditions both in vitro and in vivo.

SI-P20

Early and delayed hypoxic postconditioning in the model of birth asphyxia in 7-day-old rats

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Lack of the clinically applicable effective pharmacological neuroprotection in different forms of brain ischemia triggers increasing interest in alternative methods of therapy, including induction of brain tolerance by pre- and postconditioning. It is known for a long time that hypoxic preconditioning reduces brain damage in the rat model of perinatal asphyxia (Vannucci et al., 1998, Cantagrel et al., 2003). Recent data demonstrate that also postconditioning with moderate hypoxia delayed for 24 h after the insult results in a modest brain neuroprotection in adult mice (Leconte et al., 2009), however similar studies using the immature rats were never done. It has been suggested that similar mechanisms are involved in the induction of tolerance to brain ischemia by pre- and postconditioning, but timing may differ in both cases. Two temporal profiles of brain tolerance induced by preconditioning have been recognized: an early tolerance induced within minutes and depending on fast posttranslational modifications of proteins and a delayed one, developing after several hours to days and dependent on de novo protein synthesis (Kirino, 2002). It is not clear whether brain tolerance to hypoxia/ischemia induced by hypoxic postconditioning is also a two-phase phenomenon. The aim of this study was to evaluate efficacy of normo- and hypobaric postconditionig initiated 1, 3, or 6 hours after the insult in 7-day-old rats submitted to hypoxia-ischemia (H-I). H-I was induced by ipsilateral carotid occlusion followed by 75 min. exposure to hypoxia (7.2 - 7.4% O2 in N2). Hypoxic postconditionig was conducted under normobaric conditions at 10% O2 in N2 for 75 min, or in the hypobaric chamber set at 360 torr corresponding to 10 % O2 at the sea level. The postconditioning was repeated once a day for 3 consecutive days. The brain damage was evaluated two weeks after H-I and expressed as ipsilateral hemisphere weight deficit in percent of the contralateral hemisphere. The results of this study demonstrated that both, normobaric or hypobaric postconditioning resulted in a significant neuroprotection only if initiated 1 h or 6 h after H-I, but not after 3 h. These results demonstrate for the first time efficacy of hypoxic postconditioning in the rat model of H-I and suggest that depending on timing of the hypoxic postconditioning the early and delayed tolerance may be achieved. Experiments are in progress verifying the role of mild oxidative stress in the mechanism of hypoxic postconditioning.

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SI-P21

Influence of MAO-A inhibition on the respiratory response to hypoxia in the conscious rat

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Debrisoquine is a peripherally acting monoaminooxidase A (MAO-A) inhibitor. MAO is, in cooperation with the catechol-O-methyltransferase (COMT), the main enzyme degradating dopamine in vivo. Dopamine itself is one of the most important neurotransmitters involved in the generation of the respiratory response to hypoxia. Our study seeks to determine whether blockade of the MAO-pathway, resulting in an augmentation of the endogenous dopamine content, could influence the respiratory response to hypoxia in conscious rats. We performed the experiments in 8 male adult Wistar rats (10-11 weeks old, 290-310 g). Minute lung ventilation and its responses to 8 and 12% hypoxic stimuli were measured in a BUXCO whole body plethysmographic chamber. After the control recordings had been taken, debrisoquine (40 mg/kg, i.p.) was injected and the responses to both stimuli were reevaluated after 30 and 60 min. We found that debrisoquine, after 30 min, significantly increased the peak hypoxic ventilatory response (HVR) from 1109 ±161 and 1320±194 to 1744±265 and 2307±259 ml/min/kg for 8% and 12% hypoxia, respectively. However, this effect failed to be long-lasting: 60 min post-injection the peak respiratory response regressed to 1312±135 and 1681 ±105 ml/min/kg, for 8 and 12% hypoxia, respectively. These results indicate that a peripheral blockade of MAO-A increases the HVR. A possible explanation of this phenomenon is the putative stimulatory action of endogenous dopamine (at higher concentrations) or activation by debrisoquine some other stimulatory, non-amine neurotransmitters which do not undergo a MAO-linked oxidation, in the generation of the hypoxic response peripherally at the level of the carotid body. Yet another explanation could be by far unknown, stimulatory influence of debrisoquine on respiration.

SI-P22

Modulation of neurological deficits during EAE by glutamate receptors antagonists MPEP and amantadine

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Chronic glutamate-mediated excitotoxicity has been suggested to contribute to the pathogenesis of Multiple Sclerosis (MS). Recent data suggest that inhibition of glutamate neurotransmission via specific interaction with glutamate receptors (GluRs) might be interesting for inhibition of disease progression and early symptomatic treatment in MS. The aim of our investigation was to study the role of NMDA receptors and group I mGluRs in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), an animal model of MS. We tested the effect of MPEP (2-methyl-6-(phenylethynyl)-pyridine), the mGluR5 antagonist, in dose of 5 mg/kg b.w./day, and amantadine (the uncompetitive NMDA receptor antagonist) in dose of 100 mg/kg b.w./day on development of neurological deficits in EAE rats. Both drugs were administered intraperitoneally ones daily into EAE rats during 7 days, starting from day 5 to 12 post immunization. The neurological symptoms of EAE started at 10-11 days post immunization and peaked after 12-13 days. We noted the changes in body weight during the course of EAE. Until day 8 p.i. the body weight of rats in control and treated groups was in the same range. Starting from day 8 p.i. rats in all groups showed a progressive weight loss by about 20-30% until day 14 p.i. Application of amantadine was found to be effective and significantly reduced neurological symptoms of EAE. We did not observed any neuroprotective effects of MPEP. The level of mGluR5 protein did not increase in early phase of EAE (4 day p.i.). However, starting from day 8 p.i. to day 25 p.i. we observed its significant elevation. The difference between control and examined group reached 20% at 25 day p.i. We did not observe significant differences in mGluR5 level between three experimental groups: EAE rats (control), MPEP-treated and amantadine-treated rats. Our results confirm the involvement of glutamate into pathogenesis of EAE. Although we noted changes in the expression of mGluR 5 during the course of EAE, MPEP was ineffective in reducing the symptoms of the disease. Results suggest the main role of NMDA glutamate receptors in the pathogenesis of EAE.

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SI-P23

NO-sGC signaling in rat cervical spinal cord after unilateral spinal cord injury

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The NO-sensitive guanylyl cyclase (sGC) as a major type of cyclic guanosine monophosphate (cGMP) - forming enzymes represents the best characterized receptor for the signal molecule nitric oxide (NO) produced by the NO synthases. Relatively high levels of the sGC are found in lung, brain and platelets, but little is known about the NO-cGMP signalling in the spinal cord. Our study was focused on NO-sGC signalization in bulbospinal respiratory pathway. We used a model of spinal cord hemisection performed at C2-C3 segmental level. After spinal cord injury the animals (adult rats) survived for eight days. The expression of neuronal nitric oxide synthase (nNOS) and β1 subunit of sGC was identified by immunohistochemical fluorescent method in ventral respiratory group (VRG) of medulla and in phrenic nucleus (PN) located at C4-C5 segmental level. In addition, the retrograde tracer Fluorogold (FG) was used to label neurons of VRG. These neurons were nNOS-immunoreactive. Two days after FG injection into the phrenic nucleus (PN), we revealed many FG fluorescent neurons mostly contralaterally to the site of injection. In control group, we noted nNOS-fluorescent terminals of VRG neurons around beta 1 soluble guanylyl cyclase (ß1sGC) fluorescent motoneurons in PN. Spinal cord hemisection caused a significant decrease of the nNOS- and Synaptophysin (SYN) fluorescent terminals around alfa motoneurons in contralateral PN 8 days after hemisection. On the side of injury, nNOS/SYN fluorescent puncta were detected around phrenic motoneurons only sporadically. Phrenic alfa motoneurons responded to C2-C3 hemisection by a loss of ß1sGC positivity. These results together suggest that bulbospinal respiratory pathway is acting through NO-sGC.

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SI-P24

Decreased expression of the Kir4.1 channel in the brain of rats with hepatic encephalopathy and in ammonia- and glutamine-treated cultured astrocytes

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Hepatic encephalopathy (HE) is a neuropsychiatric disorder associated with hyperammonemia. Swelling of astrocytes is a primary cause of brain edema in patients and in animal models of HE. Hyperammonemic brain edema is aggravated by hypoosmotic imbalance due to hyponatremia, and is thought to be related not only to ammonia itself, but in the first instance to glutamine (Gln), which is synthesized in astrocytes in excess from ammonia and glutamate (Albrecht and Norenberg, 2006).

This study addressed two questions 1) whether and in what degree is the impairment of ion and water homeostasis in astrocytes re-

lated to altered potassium transport, 2) if such disturbances occur, in what degree can they be ascribed to Gln accumulation? To this end we investigated the expression of the major potassium transport vehicles: glial inwardly rectifying potassium channels (Kir4.1, Kir2.1) and the Na+-K+-2Cl- cotransporter-1 (NKCC1), in brain of rats with experimentally induced HE and in rat cortical astrocytes treated in culture with ammonia or Gln.

Thioacetamide-induced HE decreased the Kir4.1 expression in the rat cerebral cortex at both the mRNA and protein level, while the mRNA level of Kir2.1 and NKCC1 remained unaltered. In primary cortical astrocytes, Gln, but not ammonia, induced a decrease in the levels of Kir4.1 mRNA and protein. Kir2.1 and NKCC1 mRNA levels in cultured astrocytes were unchanged upon Gln administration. Treatment of cultured astrocytes with ammonia or Gln resulted in the reduction of the hypoosmolarity-induced potasium (86Rb) efflux from the cells. Treatment with Gln induced also a decrease in the astrocytic uptake of glutamate (D-[3H]-aspartate).

The results suggest that decreased expression of Kir4.1 contributes to disturbances in ion and water homeostasis and impaired Glu clearance accompanying HE, and further bespeak the role of Gln in the pathogenesis of HE. However, direct correlation between Kir4.1 downregulation and impairment of potassium fluxes awaits to be confirmed. Experiments using siRNA duplexes, selectively downregulating Kir4.1 in cultured astrocytes, are conducted to further investigate the involvement of Kir4.1 in astrocytic cell swelling and volume regulation.

Session II
NEUROPEPTIDES – ENDOGENOUS FUNCTIONS AND PROSPECTIVE CLINICAL APPLICATIONS

SII-L1
In vitro models of the blood-brain barrier

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The blood-brain barrier (BBB) is an active interface between the circulation and the central nervous system with a dual function: the barrier function restricts the transport from blood to the brain of potentially toxic or harmful substances the carrier function is responsible for the transport of nutrients to the brain and removal of metabolites.

The BBB plays a crucial role in the clinical practice as well. On the one side there are a large number of neurological disorders including cerebral ischemia, brain trauma and tumors, neurodegenerative disorders in which the permeability of the BBB is increased. On the other hand due to the relative impermeability of the barrier many drugs are unable to reach the CNS in therapeutically relevant concentration making the BBB one of the major impediments in the treatment of CNS disorders. The significant scientific and industrial interest in the physiology and pathology of the BBB led to the development of several in vitro models of the BBB. These models are mainly based on the culture of cerebral endothelial cells. The best in vitro models which mimic in the best way the in vivo anatomical conditions are the co-culture models in which the brain endothelial cells are co-cultured with astrocytes and/or pericytes. Our in vitro BBB model is characterized by high transendothelial electrical resistance (TEER regularily between 200-400 Ohm· cm^2), low permeability and expression of different transporters. In our experiments we have investigated the effect of different extracellular factors including oxidative stress and osmotic stress on functional characteristics of the BBB.

Our experiments have proven that the model is suitable for basic research and for testing the interaction between the BBB and potential drug candidates (toxicity, permeability, interaction with efflux transporters) as well.

SII-L2
Peptide-based cancer vaccines: Adjuvant vaccination with melanoma peptide-pulsed dendritic cells (DCs) in stage III melanoma patients

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Background: This is a pilot study evaluating of high-risk melanoma patients (pts) treated with peptide-DC vaccine after lymphadenectomy (LND). DC vaccination was designed to induce the immune response against melanoma antigens in melanoma pts who remain at high risk of dissemination after LND.

Methods: DCs were generated from the bone marrow cultured with GM-CSF, SCF, FLT3-L and TNF-a or from peripheral blood adherent monocytes cultured with GM-CSF and IL-4. DCs pulsed with HLA-A2-binding TYR, MART-1 and gp100 peptides and/or HLA-A1-binding MAGE-1, MAGE-3 peptides, tumor lysate if available, or with tracer antigen keyhole limpet hemocyanin (KLH), were injected subcutaneously 9 times within 8 months (mos). Boost injections were performed after 12 and 24 mos. Vaccinated pts were matched to unvaccinated controls (22 of 869) by sex, number of metastatic lymph nodes, extracapsular involvement, completion or therapeutic LND, Breslow stage (T), ulceration, and lactate dehydrogenase (LDH) level prior to LND.

