MOLECULAR BASIS OF PATHOLOGY AND THERAPY IN NEUROLOGICAL DISORDERS

11th International Symposium

Mossakowski Medical Research Centre
Polish Academy of Sciences
Warsaw
November 22–23, 2012

Guest Editors:
Barbara Lukomska
Teresa Zalewska

MOLECULAR BASIS OF PATHOLOGY
AND THERAPY IN NEUROLOGICAL DISORDERS
11th International Symposium

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PROGRAMME

Thursday, November 22, 2012

09:15 Opening of the Conference

Session I
GLUTAMATE IN CENTRAL NERVOUS SYSTEM

Chairs: **Jan Albrecht (Warsaw, Poland)**
Ursula Sonnewald (Trondheim, Norway)

09:30–10:00 **Farrukh A. Chaudhry (Oslo)**
Pathofunctional role of glutamine transporters in neurotransmission, insulin secretion and pH regulation

10.00–10.30 **Ursula Sonnewald (Trondheim)**
Using ^{13}C labeled glucose to investigate glutamate metabolism

10.30–11.00 **Coffee break**

11.00–11.30 **Wojciech Danysz (Frankfurt)**
mGluR5 NAMs as a potential treatments for L-DOPA-induced dyskinesia, Fragile X and gastroesophageal reflux

11:30–12:00 **Andrzej Pilc (Cracow)**
Metabotropic glutamate receptors as targets for psychotropic drugs

12:00–13:00 **Lunch**

13:00–14:30 **Poster session**

Session II
GLIA-COMMITED CELLS – THEIR PROPERTIES AND THERAPEUTIC POTENTIAL

Chairs: **Joanna Sypecka (Warsaw, Poland)**
Bernard Zalc (Paris, France)

14.30–15.00 **Bernard Zalc (Paris)**
Live imaging and monitoring of demyelination and remyelination

15.00–15.30 **Malgorzata Skup (Warsaw)**
Neurotrophins in CNS regeneration: BDNF up-regulation recruits neurons and glia, and promotes recovery after spinal cord transection

15.30–16.00 **Joanna Sypecka (Warsaw)**
The fate of glial progenitors is dictated by the local tissue microenvironment

16.00–16.30 **Pavla Jendelová (Prague)**
Experimental reconstruction of the injured spinal cord

Friday, November 23, 2012

Session III

RECENT ADVANCES IN BASIC AND TRANSLATIONAL CEREBRAL ISCHEMIA RESEARCH

Chairs:	**Barbara Lukomska (Warsaw, Poland)** **Satoshi Kuroda (Toyama, Japan)**
9.30–10.00	**Alexander Kranz (Leipzig)** Clearing the fog: Changes in T2 relaxation time after stroke reflect clearing processes
10.00–10.30	**Jukka Jolkkonen (Kuopio)** Experimental approaches to enhance functional recovery following cerebral ischemia
10.30–11.00	**Coffee break**
11.00–11.30	**Satoshi Kuroda (Toyama)** Transplantation of autologous bone marrow stromal cells (BMSC) for ischemic stroke – strategy and tactics for clinical application
11:30–12:00	**Miroslaw Janowski (Warsaw, Baltimore)** Preclinical and clinical experience with non-invasive imaging and homing of stem cells
12:00–13:00	**Lunch**
13:00–14:30	**Poster session**

Session IV
INSIGHT INTO CNS EPIGENETIC REGULATION

Chairs:	**Krystyna Domanska-Janik Warsaw, Poland)** **Jozef Dulak (Cracow, Poland)**
14.30–15.00	**Anne C. Ferguson-Smith (Cambridge)** Genomic imprinting and the epigenetic regulation of adult neurogenesis
15.00–15.30	**Jozef Dulak (Cracow)** The role of microRNAs in neurological disorders
15.30–16.00	**Monika Gos (Warsaw)** Epigenetic mechanisms of gene expression regulation in neurological diseases
16.00–16.30	**Leonora Buzanska (Warsaw)** Epigenetic modulation of neural stem cells: The influence on reprogramming and differentiation
16.30	**Closing remarks**

Thursday, November 22, 2012
Session I

GLUTAMATE IN CENTRAL NERVOUS SYSTEM

SI-L1
Pathofunctional roles of glutamine transporters in neurotransmission, insulin secretion and pH regulation