Results: HLA-A2+, -A1+ or -A3+ melanoma pts (n=22), stage III, N1b-N3, enrolled between Sept. 2002 and Apr. 2004, received 5-16 vaccinations (median: 11) within 2 yrs. Cutaneous delayed type hypersensitivity (DTH) to melanoma peptides was induced in 12 of 22 pts. Peptide-specific IFN-g; producing CD8+ cells were detected in peripheral blood of 13 of 19 pts after vaccination. At least one of these responses to melanoma antigens was elicited in 17 of 22 pts. DTH to KLH was positive in 15 of 22 pts. Eight

vaccinated pts are free of disease (follow up is 77-97 mos after LND), and 1 in progression is lost from follow-up by Aug 30, 2010. Survival analysis of vaccinated pts and matched controls is presented in Table 1.
Conclusions: The DC/peptide vaccine elicited immune responses to melanoma antigens. Vaccinated pts had clinically substantially longer overall survival (OS) and disease free survival (DFS) than matched control. OS was associated with the immune responsiveness to melanoma antigens and to KLH.
Table 1: Survival analysis of vaccinated pts and matched controls.
Vaccinated pts (n=22) 3-year OS [%]: 68.2
Matched control (n=22) 3-year OS [%]: 25.7
p-value accounting for matching 0.0290
HR (95% CI)* 3.25 (1.06-9.97)
Vaccinated pts (n=19**) 3-year DFS [%]: 40.9
Matched control (n=22) 3-year DFS [%]: 14.5
p-value accounting for matching 0.1083
HR (95% CI)* 2.16 (0.82-5.7)
*Cox Proportional Model - Hazard Ratio (HR) of unvaccinated pts
**3 pts with recurrence before LND were excluded

SII-L3
Mild traumatic brain injury (mTBI) mouse model

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Survivals of minimal traumatic brain injury (mTBI) do not show clear structural brain defects, but frequently suffer from long-lasting cognitive deficits, emotional difficulties and behavioral disturbances. In the present study we investigated the effects of experimental closed head mTBI induced by a weight drop device on spatial and non-spatial learning and the anxiety-like behaviors in mice. Mice were tested 7 or 30 days post-injury for cognitive function and anxiety-like behaviors. Some of these tests have never been used in the past for mTBI research. In the elevated plus maze not behavior change was shown 7 days post injury. However, 30 days post injury the mTBI induced significant anxiety like behavior in the mice. In the dry maze, the mTBI mice demonstrated significant difficulties in their learning trials as well as in the probe part, 7 and 30 days post injury. The mice's preference in the Y- maze was also significantly affected by the injury. The mTBI animals showed impaired learning compared with the sham group both 7 and 30 days post injury. In an additional, non-spatial test, measured by the novel object recognition test, revealing a significantly higher objects-exploration time by the control mice, 7-days post injury. Thirty days post mTBI, the injured mice spent significantly less time next to the new objects compared to the control mice. The results of this study further demonstrate that mTBI cause a cognitive impairment and anxiety-like behaviors in the injured mice that are similar to symptoms reported for the human "post-concussion syndrome".

SII-L4
Opioid peptides in peripheral pain control

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Malignant, long-lasting pain is an imminent component in advanced cancer and as such sufficiently decreases the quality of life of patients. Cancer patients are equally subjected to physical as well as emotional suffering. For many decades opioids have been the mainstay analgesics for treatment of moderate to severe pain conditions. Classical opioids by exerting their analgesic action in the CNS apart from producing analgesia simultaneously cause the onset of undesirable side effects such as constipation, nausea or sedation. The major advantage is a possibility to treat pain symptoms with avoidance of minor side-effects, particularly tolerance occurring in morphine-treated patients. In clinical practice it is important for a drug to possess both a peripheral and central action. In our studies we examined the analgesic effect of a dimeric enkephalin analog-biphalin in a murine skin cancer pain model developed by an intraplantar inoculation of B16T0 melanoma cells. Animals developed robust thermal hypersensitivity in the tumor-bearing paw compared to saline-injected individuals. Biphalin produced a more robust unilateral attenuation of thermal hyperalgesia in the tumor-bearing paw as compared to the classical non-peptidic drug-morphine. Results suggest a probable involvement of the peripheral opioid receptor-mediated analgesia. Thus, biphalin, may become a useful drug in cancer pain treatment because it also shows low tolerance liability.
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SII-O1
Gene expression profiling of psychotropic drugs effects in the brain

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Psychotropic drugs activate synchronized patterns of gene expression in the brain. These patterns are connected to biological processes involved in therapeutic as well as adverse drug effects. To reveal the transcriptional networks activated by different classes of psychotropic drugs we compared the effects of antidepressants (e.g. mianserine, fluoxetine), analgesics (e.g. morphine, heroin), psychostimulants (e.g. methamphetamine, cocaine) and antipsychotics (e.g. clozapine, haloperidol) on genomic profile in mouse (C57BL/6J) striatum. We applied a whole-genome microarray (Illumina WG-6) profiling to characterize time-course of transcrip-

tome alterations following acute drug administration (1, 2, 4 and 8h after injection). We identified major drug-regulated expression patterns that are formed by inducible transcriptional networks, as for example: (1) CREB/SRF-dependent genes that appears to be related to dopaminergic activity the striatum, (2) the group of genes controlled at least in part via release of steroid hormones. The data were stored as raw values, fold of change versus saline and P value of drug versus saline comparison in the database (available at www.genes2mind.org). The database interface allows for multidimensional data analysis (PCA), search for drug using genomic signatures and visualize drug-regulated gene transcription patterns. Our results elucidates the networks of drug-induced genes that share common regulatory elements, functional relations and may provide novel diagnostic tools for prediction of drug effectiveness.
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SII-O2

μ-opioid receptors' share in cardiorespiratory response to systemic injection of the analgesic chimeric peptide in anaesthetized rats

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Analgesic activity of the opioid agonists is apparently associated with a variety of side effects like depression of ventilation, arterial hypotension and drug dependence which limit their clinical use.
Neuropeptide - substance P (SP) partakes in pain transmission and acts as an excitatory mediator in the control of breathing. Moreover, it has been observed that low doses of substance P when coadministred with marginally effective doses of morphine markedly potentiate its antinociceptive effects.
Chimeric peptide recently synthesized in the Department of Neuropeptides of our Institute, signed AWL3106, comprises two pharmacophores: dermorphin and SP7-11, agonists to μ-opioid and NK1-tachykinin receptors (respectively) and shows high analgesic activity.
The aim of this study was to reveal how much the opioid receptors pertain to the peripheral vascular and respiratory effects induced by AWL3106.
Cardiorespiratory parameters were measured in 19 anaesthetized rats breathing spontaneously room air, treated by an intravenous injection of AWL3016 (0.3 μmol/kg) before (n=9) and after (n=10) opioid receptors blockade with naloxone (2mg/kg).
Control bolus injection of AWL3106 induced an apnoea of mean duration of 5s, followed by breathing at the frequency (f) reduced by 19% and of increased by 22% tidal volume (VT) which resulted in no signs of ventilatory depression.
Treatment with naloxone 2 min prior to AWL3106 challenge prevented the occurrence of apnoea, significantly reduced the maximal fall in breathing rate by 8% and maximal VT increase by 5% compared with the control injection. Minute ventilation was still left unchanged.
Mean arterial pressure (MAP) decreased in both experimental groups showing the lowest values at 1min 30s post-drug, and achieved 39% of the baseline before and 48% after opioid receptor blockade. The drops in MAP were statistically indistinguishable between the two groups.
This study evidences that concomitant activation of opioid and tachykinin receptors with AWL3106 might have an additive effect on VT and f, while only the opioid pharmacophore of this peptide is essential for the occurrence of apnoea. Arterial hypotension seems to be predominantly associated with the activation of tachykinin NK1 receptors.
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SII-P1

LITAF and TNF alpha genes sequence variants in the patients with Charcot-Marie-Tooth disease

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Recently it has been reported that Charcot-Marie-Tooth disease may coexist with chronic inflammatory neuropathy and central demyelination.
There is a question, whether CMT and inflammatory disease detected in one family share
a common pathogenesis or result from the random coincidence of two disorders.
There is a possibility that mutations/sequence variants in the gene coding for immune response mediators (LITAF, TNF alpha) may modify the CMT phenotype.
To test this hypothesis, we have sequenced the coding sequence of LITAF gene and the promoter sequence of TNF alpha gene in two families with Charcot-Marie-Tooth disease coexisting with chronic inflammatory demyelinating polyneuropathy (CIDP) and primary progressive multiple sclerosis (PPMS).
The genetic analysis has revealed three sequence variants: c.274A>G (Ile92Val) and in c.334G>A (Gly112Ser) in the LITAF gene and one SNP -308G>A in the promoter region of TNF alpha gene.
The sequence variants c.334G>A (Gly112Ser) in the LITAF gene and -308G>A in the TNF alpha gene were detected in family with Charcot-Marie-Tooth type 1C and primary progressive multiple sclerosis (PPMS). The sequence variants c.274A>G (Ile92Val) in the LITAF gene and -308G>A in the TNF alpha gene were detected in family with Charcot-Marie-Tooth type 1A and chronic inflammatory polyradiculoneuropathy (CIDP).
In agreement with previously published data we suggest that the sequence variants in the genes coding for inflammatory mediators may contribute to the clinical variability of CMT.

SII-P2

Association of the CHRNA4 1674+11C>T polymorphism with juvenile myoclonic epilepsy

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The α-4 subunit gene (CHRNA4) of the neuronal nicotinic acetylcholine receptor (nAChR), linked to an idiopathic partial epilepsy, autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), may also play a key role in the development of the idiopathic generalized epilepsy syndrome (IGE), juvenile myoclonic epilepsy (JME). This study was designed to explore an association of four polymorphisms of the CHRNA4 with JME in Polish children and young patients. The study included 92 JME patients and 222 unrelated healthy individuals. In each group the frequencies of the CHRNA4 c.555C>T, c.594C>T, 1674+11C>T, and 1674+14A>G polymorphisms were determined using PCR-RFLP analyses. An association between the 1674+11C>T polymorphism of the CHRNA4 and JME was evidenced. Allele T (the risk factor) appeared with a significantly higher frequency in the JME patients than in the controls (p=0.0299). The patients harboring the 1674+11CT+TT genotypes showed an increased risk of JME (CT+TT versus CC: OR=1.925; 95% CI=1.021-3.629; p=0.0408). No association was found for the other CHRNA4 polymorphisms tested. The CHRNA4 1674+11C>T polymorphism may be a susceptibility factor for epilepsy, and its higher frequency in patients with juvenile myoclonic epilepsy suggests that the CHRNA4 may be one of the candidate genes for this epileptic syndrome.

SII-P3

Cytogenetic analysis of multiple sclerosis-associated retrovirus sequences (MSRV), TCRB genes and karyotypes in patients with multiple sclerosis (MS)

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Introduction: The scientists look for etiopatogenic factors of multiple sclerosis among viruses and bacteria. Genetic and immunologic agents are being taken into consideration. It is more than likely that there are numerous genes that take part in a development of the disease. The influence of various factors at the same time should be considered on account of possible multifactorial etiology of the disease.
Aim and material: The aim of the studies was to analyse of MSRV pol, gag, env sequences, TCRB genes and karyotypes in patients with multiple sclerosis. The experimental material was peripheral blood lymphocytes from 130 MS patients and 50 healthy individuals.
Methods: Classical (GTG) and molecular cytogenetic techniques (FISH) were used in the experiments.
Results: 1) MSRV pol, gag and env sequences were found in both MS patients and controls. 2) The copy number of MSRV sequences was significantly greater in MS patients than in normal individuals. 3) The number of spontaneous micronuclei was significantly greater in MS patients compared to control. 4) Various chromosomal aberrations including translocations between chromosomes 7 and 14 were observed in patients with MS. 5) Translocation of constant and variable TCRB regions, deletion of constant TCRB region at 7 chromosome, duplication of constant TCRB region at chromosome 10, amplification of constant and variable TCRB regions in MS patients with aberrant chromosomes 7 and 14 were also found.
Conclusions: 1) Evident difference in MSRV pol, gag and env copy number between MS patients and control suggests that MSRV may play some role in the etiology of multiple sclerosis (latent viral infection). 2) The presence of chromosome aberrations and high amount of micronuclei in MS patients shows that the instability in MS genome often occurs.

SII-P4

Prevention of ammonia-induced oxidative/nitrosative stress in cultured astrocytes by stimulation of the natriuretic peptide clearance receptor (NPR-C)

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Oxidative and nitrosative stress contribute to ammonia-induced astrocytic dysfunction and swelling, the primary causes of lethal brain edema associated with hepatic encephalopathy (HE). Treatment of cultured astrocytes with 5 mmol/L ammonium chloride ("ammonia") increased the production of reactive oxygen species (ROS), including the NADPH oxidase reaction product, .O2-. Atrial natriuretic peptide (ANP), natriuretic peptide C (CNP) and a selective NPR-C ligand, cANP(4-23), each decreased formation of ROS measured as the sum of different species both in control cells and cells treated with ammonia. However, attenuation of .O2- by ANP and cANP(4-23) was observed in ammonia-treated cells only. In contrast to ANP, cANP(4-23) did not elevate the cGMP content in astrocytes, indicating the absence of its interaction with the NPR-A or NPR-B receptors, which are coupled to guanylate cyclase activity. However, cANP(4-23) decreased cAMP content and reduced the expression of Giα-2, the NADPH oxidase-regulatory protein. The results show the presence of functional NPR-C in astrocytes, activation of which i) attenuates basal ROS production, ii) prevents excessive accumulation of the toxic ROS species, .O2- by ammonia. Ammonia, ANP and cANP(4-23) added separately, each stimulated formation of NO (nitrates+nitrites). However, the effect of ammonia on NO was not augmented by co-addition of ANP, and was reduced

to the control level by co-addition of cANP(4-23), indicating that activation of NPR-C may also reduce nitrosative stress. Preliminary results indicate that cANP(4-23) attenuates ammonia-induced cell volume increase of astrocytes, which indicates the potential future use of cANP(4-23) or other NPR-C agonists in HE therapy.
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SII-P5
Disturbed regulation of the rat middle cerebral artery during hypo-osmotic hyponatremia

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Objectives: Hyponatremia is diagnosed in approximately 30% of neurosurgical
patients after head trauma, subarachnoid or intracerebral haemorrhage. It is well documented that hyponatremia deteriorates the general state of the patients, aggravates brain damage and increases mortality. Its influence on the regulatory mechanisms of cerebral blood vessels is unknown. The aim of our experiments was to study the impact of acute and chronic hyponatremia on the regulation of the isolated middle cerebral artery (MCA) of the rat.
Material and Methods: Seventy five vessels were studied in the organ chamber.
Acute hyponatremia (AH, 120mM Na+) was induced in vitro 1 hour prior to the study of MCA reactivity. Chronic hyponatremia (CH) was induced in vivo with a help of vasopressin and liquid diet (AIN-76). Vasopressin was delivered continuously from subcutaneously implanted ALZET osmotic minipumps. After 3.5 days, plasma Na+ concentration ranged from 114 to 122 mM in these rats. MCAs were isolated and studied in the organ chamber containing 120 mM Na+ buffer. MCAs placed in normonatremic bath (Na+=144 mM) served as a reference group. The reactivity tests comprised responses to acidosis (pH=7.0), to hyperkalemia (20 mM K+) and to changes in perfusion pressure.
Results: In normonatremia MCA dilated on average by 19±2% ($p<0.05$) during decrease of pH from 7.4 to 7.0 and by 30±3% ($p<0.05$) during increase of K+ concentration in the bath from 3.5 to 20 mM. Hyponatremia impaired reactivity of the MCA to both acidosis and hyperkalcemia. There were differences in the severity of this impairment depending on the duration of hyponatremia. During AH decrease of pH induced constriction of the MCA by 13±3% ($p<0.05$) whereas during CH constriction was only 4±1% ($p<0.05$ vs. AC and normonatremia). The impairment of the response to 20 mM K+ (constriction by 18±2%, $p<0.05$ vs. normonatremia) was observed only during AH. The response to changes in perfusion pressure was well preserved both during AH and CH.
Conclusion: Our results demonstrate for the first time that hyponatremia selectively disturbs the regulatory mechanisms of cerebral blood vessels. They also show that acute hyponatremia impairs regulation of the MCA more than chronic hyponatremia.
In conclusion, vascular impairment may be an important component of intracranial pathology during hyponatremia.
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SII-P6
STIM1 and STIM2 behave differently in neurons during store operated calcium entry

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Store Operated Calcium Entry (SOCE) is a common phenomenon in non-excitable cells. The process relies on extracellular calcium influx through the plasma membrane (PM) channels, tightly regulated by endoplasmic reticulum (ER) calcium concentration. This influx allows refilling of the ER after Ca2+ release to the cytoplasm. The proteins involved in this process are calcium sensors STIM1 and STIM2 (located in ER), and calcium channel forming protein called ORAI1 (located in PM). Complexes of the STIM proteins with ORAI1 were identified in the fluorescent microscopy and called \"puncta\". In neurons the molecular mechanism of SOCE is unclear. Our previous research led to the identification and characterization of STIM1 in the brain and neurons (Acta Neurobiol. Exp. 2009, 69:413-28; Neurochem. Int. 2009, 54:49-55). In this study we found that also STIM2 is expressed in neurons and aimed our work to compare the function of STIM proteins. In cultured cortical neurons, overexpressing YFP-STIM1/YFP-STIM2 and ORAI1, we observed changes of the fluorescence distribution from dispersed before to aggregated complexes of STIM1-ORAI1 and STIM2-ORAI1 after treatment with thapsigargin (TG). We also found that depletion of calcium from ER increased the number of STIM1-ORAI1 puncta much more than of STIM2-ORAI1 puncta. Then we analyzed the effects of STIM1/ORAI1 or STIM2/ORAI1 expression on intracellular calcium level during SOCE using Ca2+ imaging in two types of experiments. The first one was an analysis of SOCE after depletion of intracellular Ca2+ stores by TG and subsequent incubation of cells in 2 mM Ca2+ media. These measurements were performed also in the presence of SOCE inhibitors (ML-9 or 2-APB). SOCE was enhanced in neurons transfected with STIM1/ORAI1, but not with STIM2/ORAI1. Moreover, both inhibitors reduced calcium influx by about 70% in neurons expressing STIM1/ORAI1, while produced no significant change in neurons transfected with STIM2/ORAI1. In the second type of experiments a removal of extracellular Ca2+ caused a sustained decrease in intracellular calcium in all experimental setups, however the highest decrease was observed in neurons transfected with STIM2/ORAI1. In store-repleted cells, an increase in constitutive Ca2+ entry was observed with STIM1/ORAI1 and STIM2/ORAI1 expression, but not with STIM expres-

sion alone. STIM2/ORAI1-mediated constitutive Ca2+ level was raised by 50 μM 2-APB, but not in case of STIM1/ORAI1 transfectants. Based on these observations we suggest that in neurons STIM1 and STIM2 proteins have distinct role in SOCE.

SII-P7

Nuclear β-catenin is constitutively present in postmitotic thalamic neurons due to reduced activity of β-catenin degradation complex

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Wnt activation promotes β-catenin accumulation upon inhibition of β-catenin degradation. Stabilized β-catenin translocates to the nucleus where it triggers transcription of the Lef1/Tcf target genes. Wnt/β-catenin signaling is essential for nervous system development as well as division and maturation of neuronal progenitors in adult brain. We showed recently that nuclear β-catenin is abundant in vivo in non-dividing neurons of adult thalamus, where it is involved in gene transcription of CACNA1G gene (Wisniewska et al, J Neurosci, 2010). Here we demonstrate spontaneous accumulation of β-catenin in 40% of cultured thalamic neurons and lack of such accumulation in cortical neurons. This phenomenon does not depend on soluble factors produced by glia or cortical neurons, since neither conditioned medium of cortical cells nor glial cells co-culture affect the number of β-catenin positive cells. This suggests that nuclear localization of β-catenin in thalamic neurons is not a consequence of paracrine stimulation. We also observed that Wnt signaling inhibitor DKK1 had no major effect on the number of β-catenin positive thalamic neurons. Thus, autocrine Wnt stimulation is not responsible for nuclear β-catenin accumulation in these neurons. We analyzed expression of APC, AXIN1 and GSK3β that are involved in degradation of β-catenin and detected lower level of APC and GSK3β in thalamus when compared to other brain regions. Our observations support an idea that β-catenin accumulation is an intrinsic feature of thalamic neurons, independent on cellular environment of thalamic neurons and on Wnt stimulation. We propose that accumulation of β-catenin in thalamus is a result of reduced β-catenin degradation rate.
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SII-P8

Competitive interactions between N-acetyl-L-aspartate and acetylcholine for acetyl-CoA in cholinergic neuroblastoma cells?

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Cholinergic neurons like the other ones may synthesize N-acetyl-L-aspartate (NAA), which serves as a source for acetyl units for lipid synthesis in oligodendroglial cells. Pyruvate-derived acetyl-CoA is a substrate for NAA synthesis in aspartoacylase reaction in the mitochondrial and cytoplasmic compartments of neuronal cells. Highly differentiated cholinergic neurons were found to be more susceptible to neurodegenerative signals due to relative shortage of acetyl-CoA in the former ones. It gives rise to hypothesis that in cholinergic neurons, NAA synthesizing pathway might compete for acetyl-CoA with acetylcholine synthesis and energy producing pathways in their cytoplasmic and mitochondrial compartments, respectively. Therefore, the aim of this work was to investigate whether neurotoxic conditions that inhibit acetyl-CoA synthesis alter interactions between acetylcholine and NAA metabolism in neurons of low and high expression of the cholinergic phenotype. The differentiation of SN56 cholinergic neuroblastoma cells with cAMP and retinoic acid caused 30% increase of NAA content and 100% elevation of intracellular acetylcholine content and its synthesis. Simultaneously, the decrease of mitochondrial and increase of cytoplasmic acetyl-CoA levels were observed, respectively. Inhibition of pyruvate dehydrogenase activity by amprolium-evoked thiamine pyrophosphate deficit, brought about concentration-dependent suppression of acetyl-CoA content both in mitochondrial and cytoplasmic compartments along with inhibition of acetylcholine synthesis/release. However, NAA content was affected by these conditions neither in nondifferentiated nor in differentiated cells. On the other hand, acetylcholine synthesis was stronger inhibited in differentiated than in nondifferentiated cells. Zinc (0.15 mM) and L-aspartate (1.0 mM) increased NAA level but inhibited acetylcholine synthesis and decreased cell viability and their acetyl-CoA content. These alterations were more evident in differentiated than in nondifferentiated cells. These data indicate that NAA metabolism may compete with acetylcholine sythesis for common precursor - acetyl-CoA thereby negatively affecting their chance for survival in different neurodegenerative conditions. Work was supported by MNiSW projects NN401233333, NN401029937 and GUMed St57 fund.

SII-P9

Voltage-gated potassium channel in hippocampus mitochondria.

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Transient cerebral ischemia is known to induce endogenous adaptive mechanisms such as the activation of mitochondrial ATP regulated or Ca2+ regulated large conductance potassium channels that can prevent or delay neuronal injury. However, molecular mechanism of this effect remains unclear. In this study, a single channel activity was measured with patch-clamp of the mitoplasts isolated from gerbil hippocampus. In 70% of the all patches, a potassium selective current with properties of the voltage-gated potassium channel (Kv type channel) was recorded with mean conductance 109 ± 6 pS in symmetrical 150 mM KCl solution. Detected channel

was blocked by negative voltage and margatoxin (MgTx) a specific Kv1.3 channel inhibitor. The inhibition by MgTx was irreversible. We observed that ATP/Mg2+ complex or Ca2+ ions had no effects on observed activity of ion channel. Additionally, we showed that agitoxin-2 (AgTx-2), potent inhibitor of the voltage-gated potassium channels, was without effect on channel activity. This observation suggests that mitochondrial voltage-gated potassium channel can represent different molecular structure without affinity to AgTx-2 in compare to surface membrane channels. Also, Western blot analysis of mitochondria isolated from gerbil hippocampus and immunohistochemistry on gerbil brain sections confirm the expression of Kv1.3 protein in mitochondria. All together, we conclude that gerbil hippocampal mitochondria contain voltage-gated potassium channel (mitoKv1.3 channel) with properties similar to the surface membrane Kv1.3 channel which can influence function of mitochondria in physiological and pathological conditions.
This study was supported by the grant from the Ministry of Science and Higher Education (P-N/31/2006) and Polish Mitochondrial Network MitoNet.pl. P.B. would like to acknowledge to prof. Detlef Siemen for teaching patch-clamp and more discussion during postdoc in Magdeburg supported by EMBO (2006) and DAAD (2008).

SII-P10

Glial expression of purinergic P2X7 receptor in rat brain during the course of experimental autoimmune encephalomyelitis

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Multiple sclerosis is a common neurodegenerative disease with prevalence in Poland about 15 per 10000 people. It is characterized by inflamed lesions in myelin sheaths surrounding axons in the white matter of the brain and spinal cord. These changes lead to the damage of axons and, in consequence, to a broad spectrum of neurological symptoms. Experimental autoimmune encephalomyelitis (EAE) is the well known and commonly used animal model of MS.
In the present study the temporal pattern of glial activation (microglia and astroglia) together with P2X7R expression were investigated in brain of Lewis rats during the course of EAE. This receptor, activated under pathological conditions, may participate in the regulation of inflammatory response and cell death. It was shown to induce the release of inflammatory mediators like Il-1b and TNF-a in different types of glial cells. It is also pathologically involved in the release of potentially cytotoxic substances like glutamate and ATP. Western blot analysis was used to assess the relative concentration of P2X7R protein in glial fraction whereas its cellular localization was studied by immunohistochemical method. Tissue was labeled with the specific markers (Iba1 - microglial marker, GFAP - astroglial marker) and examined in the different stages post immunization (2, 4, 6, 8, 10 days). We observed the early overexpression of P2X7R protein (2-4 d.p.i.) in glial fraction obtained from brains of EAE rats with parallel enhancement of glial markers. Double immunofluorescent labeling showed colocalization of the receptor with glial markers. The results revealed that activation of both astroglia and microglia takes place very early post immunization, well before the neurological symptoms of the disease occur and is temporary connected with the overexpression of P2X7R. This suggests the involvement of P2X7R-mediated signals into the early pathological mechanisms operating during the disease.

SII-P11

The effect of glutamic acid, N-acetylcysteine, ebselen in oxygen-glucose deprivation conditions on signaling kinases and structural and functional proteins of rat brain endothelial cells in vitro

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The expression of signaling kinases and specific junctional proteins (VE-cadherin, occludin, claudin, JAM-1) and transport proteins (MDR-1) were measured in rat's brain endothelial cells cultured in vitro and exposed to simulated ischaemic conditions (OGD, oxygen-glucose deprivation). The effects of ebselen, N-acetylcysteine and glutamic acid were evaluated either in normoxic or simulated ischaemic conditions. Endothelial cells isolated from the fragments of rat brain microvessels seeded on the cell culture plates were cultured at 37^{0}C in Dulbecco's modified Eagle's medium containing 20% fetal bovine serum, antibiotics and bFGF. OGD was obtained by incubation of cells in humidified atmosphere (3%O_2, 92% N_2, 5% CO_2) in culture medium deprived of glucose and serum. Confluent cultures of endothelial cells were exposed to: 5000µM glutamic acid, 200µM N-acetylcysteine or 20µM ebselen. Intracellular signaling kinases (Akt, ERK1/2) and junctional and transporter proteins (VE-cadherin, occludin, claudin, JAM-1 and MDR-1) were detected using Western-blot. Ischemic conditions exerted negative effects on tight junctions of brain endothelial cells. ERK kinases were involved in the transduction of signals induced by ischemic conditions or by glutamic acid. Glutamic acid receptors were involved either in activation or inhibition of this pathway. In simulated ischaemic conditions ebselen as well as ebselen in combination with N-acetylocysteine exerted negative effects on intercellular junctions between brain endothelial cells. Antiooxidant compounds: N-acetylocysteine and ebselen affected MDR-1 level in brain endothelial cells.
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SII-P12
α,β-Hybrides of opioid peptides analogues

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Endogenous opioid peptides like enkephalins endomorphin, dermorphin and deltorphins are model peptides for the development of new analgesic drugs. Since the discovery of the endogenous opioid peptides, numerous analogues have been synthesized in attempts to develop an analgesic without the serious side effects. A major problem with opioid peptides as drugs is their susceptibility to enzymatic hydrolysis when administrated *in vivo*. Different chemical approaches, as the incorporation of D-amino acids, unnatural amino acid, α,α-disubstituted amino acids, cyclization, have resulted in more stable analogues. Among the numerous suggestions for modification the substitution of β-amino acids for proteinogenic amino acids represents an interesting possibility. ²-Peptides, oligomers of ²-amino acids are a very actual subject of research. β-Peptides do not bind to the active sites of human peptidases and they are entirely stable against proteolytic degradation. On the other hand β-peptides can mimic α-peptides. This was demonstrated that small β-peptides with their strong folding preferences may be used as pharmaceutically active compounds.
In this communication we report the effect on receptor binding single replacements in β^3-hybrides of deltorphin I, μ-selective ligands, tetrapeptide Tyr-D-Ala-Phe-Phe-NH_2 (TAPP) and biphaline. *N*-protected β^3-*homo*-amino acids were synthesized using procedures reported in the literature. α-Amino acids are enantiomerically pure, commercially available compounds which have frequently used as starting materials for synthesis of β^3-*homo*-amino acids. Isomeric optically pure Fmoc and Boc β^3-*homo*-amino acids were prepared in two-step *Arndt-Eistert* homologation of *N*-protected amino acids.
The α,β-hybrides of DT I were synthesized by manual solid-phase peptide synthesis (SPPS) using standard techniques. The α,β-hybrides of tetrapeptides and biphalin were synthesized in solution. The opioid analogues containing β^3-*homo*-amino acids were for μ and δ-opioid receptors affinity.

SII-P13
Synthesis and cell cancer growth inhibiting properties of somatostatin analogs

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Somatostatin (SST) is a tetradecapeptide that was originally characterized as a physiological inhibitor of growth hormone. In addition, SST has another multiple functions. It regulates endocrine and exocrine secretion, possesses antiproliferative properties and acts as a neurotransmitter/neuromodulator. These diverse physiological effects are mediated by a family of G-protein-coupled receptors, called somatostatin receptors sst1 - sst5. Nowadays, there are several cyclic synthetic somatostatin analogues (eg octreotide, lanreotide), clinically used for cancer therapy and gastrointestinal disorders, that primarily interact with receptor sst2 and sst5.
Our goal is to synthesize a linear tetrapeptide which would have activity like somatostatin. The base of analogs' structures are, key for somatostatin activity, aminoacids residues like. Results of this experiments will be present on the poster.

SII-P14
Novel, highly antinociceptive hybrid compound in acute pain

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The clinical treatment of various types of pain relies upon opioid analgesic, however most of them produce, in addition to the analgesic effect, several side effects such as development of dependence and addiction as well as sedation, dysphoria, and constipation. One of the solutions to these problems are chimeric compounds in which opioid pharmacophore is hybridized with other type of synergically active antinociceptor. Neurotensin-induced antinociception is not mediated through the opioid system. Therefore, hybridizing neurotensin with opioid element may result in a potent synergic antinociceptor.
The opioid-neurotensin hybride analogue PK20, in which opioid and neurotensin pharmacophores partially overlapped, expresses high antinociceptive tail-flick effects after central as well as peripheral applications. The antinociceptive effects likely are result of synergistic/additive interaction of hybridized opioid and neurotensin pharmacophores.

SII-P15
Genetic correlation between analgesia, metabolism and thermoregulation in a cross between mouse lines with high and low pain sensitivity

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Exposure of an animal to stressful stimuli elicits a transient decrease in pain sensitivity, which often affects thermoregulatory

mechanisms in the threatened organism. Briefly, we found that stress-induced analgesia (SIA) is inversely related to PMR (peak metabolic rate) and thermogenic capacity. This relationship was strong enough to permit the claim of an existence of a negative genetic correlation between SIA and PMR as well as thermogenesis. In the present study we performed a genome-wide quantitative trait locus (QTL) analysis in our mouse model established from outbred parental lines divergently selected for 60 generations for high- (HA line) or low- (LA line) swim stress-induced analgesia (SSIA) elicited by 3-min swim in 20°C water. The F2 population of 267 mice was used for the QTL analysis with 40 microsatellite markers distributed across the first five chromosomes. The analysis revealed significant QTLs SIA, for peak metabolism rate (PMR) and for hypothermia (Hyp). Some of these QTLs map to regions of known gene mutations influencing SIA, PMR and Hyp traits, moreover they map to regions of previously described QTLs and candidate genes. Apart from QTLs affecting single traits we identified QTLs common for examined traits (SIA-PMR-Hyp), (SIA-PMR), (SIA-Hyp) and (PMR-SIA) parameters, which explain phenotypic correlations between these traits

SII-P16

Stimulation of angiotensin AT2 receptor fails to alleviate blood pressure (BP) increase induced by high sodium intake in normal Wistar rats

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Selective ligands of both Ang II receptor types are needed to study their relative importance or possible interaction in the control of normal or elevated arterial blood pressure (BP); the knowledge of the role of AT2 is still limited.

We examined if a newly synthetised (A. Lipkowski) agonist of vasodilator AT2 receptors (LKP) would affect increase in BP (7 mmHg) which developed during a 10-day exposure of Wistar rats to high-salt diet (HS, 4% Na w/w).

With LKP treatment (48 mg/kg/24 h orally) BP increased more (31 mm Hg) than in untreated rats. With combined treatment: LKP + AT1 receptor antagonist (oral losartan (Los), 15 mg/kg/24 h; gift from Adamed Company, Pieńków, Poland), BP increased 19 mm Hg. At the end of studies the response of the total renal blood flow (RBF, Transonic probe) and cortical blood flow (CBF, laser-Doppler superficial probe) to intrarenal infusion of acetylcholine (Ach, 5-10 µg/kg/h) or norepinephrine (NE, 10-30 µg/kg/h) was determined. In untreated HS rats intrarenal Ach increased RBF 17%, whereas in HS+LKP and HS+LKP+Los groups it decreased RBF 1 and 2%, respectively (NS). LKP treatment did not modify decreases in RBF after NE but limited the decrease in CBF.

We conclude that stimulation of AT2 receptors did not effectively oppose the increase in BP elevation, which follows increased Na intake in Wistar rats. At high AT2 activity the renal vascular bed lost its ability to dilate, which suggests a state of substantial basal vasodilation; the ability to constrict was preserved. This suggests that intrarenal microvasculature is more responsive to AT2 stimulation compared to other peripheral vessels, as it is also more responsive to stimulation of vasoconstrictor AT1 species.

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SII-P17

Antihypertensive effects of simultaneous AT2 stimulation and AT1 inhibition in anaesthetised rats - mediatory role of renal medullary vasodilatation?

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Angiotensin II acts as vasoconstrictor via AT1 and as a vasodilator via AT2 receptors.

Selective ligands of both receptor types are needed to study their relative importance or possible interaction in the control of normal or elevated arterial blood pressure (BP); the knowledge of the role of AT2 is still limited. In this study effects of a newly synthetized (A. Lipkowski) tripeptide AT2 agonist (LKP) on BP and intrarenal haemodynamics (renal artery Transonic probe, RBF; cortical-, outer- and inner medullary laser-Doppler fluxes, CBF, OMBF, IMBF) were determined in anaesthetised Wistar rats, receiving background intravenous infusion of norepinephrine (NE) which raised BP to 130±2 mmHg and in non-pretreated spontaneously hypertensive rats (SHR). LKP infusion (120 µg/kg/h i.v.) decreased BP (-4%, $p<0.01$), which was associated with a 9% increases in OMBF ($p<0.05$) and IMBF (NS) in Wistar rats. Although NE-induced hypertension was expected to inhibit endogenous angiotensin II synthesis, additional AT1 inhibition (losartan, 1 mg/kg i.v.) further decreased BP and increased OMBF, and tended to increase RBF. In SHR LKP infusion tended to decrease BP (-3%), which was associated with nonsignificant increase in RBF and IMBF. These changes were augmented by AT1 inhibition (losartan) and reach statistical significance. The findings indicate that the levels of AT1 and AT2 activity can independently influence arterial pressure in acutely hypertensive rats. A striking association of BP decrease with an increase in medullary blood flow suggests that perfusion of the renal medulla has a direct causative role in the control of arterial pressure.

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SII-P18
NAC peptide evoked oxidative stress leads to p53 mediated apoptosis

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The non-Aβ component of Alzheimer's disease (AD) amyloid (NAC) is a highly amyloidogenic peptide consisting of 35 amino acids which was first identified associated with senile plaques in AD brain. It is a fragment of the presynaptic protein alpha-synuclein and, as such, it is implicated in the etiologies of both Alzheimer's and Parkinson's (PD) disease. However the molecular mechanisms of NAC toxicity is not fully understood. Our present study focused on the role of oxidative stress mediated p53 pathway in apoptotic cell death evoked by NAC peptide. Here we found that exposure of PC12 cells to exogenous NAC peptide (10 μM) enhanced free radical generation, induced mitochondria dysfunction and cell death. We also observed free radicals-dependent enhancement of Tp53 gene expression after NAC treatment. The inhibition of p53 by pifithrin significantly protected PC12 cells against NAC peptide - evoked mitochondria failure and death. In addition, exposure to NAC peptide resulted in the higher expression of cyclin-dependent kinase 5 (Cdk5), one of the enzymes responsible for p53 phosphorylation and activation. Concomitantly, we observed the increase of expression of Cdk5r1 and Cdk5r2 genes, coding p35 and p39 peptides, that are essential co-factors in regulation of Cdk5 activity. Moreover, the specific Cdk5 inhibitor (BML-259, 10μM) protected large population of cells against NAC-evoked cell death. Our findings indicate that NAC peptide exerts its toxic effect by activation of p53/Cdk5 - dependent apoptotic signaling pathway.
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SII-P19
Mangiferin - a useful antioxidant

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Mangiferin is a poliphenolic compound used in the traditional South American and Indian medicine as a panaceum with antibacterial, antitumor, antidiabetic, and antiviral activity. Its main source is the bark and leaves of Mango tree (Magnifera indica L.). Several studies indicate mangiferin antioxidant properties. The purpose of our experiments was to study the possible inhibitory effect of mangiferin on lipid peroxidation, as an expression of its antioxidative capacity. We used the TBA-lipid peroxidation model according to Ernster and Nordenbrand in plasma from rats treated and untreated with mangiferin in both in vivo and in vitro conditions. In the in vitro non-treated rat plasma the amount of peroxidated lipids was 1.114±0.132 μmol/l, the addition of mangiferin (50 μmol/l) diminished this value to 0.720 ±0.025 μmol/l, which is a significant 35% reduction in lipid peroxidation. In plasma from the mangiferin-treated rats (300 mg/kg, i.p.), the concentration of reaction products was at the level of 0.352±0.044 μmol/l; being 68% lower than in the untreated rats. We conclude that mangiferin is able to powerfully decrease lipid peroxidation. That indicates that mangiferin's broad bioactivity should also have to do with its antioxidant properties.

SII-P20
Stability of N-oleoyl-dopamine

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N-oleoyl-dopamine (OLDA) is a newly discovered endogenous lipid derivative of dopamine. It acts in a dopamine-like manner by, e.g., diminishing the respiratory response to hypoxia, enhancing the locomotor activity of the rat, or relaxing muscles in a reserpine model of Parkinson,s disease. In the context of being a potential prodrug or dopamine carrier in Parkinson's disease, the aim of our study was to establish OLDA's penetration into the brain after systemic administration and its stability in both in vivo and in vitro conditions. Thin layer chromatography and UV/VIS spectrometry techniques were used. We found that OLDA penetrates after i.p. injections into the brain, where it binds to membranes and stays stable for at least 24 h. In inorganic buffers its stability is comparable with those of dopamine. However, in rat brain membrane solution, OLDA remains unchanged for 17 h; lack of calcium ions prolongs this period to 24 h. In cytosolic solutions, OLDA is stable for over 24 h, regardless of the presence of Ca ions. Additionally, an intact rat brain membrane system protects OLDA from oxidation. In conclusion, N-oleoyl-dopamine is a highly stable, blood-brain-barrier penetrating, bioactive compound. OLDA's stability, penetration into the nervous tissue and dopamine-like actions suggest its being a potentially useful compound in treatment of diseases linked to central dopamine deficits.

SII-P21
Changes in mRNA expression of alpha1-adrenoceptor subtypes in brain of rats reactive to the chronic mild stress

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The chronic mild stress (CMS) procedure induces depression-like symptoms in animals. In this model of animals' depression, rats subjected for a prolonged period of time to a variety of mild stressors gradually decrease their responsiveness to rewarding stimuli (e.g., consumption of sweet pellets).

Cerebral alpha1-adrenoceptors (alpha1-AR) are known to be essential for behavioral activation in rodents and changed by stress conditions. Molecular and pharmacological studies revealed the existence of three subtypes of alpha1-AR, named alpha1A, alpha1B and alpha1D. These alpha1-ARs are widely expressed in the brain, though the functional differences among individual subtypes are not clear.
We aimed to investigate the expression of alpha1A-, alpha1B- and alpha1D-AR mRNAs in the thalamus, the hippocampus and the prefrontal cortex of rats subjected to the standard CMS procedure.
Three groups of male Wistar rats were selected based on behavioral test of sucrose (1 % solution) consumption and were considered in the molecular study: sham, stress reactive and stress non-reactive in the behavioral test. The expression of alpha1-AR mRNAs was measured by quantitative real-time PCR method with the use of TaqMan probes.
We found that CMS procedure differently affected the expression of the alpha1-AR mRNAs and the changes were brain structure- and receptor subtype-dependent. No changes in expression of three subtypes of alpha1-AR mRNAs was found in the prefrontal cortex. In the thalamus of rats that developed anhedonia to sucrose consumption after the CMS, the expression of all alpha1-AR subtypes was significantly attenuated: the alpha1A mRNA was decreased by 52%, alpha1B - by 55% and alpha1D - by 57% ($p<0.05$) in the stress reactive animals, while no change in the alpha1-ARs expression was observed in the stress non-reactive animals. In the hippocampus, an opposite direction of change was observed and the effect was limited to the alpha1B-AR mRNA which was increased in the stress reactive group of animals (by 128 %, $p<0.5$, vs. sham group). Similarly to the thalamus, the alpha1-ARs level in the hippocampus was unchanged in the group of stress non-reactive animals.
Our results indicate that CMS induces changes in all subtypes of thalamic alpha1-ARs and suggest the significant impairment of noradrenergic transmission in the thalamus of stress reactive rats. In addition, the hippocampal alpha1B receptor seems to be specifically involved in the phenomenon of response of animals to CMS.
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SII-P22

Does low-threshold electrical stimulation of muscle afferent fibers affect expression of the pool of neurotrophin 3 in the lumbar spinal cord of the rat?

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Availability of brain-derived neurotrophic factor (BDNF) and neurotrophin 4 (NT-4) in the nervous system depends on the neuronal activity. We have previously shown that moderate locomotor exercise causes an increase of BDNF and NT-4 proteins but not neurotrophin 3 (NT-3) in the lumbar spinal cord. The questions arise whether NT-3 is regulated in a different way than BDNF and NT-4 or, that proprioceptive stimulation during treadmill locomotion is not sufficient to activate NT-3? To verify the latter possibility we applied direct electrical stimulation of the tibial nerve to activate the low-threshold muscle afferent fibers eliciting monosynaptic Hoffmann (H) reflex, an analog of the stretch reflex. Both the H-reflex and direct motor response (M), recorded from the soleus muscle, allowed keeping the strength of stimulus near the threshold of M response, confirming that stimulus is primarily addressed to low-threshold afferents. The cuff stimulating electrode over the nerve and recording intramuscular electrodes were implanted bilaterally. The tibial nerve was stimulated unilaterally, throughout one and four weeks, starting two weeks after implantation of electrodes. The contralateral limb served as a control. Two sessions of stimulation daily, 30 min each (rectangular pulse of 300 µs duration at 0.33 Hz) were separated by about 2 h break. The total number of stimuli delivered was about 800 per day. The expression of NT-3 in the spinal cord was evaluated immunohistochemically (IR, Santa Cruz antibody) in the lumbar L4/L5 segments. Both neuropil and numerous cell bodies expressed NT-3. Perikaryonal expression was predominantly observed in the lower dorsal horn laminae (III- VI), in the intermediate zone and in the lamina IX. However, the effects of implantation and stimulation on the expression of NT-3 was negligible. The effect will be verified quantitatively with Elisa method.
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SII-P23

Susceptibility to electroshock-induced seizures in rats following neocortical injuries at different developmental stages

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Brains injured at different developmental stages may acquire different susceptibility to epileptiform activities. Epileptogenesis appears to be triggered by age-dependent reactive processes including gliosis and formation of aberrant axonal connections in the tissue surrounding the lesion site. The present study focuses on relations between brain injuries at different developmental stages and subsequent susceptibility to seizures in adulthood.
In 6- and 30-day-old Wistar rats (P6s and P30s, respectively), a mechanical injury was performed in the left cerebral hemisphere. From the postnatal day 60, the injured rats and non-injured controls underwent 21 daily electrical stimulations to evoke seizures.
Tonic and clonic reactivity to electric stimulation in P6s and P30s showed considerably different profiles contrasting with those previously observed following pilocarpine or kainic acid administration in the same experimental paradigm. In P6s intensity of tonic seizures was significantly higher than that in controls or in P30s while clonic seizured revealed no intergroup difference. The results proved that the observed phenomena depended on the model used for experimental exploration of the problem but their structural determinants remain obscure.

SII-P23

***In vivo* transduction of spinal cord cells in adult, spinalized rats, with adeno-associated viral vectors: long-term efficiency and specificity of $AAV_{1/2}$-hSYN and AAV_5-mCMV, encoding eGFP**

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We compared the efficiency and specificity of *in vivo* transduction of spinal cord cells in adult, spinalized rats, with adeno-associated viral vectors: $AAV_{1/2}$ and AAV_5, with human synapsin (hSYN) and murine cytomegalovirus (mCMV) promoters, respectively. Both AAV vectors carried eGFP transgen, and were injected bilaterally to the lumbar L1 segment immediately after spinal transection at the Th10/11. At 5-6 weeks postlesion (1) the distribution and extent of eGFP expressing cells and fibers and (2) their phenotype (immunohistochemical identification; IHC) were determined. To evaluate virus expansion we compared distribution of eGFP signal at the microscopical reconstructions (parasagittal sections). A comparison between serotypes showed, that caudorostral range of cells expressing eGFP was comparable ($AAV_{1/2}$ – 6.8 mm; AAV_5 – 8 mm), with a core of transduced cells ($AAV_{1/2}$ – 4.2/4.6 mm; AAV_5 – 3.4/3.8 mm), surrounding the injection site. Fibers emerging from $AAV_{1/2}$-transduced cells reached the lesion border, many of them entered the lesion and occasionally went across the scar, whereas fibers of AAV_5-transduced cells faded in a proximity of 300 μm to it. Dorsoventrally, cellular eGFP signal was detected in a gray matter of the subjects transduced with both serotypes, whereas only AAV_5 - mCMV transduced cells also in a white matter. Morphology of eGFP expressing cells indicated that both serotypes transduced interneurons and large neurons of Lamina IX. IHC documented that AAV_5 and, to a lesser extent, $AAV_{1/2}$, transduced cholinergic cells (VAChT), whereas none of the transduced neurons were GABAergic (GAD67) or glutamatergic (VGLUT2). AAV_5 transduced also glial cells, some identified as astrocytes (GFAP). In conclusion, both vectors efficiently transduce neurons in spinal animals; mCMV promoter drives eGFP expression also in glia.

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SII-P26

Analysis of possible allosteric modulation of opioid signaling

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Administration of classic orthosteric ligands of opioid receptors, like morphine, apart from inducing significant therapeutic effects such as analgesia, presents many disadvantages, with drug addiction and impairment of the breathing centre on the first place. However, allosteric modulation of these receptors could offer better selectivity among receptor subtypes and preservation of the physiological pattern of activation [1]. All in all, such compounds could bring more advantageous pharmacological profile, and decrease possibility of undesirable side effects.

The aim of work was to investigate the mode of interaction of the unique ligands [2-5] with MOR and DOR opioid receptors. As premises of allosteric pockets existence appeared, establishment of a possible mode of interaction between ortho- and allosteric pocket became an consecutive aim.

Models of the receptors were obtained with the method of homology modelling, using β2-adrenergic receptor as a template. These models were initially verified by rigid docking of rigid opiod receptors' ligands (SIOM and naloxone). Best protein models were chosen for flexible dockings.

Analysis of the results revealed that investigated compounds could be bound into two different pockets on the extracellular receptor's surface near the orthosteric pocket. Location of those hypothetical binding sites suggests, that their interaction with ligands could significantly modulate function of the receptor. The hypothetical pocket located between extracellular parts of TM1, TM2 and TM7 seems to share important amino acids with orthosteric pocket. Moreover, the other hypothetical binding site is located in ECL2 region, and seems to be analogous to allosteric binding site discovered in muscarinic M2 receptor structure [6].

[1] May, L. T.; Christopoulos, A. Curr. Opin. Pharmacol. 2003,
[2] Matosiuk, D.; Fidecka, S.; Antkiewicz-Michaluk L.; Dybała, I.; Kozioł, A. E, Eur. J. Med. Chem. 2001,
[3] Matosiuk, D.; Fidecka, S.; Antkiewicz-Michaluk, L.; Lipkowski, J.; Dybala, I.; Koziol, A. E. Eur. J. Med. Chem. 2002,
[4] Matosiuk, D.; Fidecka, S.; Antkiewicz-Michaluk, L.; Dybala, I.; Koziol, A. E. Eur. J. Med. Chem. 2002,
[5] Sztanke, K.; Fidecka, S.; Kędzierska, E.; Karczmarzyk, Z.; Pihlaja, K.; Matosiuk, D. Eur. J. Med. Chem. 2005,
[6] May, L. T.; Avlani, V. A.; Langmead, C. J.; Herdon, H. J.; Wood, M. D.; Sexton, P. M.; Christopoulos, A. Mol. Pharmacol. 2007,

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SII-P27

Molecular mechanism of interactions in human $NPFF_2$ - NPAF complex

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The NPFF system represented by two receptors - $NPFF_1$ and $NPFF_2$, belongs to the class A (rhodopsin-like) G protein-coupled receptors.

These receptors are specifically activated by different peptides arising from two precursors proNPFF$_A$ and proNPFF$_B$, sharing a common PQRFamide C-terminus. Structure–activity relationship studies have revealed that NPFF$_2$ receptors exhibit highest affinity for oligopeptides derived from proNPFF$_A$ precursor, such as neuropeptide FF itself and related peptides, as well as NPAF related peptides [1]. These peptides modulate the opioid system by exerting functional anti-opioid activity on neurons, but the mechanism of it is still unknown.
Fluorescence resonance energy transfer (FRET) and co-immunoprecipitation studies suggests an association between NPFF and MOP receptors. Moreover, NPFF$_2$-MOP receptor heterooligomeric complex exists at the basal level and is differently modulated by NPFF and opioid agonists: the neuropeptide FF analog 1DMe promotes NPFF$_2$-MOP receptor association, whereas the opioid agonist DAMGO disrupts it [2].
Thus, the aim of work was investigation of the molecular mechanisms of interactions in human NPFF$_2$ - NPAF complex.
For that purpose 3D models of human receptor NPFF$_2$, as well as NPFF$_2$ receptor endogenous selective peptidic ligand NPAF (AGEGLNSQFWSLAAPQRFNH$_2$) was obtained. For modeling of the receptor protein homology modeling with YASARA STRUCTURE was used. For generating peptidic ligand structure experimental investigations on the neuropeptide AF structure [3] and *de novo* modeling method, as well as homology modeling were applied. In the following step molecular docking of the final model of ligand was performed with use of available tools: CLUSPRO and ROSETTADOCK. Complex of the ligand with the receptor protein was placed in a lipid bilayer and subjected to MD simulations.
Procedure use allowed identification of the potential mechanism of interaction in the receptor – peptidic ligand complex. The results will be used to investigate the molecular mechanisms of interaction in human NPFF$_2$ - MOP$_1$ heterooligomeric complexes as an effect of the endogenous ligand of NPFF$_2$ receptor binding.
REFERENCE
[1] Talmont, F.; Garcia, L.P.; Mazarguil, H.; Zajac, J.M.; Mollereau, C. *Neurochem. Int.* 2009, 55, 815
[2] Roumy, M.; Lorenzo, C.; Mazères, S.; Bouchet, S.; Zajac, J.M.; Mollereau, C. *J Biol Chem*. 2007, 282, 8332
[3] Miskolzie, M.; Kotovych, G. *Biopolymers*. 2003, 69, 201

Session III
DIFFERENT TYPES OF SOMATIC STEM CELLS; IMPLICATIONS IN NEUROLOGICAL DISEASE THERAPY

SIII-L1
"Key to Immortality" - the potential role of adult pluripotent stem cells (VSELs) in tissue and organ regeneration

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The main purpose of regenerative medicine is to improve irreversible damage of organs including brain and neural tissues by harnessing the capacity of stem cells in process of tissue repair and renewal. Several types of stem cells including mesenchymal stem cells, hematopoietic stem cells as well as neural cells differentiated from embryonic stem cell lines have been postulated as potential source of therapeutical cells.
Recently, it has been found that murine bone marrow (BM) contains a mobile population of Oct-4+CXCR4+SSEA-1+Sca-1+Lin-CD45- very small embryonic like stem cells (VSELs) that may be mobilized into peripheral blood due to the tissue injury including stroke. The number of these cells in circulation may be also efficiently increased after pharmacological mobilization such as administration of granulocyte colony stimulating factor (G-CSF).
VSELs have been also identified in other adult tissues and organs and interestingly, the highest number of cells resembling VSEL phenotype was found in brain tissue. Recent molecular studies investigating the genetic and epigenetic status of VSELs indicate that these cells represent a mobile population of epiblast/germ line derived stem cells and play an important role as organ-residing reserve population of pluripotent stem cells that gives rise to stem cells committed to particular organs and tissues - including neural tissue. Moreover, a similar population of very small CXCR4+CD133+CD34+SSEA-4+Oct-4+Lin-CD45- cells resides also in human bone marrow and umbilical cord blood. Such population may be also detected in peripheral blood of patients with acute myocardial infarction, stroke, unhealed wound as well as suffering with tumors, which injuries and diseases may trigger mobilization of VSELs into blood. It has been shown that VSELs reveal the regenerative potential when injected into injured tissues in vivo, in experimental model of heart ischemia and reperfusion.
We conclude that VSELs which exhibit pluripotent characteristics, are mobilized to blood due to a tissue injury and possess regenerative capacity in injured tissues, may represent promising population for future applications regenerative medicine including novel therapies for brain injury treatment.

SIII-L2

Biomimetic modulation of cord blood derived stem cells for neural fate control

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Bioactive surface domains were applied to investigate cellular developmental processes of human cord blood- derived stem cells and to direct their fate into desired neural lineages. Such domains should represent microenvironmental cues resembling those found in vivo. For that purpose we have created miniaturized cell growth platforms with defined arrays of cell attractive biomaterials serving as functional domains. Emerging technologies applied included a nano/micro-fabrication technique like microcontact printing and piezoelectric (noncontact) microspotting of biomolecules on plas-

ma deposited cell repellent surface. Human Umbilical Cord Blood Neural Stem Cell (HUCB-NSC) line was plated on biodomains at different concentrations and serum conditions. HUCB-NSCs were shown to adhere and differentiate on microarray platforms in a protein type, concentration and cell density dependent manner. Receptor-mediated interactions with extracellular proteins promote neuronal differentiation, while non-specific adhesion to polyaminoacid molecules allows maintaining of stem cells immobilized to the surface in non-differentiated stage. "Smart" functional domains were created by immobilizing to the surface small signaling molecules (e.g wnt, shh, notch or jagged) together with ECM proteins. Stimulation of selected intracellular pathways by signaling molecules resulted in differentiation of HUCB-NSC to either neuronal or astroglial lineage. Miniaturization of such bioengineered active domains combined with appropriate stem cell model may allow application of such stem cell growth platforms for the multiparameter bio-tests and can provide important, additional information on the sensitivity of certain neural stem cell molecular pathways to the selected neurotoxins. Since HUCB-NSC can be cultured and harvested at different developmental stages and was shown to be a good model for developmental toxicity testing, homogenous lineage related pluripotent population is required. For that purpose iPs cells from HUCB-NSC are produced.
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SIII-L3
"Neural" differentiation of bone marrow mesenchymal stem cells

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Therapeutic benefits of bone marrow MSCs use in the neurological disorders exist. Although, currently there is not a clear understanding of the detailed mechanisms by which MSCs mediate neural recovery. Early studies suggested that MSCs injected into the lateral ventricles of developing animals differentiated into neural cell types. However, latter data showed that appearance of GFAP positive MSCs derivatives was consequence of cells fusion. Moreover, older data suggesting transdifferentiation of mesenchymal stem cells into neural derivatives is poorly supported by the current data. Many of articles suggesting neural differentiation of bone marrow mesenchymal stem cells appeared as based on controversial, or even inconsistent data. Surprisingly, authors analyzing putative neural differentiation of bone marrow MSCc do not present corresponding results of neural differentiation of neural progenitors. Moreover, markers of neural differentiation can be misleading. To this end, neural differentiation of bone marrow mesenchymal stem cells should be considered as controversial.

SIII-L4
Transplantation of human MSC in experimental neurodegenerative diseases

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Etiology of human neurodegenerative disorders such as Parkinson`s disease (PD), Huntington`s disease (HD) and motor neuron disease (ALS) is unknown. All are characterized by progressive death of specific neuronal populations that gives rise to their particular clinical phenotypes. The ever-present problem is how to prevent further neuronal attrition and how to repair and restore this loss of neurons. Cell therapy using stem cells could be such therapeutic solution but there are still many obstacles to overcome eg source of cells-embryonal or adult. We focused on adult mesenchymal stem cells derived from human bone marrow and can now isolate such cells, expand them to large numbers and using specially-devised \"cocktails\" induce their differentiation to astrocyte-like neurotrophic factors (such as BDNF and GDNF) producing cells (NTFs). We have now transplanted human NTFs in different targets in rats and mice with various experimental models for human disorders. These included unilateral 6-OH-DA striatal lesions and systemic MPTP for PD, transgenic HD mice, SOD-1 mutated mice transgenes for ALS, and rats with schiatic nerve injury. We have been able to attenuate the relevant behavioural impairments and also neuronal losses. We propose that such adult human bone marrow-derived and differentiated NTFs can become a novel autologous therapeutic strategy in neurodegenerative diseases in the forseeable future.
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SIII-O1
Potential role of MMP-2 and -9 in ischemia-stimulated neurogenesis in the gerbil hippocampus

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Matrix metalloproteinases (MMPs) are a growing family of zinc-dependent endopeptidases that are classically recognized as matrix-remodeling enzymes implicated in various physiological and pathological processes. Apart from relatively well established detrimental role of MMPs, in particular gelatinases (MMP-2 and MMP-9), following brain injury, MMPs have been considered recently to be involved in the neurogenic response of the adult neural stem/progenitor cells after ischemic challenge. However, the role of these enzymes in the neurogenesis still remains to be clarified. The goal of the present study was to elucidate if activation of MMPs parallels the rate of neural progenitor cells proliferation and/or further dif-

ferentiation. Our results show that post-ischemic acceleration in the proliferation and differentiation of progenitors in the dentate gyrus of the adult hippocampus coincides with the remarkable elevation of MMPs activity. On the contrary, in the ischemia-damaged CA1 pyramidal cells layer the activity of MMPs fell below the control level. It should be pointed out, that in this structure neurogenesis seems to be rather elusive, as we did not found evidence for production of a new matured neurons. In an effort to further check the potential participation of MMPs in neurogenesis-associated processes we have tested the effect of MMPs inhibitors (GM6001 and doxycycline) on neural stem cells line. We observed that the addition of these agents decreased the rate of proliferation and differentiation toward neurons.

In conclusion, the spatial and temporal profile of MMPs activity during reperfusion following transient forebrain ischemia suggest that these proteinases might belong to the discussed mechanism(s) which govern neurogenesis-associated processes in ischemic brain hippocampus.

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SIII-O2

MRI-monitored cord blood-derived cell transplantation to the ventricular system of child with global cerebral ischemia - a case report

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Introduction: Neurological disorders are the most common cause of serious disability and have a major impact on financial health-related burden to society. Most of them are definitely associated with cell death: sudden or chronic. Conventional treatment methods yield disappointing results. Thus the discoveries in stem cell biology have fueled the interest in cell-based therapeutical approach. Based on experimental data cord blood has been proposed as a novel, autologous cell source for pediatric population. Non-invasive monitoring of cell fate following transplantation has been recently recommended as a basis for rational stem cell therapy. Subject: One year old child experienced devastating, cardiac arrest-induced cerebral ischemia. Despite a broad rehabilitation program diagnose of vegetative state has been established three months later. After next three months of continued rehabilitation no noticeable improvement has also been found and the child has been included into study. The protocol has been approved by the ethical commission of The Children's Memorial Health Institute in Warsaw, Poland. Then the child's own cord blood cells have been neurally-converted over 10 days in culture within GMP facility. Prior to transplantation cells were labeled with iron oxide (SPIO) for MR imaging. For scaling sensitivity of MR signal different concentrations of SPIO-labeled cells were scanned in the phantom. Then patient received monthly 3 subsequent cell infusions (1.2 x 10^7 cells each) to lateral ventricles. The follow up continued up to 6 months and included both clinical assessment and MR examinations. Results: High efficiency of neural cell conversion and SPIO labeling as well as no cytotoxicity were observed. The employed method of cell transplantation was found to be efficient to deliver cells to CNS as confirmed by MR imaging. Gradual decrease of SPIO signal intensity was observed over the period of follow up. No adverse events or abnormal reaction to cell implantation was detected. The follow up revealed mild functional improvement - decreased nystagmus, spasticity and the number of epileptic seizures. Moreover, the features of the child contact with parents has appeared, thus vegetative state can not be diagnosed any more. Conclusions: This report indicates that transplantation of autologous, neurally-committed cord blood-derived cells to the ventricular system of child is safe, feasible and able to result with mild functional improvement. Additionally cell-related MRI signal can be monitored for more than 4 months in transplanted brain hemisphere.

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SIII-P1

Molecular and phenotypic characterization of mesenchymal stem cells derived from human umbilical cord

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Mesenchymal stem cells (MSC) are of clinical interest because of their potential use in autologous transplantation. The ability of MSC to differentiate into multiple different cells of mesodermal origin has offered therapeutic tool for the treatment of hematopoietic malignancies and graft versus host disease. Recently, MSC have been shown to ameliorate a variety of neurological dysfunction. This effect is believed to be mediated to their paracrine functions since it is known that MSC produce bioactive substances that promote endogenous neurogenesis. However, the critical question that remains unanswered is whether MSC can transdifferentiate into neural cells. To be "primed" toward a neuronal fate, MSC have to express neural antigens. In an attempt to clarify this issue we explored the constitutive expression of different markers by mesenchymal cells isolated from human umbilical cord Wharton jelly (HUC-MSC) and compare their expression with neural stem cell line derived from human umbilical cord blood (HUCB-NSC) established in our laboratory. *Materials and methods*: Gene expression pattern in HUC-MSC (passage 5 of cells cultured in MSCGM hMSC Lonza medium) and HUCB-NSC (cultured in DMEM/F12 medium+2% FBS) was performed by real time PCR and RT-PCR reactions. Total RNA was extracted using TRIzol (Invitrogen). Then cDNA was synthesized

from total RNA, using High Capacity RNA-to-cDNA kit (Applied Biosystems). PCR reactions were carried out using template cDNA in the presence of specific primers. Concomitantly immunocytochemical analysis of gene-related proteins was employed. *The results* of our studies have demonstrated that HUC-MSC, in addition to pluripotent (Oct3/4, Nanog1), mesenchymal (CD73, CD90, CD105, CD166) and extracellular matrix (Fibronectin, Vimentin, Collagen1) genes, spontaneously express neural genes i.e. Nestin, NF200, βIIITubulin, MAP2 and GFAP. The initially expressed neuroectodermal genes were comparable with mRNA level of the same neural genes in HUCB-NSC. In addition to neural genes non-induced expression of neural proteins was found. Subsets of HUC-MSC were positive for several neural markers, including: Nestin, NF200, βIIITubulin and GFAP. *Summary and conclusion*: We have demonstrated that MSC derived from human umbilical cord Wharton jelly acquire neural progenitor-like properties by expressing neuronal and astrocytic specific markers. However, it is of clinical interest whether transplanted MSC respond with an appropriate neural pattern of differentiation when exposed to the environment of the host brain.

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SIII-P2

Changes in pattern of DNA methylation in DE promoter region of Oct3/4 gene before and after neural differentiation of HUCB-NSC

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Umbilical cord blood is considered as a promising source of stem cells capable of self-renewal and differentiation into different cell types, including neural. Differentiation processes are governed by microenvironmental cues and by unique molecular mechanisms, where epigenetic changes of the chromatin play an important role. Emerging evidence suggests, that changes in expression of so called "stemness" gene, like Oct3/4, are associated with the specific epigenetic modifications of gene promoter. Methylation status of Oct3/4 and Nanog promoters correlates strongly with their ability to be expressed. The promoters are unmethylated in pluripotent stem cells, where those genes are expressed, and almost fully methylated in differentiated cells, where Oct3/4 and Nanog are silenced. The aim of the study was to analyze the DE (Distal Enhancer) promoter region's methylation pattern in Oct3/4 gene in HUCB-NSC (Human Umbilical Cord Blood - Neural Stem Cells) line comparing to hESC (Human Embryonic Stem Cells) and also changes caused by neural differentiation of HUCB-NSC. Materials and Methods. HUCB-NSC were cultured in serum free, low serum (2% FBS) and in differentiating medium containing dBcAMP (300 μM) in the density of $5x10^4$ cells per cm^2 in standard conditions. After 14 DIV DNA from harvested cells was isolated. Methylation status of gene DE promoter region was analyzed by sodium bisulfite reaction. To analyze sequence of obtained PCR fragment subcloning into pGEM-T easy vector and sequencing was performed (at least 10 individual clones). DNA of hESC was received from Prof. Dvorak laboratory in Brno. Results. Methylation pattern of Oct3/4 DE promoter region was changing along differentiation process. HUCB-NSC after neural differentiation revealed higher methylation status in promoter region than in undifferentiated cells. Those changes correlate with the expression of Oct3/4 gene.

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SIII-P3

Influence of low oxygen tensions on expression of pluripotency genes in HUCB-NSC

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The Embryonic Stem Cells (ESCs) are characterized by unlimited self-renewal ability and potential to differentiate into all cell types of the body. Those cells are derived from embryos which reside in 3-5 % oxygen environment. This hypoxic condition is physiologically normal not only for ES cells but also for many other types of stem cells, for example Neural Stem Cells. These observations suggest that hypoxic condition plays a very important role in the maintenance of cell stemness. It was also demonstrated that low oxygen tensions are preferential for maintenance of a highly proliferative, pluripotent population of hES cells. Stemness is regulated by Hypoxia Inducible Factors (HIF), which depend on oxygen tensions. HIF2A (HIF-2 alpha) is an upstream regulator of Oct4, which is the main transcription factor used by Yamanaka and his group to generate the first iPSCs (induced Pluripotent Stem Cells). It has been shown that knock-down of HIF-2 alpha or HIF-3 alpha but not HIF-1 alpha, leads to a decrease in the expression of Oct4, Nanog and Sox2, which are important stem cells markers.

In this study we are trying to find out the best oxygen conditions for HUCB-NSC (human umbilical cord blood neural stem cells), from which iPS cells will be generated. We investigated the difference between the level of expression of chosen genes in HUCB-NSCs cultured under atmospheric air (21% oxygen) and 5% oxygen (low oxygen tensions). The cells were cultured for two weeks in two incubators with two different oxygen concentrations. HUCB-NSCs were grown in medium containing: DMEM/F12, 1%ITS, 2%FBS, 1%AAS. For comparison of expression levels of Oct4, Sox2 and Nanog from two different oxygen environments Real-Time RT-PCR was used.

In summary, the cells from low oxygen conditions had higher expression of genes: Oct4, Sox2, and Nanog compared to that of cells cultivated under atmospheric air, which is in agreement with previous observations. These outcomes indicate, that the cells from 5%

oxygen conditions are the better source of cells for iPS generation than those which grow in 21% of oxygen. This is due to the higher endogenous expression genes of pluripotency what suggests possible easier generation of iPS cells and more efficient responses to reprogramming program. Thus in our further investigation on reprogramming of HUCB-NSC we will apply low oxygen conditions and epigenetic modifications in order to obtain iPS cells from HUCB-NSC cell line.
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SIII-P4
Generation of stable HEK293 cell lines expressing cell reprogramming factors

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Induced pluripotent stem cells (iPSCs) are the product of somatic cell reprogramming into an undifferentiated embryonic-like state. These pluripotent cells might adopt various phenotypes by means of bioengineering methods and therefore might serve for disease modeling, pharmaceutical screening and cellular replacement therapies. Transcription factors such as Oct4, Sox2, Klf4 and Myc play the crucial role in the cell converting. The aim of our study was to obtain the protein extracts for the purpose of cell reprogramming experiments. Methods The cells of HEK 293 (Human Embryonic Kidney) line (ATTC/CRL15-73) have been transfected by non-viral, HiFect method with the pCMV cDNA-9R-myc plasmid, coding one of the selected factors: Oct4, Sox2 or Klf4. After transfections, cells were cultured in low density for 2-3 weeks in the presence of neomycin to select the resistant (i.e.transfected) colonies. The expression of c-myc as a marker of stable transfectants was determined by western blot analysis. The overexpressed reprogramming proteins were gently extracted with non-denaturating CellLytic buffer supplemented with protease inhibitor cocktail and stored for the future application. Results. Isolation and propagation of an individual cells from neomycin-resistant colonies allowed us to obtain about 20-30 clones for each transcription factor. The c-myc positive clones have been selected for further in vitro culturing with the purpose of continual generation of Oct4, Sox2 or Klf4 proteins. Conclusions. The presented study resulted in successful generation of stable HEK293 cell lines that could express each of the three human reprogramming factors fused with the myc tag and with polyarginine (9R) to facilitate intracellular trafficking. The extracted proteins might therefore be used in induction of cell reprogramming experiment with the aim of generating IPSCs for potential neurorestorative therapies.
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SIII-P5
Heterogeneity of the local tissue microenvironment influences differentiation of NG2 cells

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The NG2 cells are the oligodendrocyte precursors that terminally differentiated are capable for myelination of central nervous system (CNS). They exhibit many features of neural stem cells and constitute the abundant population of dividing progenitors in the young and adult brain. A question arises if their commitment could be modulated by local tissue-specific or neuropathological signals. The aim of our study therefore was to evaluate the effect of distinct microenvironments (provided by either the spinal cord or the hippocampal slices) on the differentiation of neonatal NG2 cells. Subsequently, hippocampal slice culture subjected to an ischemic injury (the glucose-oxygen deprivation, OGD) was used in order to evaluate the cell development in microenvironment conditioned by traumatized tissue. Methods. Both the hippocampal and spinal cord slice cultures were established from the same 7-day old rats. The model of an indirect contact (i.e. exclusively by the culture media) in co-culture system was chosen to eliminate the influence of cell-cell contact. The NG2 cells were obtained from 10-day old mixed primary culture of neonatal rat hemispheres. After 7 days in co-culture, the cells were either stained with neural markers or collected for the RNA isolation and real-time PCR. Results. The medium conditioned by hippocampal slices effectively promoted neurogenesis: ~30 % of NG2 cells differentiated into TUJ 1-positive neurons. The remaining fraction mostly formed premyelinating and mature oligodendrocytes. The exposition of hippocampal slices to the OGD injury abolished the effect of pro-neuronal induction in co-cultures. In media conditioned by spinal cord slices, neurogenesis was less pronounced (20% neurons) and the oligodendrocyte differentiation was significantly slowed-down. Conclusions. The NG2 cells were shown to have intrinsic potency for neurogenesis. Heterogeneity of local microenvironment might modify the fate of endogenous or transplanted NG2 cells what should be taken into consideration in potential neurorepair strategies.
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SIII-P6
Transplantation of human cord blood-derived neural stem cells modify endogenous ischemia-induced neurogenesis in post-stroke rats

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Studies in experimental stroke demonstrate that cerebral ischemic injury promotes neurogenesis in the subventricular zone (SVZ) and

subgranular zone (SGZ) of the dentate gyrus. Spontaneously occurring injury-induced neurogenesis is insufficient to fully reverse disease pathophysiology. Exogenous neural progenitors transplanted into damaged brain might be useful for facilitating the repair of damaged tissue by instructing several endogenous processes. However, the molecular and cellular mechanisms stimulating cell proliferation and mediating the migration of arising neuroblasts towards the ischemic boundary still remain to be characterized. Building evidence suggests that matrix metalloproteinases (MMPs) seem to play a role in neurogenesis-associated processes, providing an environment which may be instructive or permissive to stem cells activation. The overall goal of our present studies was to examine whether HUCB-NSC transplantation modulates migration of endogenous progenitor cells and MMPs activity in adult rat brain after focal ischemia. Methods: $2x10^4$ neural stem cells from human cord blood (HUCB-NSC) were transplanted into corpus callosum of naive or focally injured (induced by 1µl/50nmol OUA injection) rat brain. At 1, 3, 7 and 14 days rat brains were removed. Then immunocytochemical analysis of doublecortin (DCX) (marker expressed by immature migratory neuroblasts) and in situ zymography of MMPs activity was performed. Results: OUA-induced brain lesion resulted in increase of DCX+ cells in SVZ and SGZ in comparison to intact rats. This response has been potentiated by HUCB-NSC transplantation. Moreover, the activation of MMPs in cells was visible in SVZ. At 7th day after HUCB-NSC transplantation the intense migration of DCX+ cells from SVZ towards ischemic boundary regions of the striatum was observed. Double-labeling showed co-localization of DCX marker with MMPs activity. The presence of MMPs appeared to be associated with cell nuclei and cytoplasm but interestingly it was also seen outside the cell bodies and in the neuronal protrusions. Conclusions: Proteolytic activity of MMPs in extracellular compartment suggests its ability to remodel extracellular matrix and facilitate migration of neuroblasts to the damaged brain areas. The localization of MMPs in cell nuclei implies the involvement of these proteases in proteolytical activation of pro-neural gene transcription.
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SIII-P7

Human umbilical cord wharton jelly grafts: preliminary characterization and effect of transplantation in a rodent model of stroke

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Over the last decade a large number of studies explored the use of cord-blood-derived stem cells for treatment of neurological disorders. Despite of some positive preclinical results low survival of transplanted cells was noticed in the host brain. It seems that transplantation of the donor cells in their own milieu might be more effective due to the natural cell-cell contact and the presence of growth factors and cytokines. The concept of our studies was to deliver human stem cells resided in Wharton jelly (WJ) of umbilical cord tissue into rat brain. 3D tissue implant contains mesenchymal stem cells (MSCs) capable of multilineage differentiation. In the current study we performed in situ analysis of entire WJ-MSCs after their transplantation into: hippocampal organotypic slices isolated from neonatal rats (i); the striatum of normal (ii) or focally injured (iii) adult Wistar rats. Results: Seven days after grafting on hippocampal slices intense migration of the donor cells (NuMa+) from 3D WJ implant was noticed. Double-labeling showed co-localization of NuMa marker with NF200 or GFAP activity in these cells whereas donor cells residing in 3D WJ implant did not express any neural markers. Interestingly, in intact brain all WJ-MSCs (NuMa+) cells remained in 3D tissue implant but differentiated into NF200+ and GFAP+ phenotypes during the time of observation. In contrast, after WJ implantation into injured rat brain the donor MSCs proclaimed migration towards ischemic boundary regions. Moreover, NF200 or GFAP markers were localized among both, migrating as well as remaining in the graft NuMa+ cells. Conclusions: Transplantation of WJ-MSCs in 3D tissue implant into adult rat brain improves cells survival within graft and induces their spontaneous transition into cell of neural lineage. Brain injury additionally stimulates migration of these donor cells out of WJ implant with their further differentiation in the host tissue.
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SIII-P8

FAK- and Pyk2- coupled pathway may contribute to the neurogenesis in gerbil hippocampus after ischemia

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Recently published data indicate that in physiological conditions proteolytic remodeling of extracellular matrix (ECM) by matrix metalloproteinases (MMPs) participates in the stem cells development. Signal derived from ECM may activate specific intracellular signaling pathways which involve non-receptor tyrosine kinases such focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2 (Pyk2), key components responsible for their flow of information to the cell. The function of these enzymes is believed to be tightly linked to its autophosphorylation and association with Src kinase necessary for reciprocal activation/phosphorylation of both enzymes in response to adhesion-dependent signals. FAK and Pyk2 might act through a diverse array of downstream molecules and may regulate biological functions of the cell. These prompted us to evaluate the possible involvement of FAK/PYK2-coupled pathway in the regulation of neurogenesis-associated processes stimulated by transient global ischemia in gerbil hippocampus. For this pur-

pose we checked if there is temporal relationship between activation/phosphorylation of both kinases and proliferation and/or determination of neural progenitor cells.
We found that short-term (5 min) ischemia increased Pyk-2 phosphorylation level in dentate gyrus (neurogenic part of hippocampus) after 2 and 4 weeks of recovery, the time when we observed the intensive proliferation rate and differentiation of progenitors toward neuronal phenotypes. In contrast, in the CA1 region of the hippocampus the level of phosphorylated Pyk-2 was slightly reduced after 2, 4 and 6 weeks of reperfusion. At the same time the level of phosphorylated FAK was significantly increased in both investigated hippocampal regions. The elevation of PYK-2 activity in dentate gyrus might suggest the involvement of this kinase in the post-ischemic stimulation of neurogenesis after global ischemia.
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SIII-P9

Bystander effect of Human Umbilical Cord Blood Mononuclear Cells administered systemically in long-term repair processes after brain injury

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Stem cell transplantation offers an exciting new therapeutic avenue for stroke not only to prevent damage, which has been the focus of conventional therapeutic strategies, but also to actually repair the injured brain. Indeed, exogenous stem cell grafting in animal models of CNS damage improves function by replacing the lost neurons. However, therapeutic mechanism different from the expected contribution of cell replacement have been also postulated. Many studies applying systemic delivery of cells in ischemic stroke disorders have shown significant functional recovery with very few or frequently no cells entering brain. It seems that transplanted cells could propel local micro-environmental signals to sustain active endeavors for damaged neurons substitution. The question arises if systemic infusion of cells enhances endogenous neurogenesis previously activated by focal ischemic brain injury. Materials and methods: Experimental model of focal ischemic brain injury was performed by local application of Na/K ATP-ase pump inhibitor - ouabain (OUA) (1 µl/50nmol) into the striatum of CsA-immunosuppressed adult Wistar rats. Three days later 10^7 human umbilical cord blood CD34- mononuclear cells (HUCB-MNC) were infused into internal carotic artery. At 30 day thereafter rat brains were removed and the neurogenic regions and tissue around the damaged areas were analyzed immunohistochemically. Results: Analysis of brain tissue in OUA injured rats transplanted with HUCB-MNC revealed augmentation of proliferative cells (Ki-67+) in subventricular zone (SVZ) of ipsilateral hemisphere and at the border of the lesion area as well as higher number of DCX+ cells in SVZ. Moreover, the extensive neuroblast migration and their accumulation in the peri-infarct striatum were observed in comparison to non-transplanted rats after OUA injury onset. HUCB-MNC injection into rats with brain infarct showed a significant increase of cells with immature (Nestin+) or more mature (NF-200+) neuronal phenotypes observed in the tissue alongside OUA lesion. The intensive staining of GFAP at the border of injured area in HUCB-MNC transplanted and non-transplanted rats reflected gliosis however, the increased expression of GFAP in brain tissue of the former ones may point to the possible expansion of endogenous progenitors. In conclusions, HUCB-MNC transplanted systemically into OUA focal ischemic brain injured rats activate the endogenous stem cell compartment where the newly arisen cells adopt a neuronal or astrocytic fate. This effect may prove applicable for future clinical therapy.
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SIII-P10

Functional effect of human umbilical cord blood-derived mononuclear cells (HUCB-MNC) transplanted systemically into focal brain injured rats

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Cerebral ischemia causes severe functional deficits due to the death of neuronal and glial cells in the cortex and sub-cortical regions. Stem cell-based therapy could be used to restore lost cells and thus may enhance functional recovery. The aim of the study was to compare therapeutic effectiveness of intra-arterial infusion of human umbilical cord-blood derived mononuclear cells (HUCB-MNC) at different stages of their neural conversion in vitro. Materials and methods. Freshly isolated HUCB-MNC (D-0) neurally directed progenitors (D-3) obtained during 3 days culture of HUCB-MNC and neural-like stem cells (HUCB-NSC) line derived from human cord blood cells were assessed. Focal brain damage was induced in Wistar rats by stereotactic injection of previously established low dose of ouabain into dorsolateral striatum Three days later 10^7 HUCB cells were infused into internal carotid artery. Following surgery rats were housed in large enriched environment cages, in groups of 7-8 animals per cage, for 30 days observation period. Behavioral assessment consisted of tests for sensorimotor deficits (walking beam task, rotarod, vibrissae elicited forelimb placing), cognitive impairments (habit learning task and object recognition test), exploratory behavior (open field test) and apomorphine induced rotations. At the end of 30 days observation the lesion volume was measured and the presence of donor cells visualized by the expression of mRNA of human reference gene β-2-microglobulin. Results. Functional effects of different subsets of HUCB-MNC treatment shared substantial diversity in various behavioral tests. In walking beam test the most effective in recovery the impaired sensomotor functions in focal brain injured rats were freshly isolated HUCB-MNC

(D-0). Also, in rotarod task and in apomorphine induced rotations the tendency to improve scores was observed 30 days following HUCB-MNC (D-0) treatment. In parameters describing open field exploratory behavior the positive effects of HUCB-MNC (D-0) as well as HUCB-NSC cells treatment were observed. However, in cognitive tasks none of tested cell subsets reduced the functional deficits induced by ouabain injection. Thirty days after HUCB cell transplantation we did not observed any mRNA expression of human reference gene in the rat brain samples.
Conclusions. Our observation reveals that freshly isolated D-0 HUCB-MNC are the most effective in functional recovery of injured rats. These cells are also the most potent in reducing the ouabain-induced brain lesion volume. The best functional outcome observed after transplantation of HUCB-MNC (D-0) is probably due to the positive effect of therapeutic molecules secreted by these cells than the persistence of donor per se in the host since we did not detect systemically infused human cells in rat brains.
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SIII-P11
Anisotropic development of neurons transplanted into the mouse somatosensory cortex

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Transplantation of neuronal precursors and immature neurons (neuroblasts) is one of highly potential approaches towards regeneration of injured and degenerating neuronal circuits in the central nervous system. Appropriate interaction of donor cells with existing network is a critical factor for successful repopulation therapy. The aim of the study was to investigate whether newly introduced neuronal precursors and neuroblasts may survive, differentiate, and form proper neuronal projections and dendritic network to target areas within the barrel cortex of postnatal mice. Additionally, we extended our evaluation to systemic spatial comparison in reference to the barrel cortex cytoarchitecture.
We isolated immature neuroblasts from late-stage embryonic somatosensory cortex (>E16) from mice expressing green fluorescent protein (eGFP) in order to unambiguously track developing population of reconstituted neurons. To determine a real specificity of transplanted neuroblasts to survival and neuronal arborization, cells were injected into different compartments of the barrel cortex, such as barrel hollow and interbarrel septae. Each mouse received a single injection of 1000-3000 cells throughout the cortical depth. After three to eight weeks of survival time (and additional group with 6-month-survival), brains were processed for immunohistochemistry for detection of eGFP-expressing cells and neuron-specific markers. A delicate neuronal network extending from transplanted cells within the recipient cortex was evaluated in reference to the barrel field cytoarchitecture visualized by the cytochrome oxidase staining in selected alternative sections.
Transplanted GFP-expressing neurons were mostly observed in interbarrel regions. The cells expressed mature neuronal markers MAP2, NeuN and presented morphology of typical neurons. The local fiber outhgrowth was highly anisotropic throughout cortical layers—in layer IV it was mostly limited to interbarrel septa while was almost symmetrically radiating in supragranular and subgranular layers. Our results provide evidence that limiting cues exists in the barrel cortex, and that they are layer-specific. We confirmed that such anisotropy has a long-lasting effect. Our studies allow investigation of survival, differentiation, and integration of transplanted neurons in the mouse cortex in the area- and layer-specific manner.
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SIII-P12
Involvement of proBDNF in neuronal cell death induced by trimethyltin

Figiel I.
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It is well established that in response to neuronal injury glial cells produce various pro-inflammatory cytokines and neurotrophic factors. These immunoregulatory molecules have been shown to play an important role in sustaining and modulating neurodegenerative events or to promote cell survival.
To address question about molecular mechanisms of interactions between neurons and glial cells in pathogenic conditions, we used the in vitro model of mixed neuronal-glial cultures of rat hippocampal dentate gyrus treated with trimethyltin (TMT). In previous studies we demonstrated that TMT induced neuronal apoptosis, which was accompanied by an enhanced production of tumor necrosis factor (TNF-α) in microglial cells and a strong increase in brain-derived neurotrophic factor (BDNF) expression in astrocytes. Since evidence has been provided that BDNF exerts growth promoting actions on hippocampal dentate granule neurons and is implicated in resistance of these cells to various insults, the current study was dedicated to elucidate whether BDNF may promote beneficial effect on TMT-injured dentate neurons. Immunocytochemical studies of active caspase-3 expression revealed that pretreatment of the cultures with recombinant BDNF did not diminish neuronal injury. On the other hand, using western blot analysis it was demonstrated that TMT evoked increased expression of proBDNF (32 kDa) as well as p75 neurotrophin receptor (p75NTR). Moreover, addition of anti-p75NTR neutralizing antibody to the cultures suppressed TMT-induced apoptosis. These results indicate that neither endogenous nor exogenous BDNF is able to provide neurotrophic support for TMT-injured dentate granule neurons and suggest potential deleterious effect of proBDNF in this model of neurodegeneration.

SIII-P13

In vitro cultured 3D aggregates of glioblastoma cells preserve in vivo observed phenotype and genotype of this tumour

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Surprisingly in vitro conditions allowing to culture glioblastoma cells presenting EGFR amplification were not known until now. Our evaluation of EGFR amplification status in glioblastoma (GBM) culture demonstrated that this anomaly was preserved for months in spheroids (aggregated glioblastoma cells) at a level comparable to the earliest passage of cell culture. In contrast, and in accordance with already published data we detected it as completely lost in the adherent culture. Apparently glioblastoma cells presenting EGFR amplification become apoptotic in the regular cell culture conditions.
In addition discrepant expression of SOX2 and multilineage phenotype recognized as a markers of neural progenitors was observed in monolayer and 3D culture.
Moreover, our analyses showed a decreased invasion potential of adherent GBM loosing EGFR amplification, and spheroids maintaining EGFR amplification. In conclusion, our findings confirm that GBM-derived spheroids seem to be a promising tool to preserve original molecular features of the tumor in vitro, with a special emphasis on EGFR gene aberrations, including EGFRvIII, regarded as novel therapeutic target.
Our last unpublished and preliminary data suggest that mechanism responsible for in vitro death of adherent glioblastoma cells showing EGFR amplification seems to be linked to the artificial in vitro cell-cell interaction rather than to the lack of proper autocrine effects. Those suggestion came from analysis of glioblastoma cells in artificial brain tissues system and analysis of aggregated glioblastoma cells only.
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SIII-P14

Neurovascular unit and glial scar formation following the surgical brain injury

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Normal functioning of both the CNS and the blood-brain barrier depends on proper functioning of the neurovascular unit (NVU) - a dynamic structure made of neurons, capillary vessel (consisted of endothelial cells, pericytes and basement mebrane), extracellular matrix and vessel-bound astrocytes.
Human brain trauma occurs during numerous life-saving neurosurgical procedures (e.g. removal of a brain tumor) associated with disrupted continuity of the meninges followed by interventions within the cerebral parenchyma. Such interventions result in damage to all morphological components of NVU.
Our rat model of cerebral cortex injury imitates quite well the respective human neurosurgery situation in that it involves the most typical early and delayed consequences of neurosurgical procedures. This model, enables studying the cortical response to the lesion at cellular and subcellular levels and relating them to the underlying biochemical changes. The injury is being made by excising of a moderate-sized (about 2.5 mm × 2.5 mm × 1.5 mm, length × width × thickness) piece of sensorimotor cortex in the frontotemporal region and resulting in the massive damage of that area.
Within first few hours following the lesion the border zone of the damage area showed a perivascular astrocytic edema. Two days after the injury, a massive angiogenesis was observe in this region. Formation of new blood vessels occurred even 30 days after the lesion. Beginning on postinjury day 4, the area around the wound showed an increase in both the number and hypertrophy of astrocytes, that showed an enhance of immunoreactivity for the main astrocytic markers: vimentin and GFAP. Fifth postlesion week a well-formed scar was observed within the operated area. However, 3 months after the operation astrocytic processes began to show an edema, and shortly thereafter the scar presented signs of lysis and dissolution. Beginning 24 hours after the injury, the cortex adjacent to the injury showed the presence of degenerating necrotic and, particularly at later time points, of apoptotic neurons.
Our studies reveal that the damage and remodeling of the surgical brain injury zone and its vicinity, as well as forming of the glial scar do not mark an end of the process initiated by the cortical injury. Despite completion of these processes, the area adjacent to the damage was always subject to a secondary damage resulting in brain parenchyma loss that reached far beyond the primary injury zone.
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SIII-P15

Electrospun nanofibrous nets as a potential materials for neurology

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Electrospun nanofibers are very promising material to be used in biomedicine. Electrospinning (electrical spinning) is a method of producing non-woven fibers of diameters down to 2 nm (and

length of many cm) in contrast to a classical spinning - not thinner than 5µm. Main feature of the nanofibers is a very high surface to volume ratio of the material and lack of crystalline defects. The electrospinning process is usually conducted in solvents, even water can be applied, the process conditions are in favor to a very soft molecules and species. Such made fibers contain undamaged polymers or drugs, the proteins are not denaturated even living cells can survive the process. Mats made of the fibers are done of variety of polymers, including biodegradable polyesters and proteins. They can be used as scaffolds for the tissue engineering, wound dressings, barrier materials or Drug Delivery Systems (DDS). The main advantage for the use of mats for the tissue engineering is size similarity of the nanofibers and the fibers of Extracellular Collagen Matrix (ECM). For the use as DDS the fibers act as "nanodiffusion pump" releasing constant amount of drug in a controlled and tailored manner. Electrospun nanofibers made of biodegradable and biocompatible polymers(materials) are harmless and safe nanomaterials. They don't cause inflammatory reaction when implanted. They can be used either to help guiding cells to produce properly formed tissues or inhibit cells growth to prevent liaisons. Depending of the type of material used, processing, surface modification, even the way of sterilization material of desired properties may be produced. The fibers already tested in our laboratories were successfully used as a scaffolds for cells growth (human: UCSC, MSC, hepatocytes). Other applications included: coatings for Bioglass bone implants, nanofibrous sensors made of BSA surface modified by FITC and conductive nanofibers. Research on anti -liasions, wound dressing, barrier materials and tubular scaffolds of enhanced vascularization are being conducted.

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SIII-P16

Is pig spinal cord hydrolysate safe for treatment Experimental Allergic Encephalomyelitis in rats?

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EAE (Experimental Allergic Encephalomyelitis) is the animal model of Multiple Sclerosis, an autoimmune and neurodegenerative human disease. Proposed method for autoimmune diseases treatment is to evoke oral tolerance, which is lack of response to fed antigen. Our previous experiments showed that pig spinal cord hydrolysate, which is source of myelin antigens is able to evoke oral tolerance in EAE rats.

The aim of our study was to investigate, if treatement with pig spinal cord hydrolysate has any toxic effects on rats.

Twelve female Lewis rats were fed with pig spinal cord hydrolysate (500mg/kg) or PBS (control group, 0,5ml per rat) each day for one month. One day after the last day of feeding animals were sacrified, and the main organs were collected. Organs were fixed in 10% formalin, postfixed in paraffin, cut into slices and stained with hematoxylin and eosin. Slices were analysed using the light microscope. Every spleen, thymus and adrenal gland was weighted to count relative mass. Number of spleen megakaryocytes in 1mm^2 was counted.

There were no changes observed in any brain, heart, stomach, intestine, uterus and ovary preparations. We observed single changes in rats liver, kidney, thymus, mesenteric lymph node, adrenal gland, and in the number of spleen megakaryocytes, but not corelated with experimental group.

The fact that we did not find any significant changes in rat organs can confirm that pig spinal cord hydrolysate is safe for rats.

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SIII-P17

Spinal cord hydrolisate epitopes ameliorate immunological reaction in experimental allergic encephalomyelitis/EAE/

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The aim of our study was to evoke oral tolerance with hydrolisate of spinal cord of pig used for feeding the experimental animals/ rats/After induction of tolerance animals were immunised by injection of guine pig spinal cord homogenate with Freund's adjuvant to induce of EAE, which is an animal model of sclerosis multiplex. Clinical course was have been observed, histopatological study ,ultramicroscopic study and metalloproteinases determination in serum were done. Study of lymphocytes proliferation and level of cytokines IL-4,Il-6, Inf-gamma and TGF-beta were also performed. Clinical course of EAE after hydrolisate treatment has been milder than control. TNF in brains was decreased. Metalloproteases increased in EAE, after hydrolisate treatment were diminished by 30%. Some changes in blood brain barrier/BBB/ as opened tight junction and other changes in early phase of EAE as karioskeletal damage with vesicular structures in karioplasm, compartmentalisation of the endoplasmic reticulum in numbers of phagolisosomes, desorganisation of sheets myelin, neoangiogenesis of parenchyma of the cerebral cortex has been diminished. Mechanism of this effects is probably through active suppression involving diminishing production of IL-4 and interferon gamma as well as increasing production of Il-10 and TGF alfa. All above results indicate that mixture of neuropeptides in spinal cord hydrolisate given orally decrease immunological response to myelin antigens and gave an implication for using oral tolerance to support multiple sclerosis/ MS/ treatment.