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Glutamine is involved in many metabolic pathways such as generation of amino acids, nucleotides and glutathione. Glutamine also serves in pH homeostasis, urea formation, immune response and wound healing. In addition, glutamine is considered to be the primary precursor of the fast neurotransmitters glutamate and GABA in the central nervous system (CNS). The prevailing hypothesis of a glutamate/GABA-glutamine cycle suggests that a large amount of released glutamate and GABA are translocated into perisynaptic astroglial cells, converted into glutamine, and subsequently shuttled back to neurons for regeneration of the neurotransmitters. This mechanistic view is supported by differential localization of glutamate and GABA transporters on perisynaptic glial processes, and demonstration of the key glutamine metabolizing enzymes glutamine synthetase (GS) and phosphate-activated glutaminase (PAG) in glial cells and nerve terminals, respectively. However, the molecular mechanisms involved in glutamine extrusion from glial cells and its transport into neurons have until recently eluded characterization. We have molecularly identified a family of amino acid transporters (Slc38) with isoform specific characteristics. We show that the system A transporters (SATs) mediate neuronal transport of glutamine. SAT1 is enriched in GAD67 expressing GABAergic neurons suggesting a role in GABA formation. SAT2 expression is pronounced in the somatodendritic domains of glutamatergic neurons where it sustains formation of glutamate and is intrinsic for retrograde signaling. Activity of the homologous system N transporter SN1 – expressed exclusively on astroglial cell membranes – is dynamically regulated by intracellular protein kinases and may fine-tune extracellular levels of glutamine accessible for neuronal uptake. SN2 – also expressed in the astroglial cells, but with differential subcellular localization – mediates glutamine release for neurotransmitter synthesis and glycine release to regulate NMDA receptors. Finally, we have shown that these transporters also contribute to pH restoration during chronic metabolic acidosis and regulation of insulin secretion. Recently, I have also contributed to the investigation of a child with congenital glutamine synthetase deficiency, who developed generalized hypotonia and hyperreflexia and treatment-resistant seizures postpartum and had very low serum and cerebrospinal fluid concentrations of glutamine and glutamate (Häberle et al. 2012). Glutamine supplementation restored serum levels of glutamine and glutamate, while corresponding values in the CNS approached normal. Ammonia toxicity was also prevented. The frequency of seizures abated and EEG showed significant improvement. Altogether, our data show the importance of glutamine and glutamine transporters in normal physiology and pathophysiology and bolster existence of a glutamate/GABA-glutamine cycle.

SI-L2
Using ^{13}C labeled glucose to investigate glutamate metabolism

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Using ^{13}C labeled compounds and ^{13}C magnetic resonance spectroscopy (MRS) it is possible to monitor cellular metabolism and astrocyte-neuronal interactions. Various ^{13}C labeled substrates are used to unravel different aspects of cerebral metabolism. This presentation will focus on [1-^{13}C]glucose, [U-^{13}C] glucose, [2-^{13}C]glucose and [3-^{13}C]glucose metabolism in cerebellar and cerebro-cortical neurons and astrocytes in culture. [1-^{13}C]Glucose is metabolized by both astrocytes and neurons and labeling of metabolites from this isotopomer of glucose will not be affected by the pentose phosphate pathway (PPP). Using [U-^{13}C]glucose and 3-nitropropionic acid it could be confirmed that pyruvate carboxylation takes place in cortical astrocytes but not neurons. This carboxylation leads to the formation of oxaloacetate, which condenses with acetyl coenzyme A to form citrate. However, oxaloacetate may also be converted to malate and fumarate before being regenerated. This redundant pathway is termed the oxaloacetate-fumarate-flux, or backflux and has been shown to be extensive using [2-^{13}C]- and [3-^{13}C]glucose in cultured cerebral cortical and cerebellar cultures. It could also be calculated to be present *in vivo*. [2-^{13}C]- and [3-^{13}C]glucose can also be used to probe the PPP in neurons where pyruvate carboxylation is not present. Indeed, the PPP contributed to labeling of glutamate and other metabolites.

SI-L3
mGluR5 NAMs as a potential treatments for L-DOPA-induced dyskinesia, Fragile X and gastroesophageal reflux

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Subtype 5 metabotropic glutamate receptors (mGluR5) have been implicated in the control of movement, mood, cognition,

and nociception. Correspondingly, different mGluR5 antagonists have been shown to alleviate L-DOPA-induced dyskinesia (LID), anxiety, and pain in experimental animals. A novel proprietary mGluR5 antagonist 6,6-dimethyl-2-phenylethynyl-7,8-dihydro-6H-quinolin-5-one (MRZ-8676) having ~20 nM affinity to mGluR5, was tested in the rat models of LID, persistent pain, and anxiety. Effects of MRZ-8676 on motor performance and on learning were investigated. Presence of MRZ-8676 at target receptor in the brain was ascertained by measuring its extracellular concentrations and mGluR5 occupancy *in vivo*. MRZ-8676 had 20–25% bioavailability after oral treatment reaching Tmax at 2.9 h while T1/2 was 11.2 h. At 25 mg/kg p.o. Cmax was 348 ng/ml and AUC 2045 ng*h/ml). In *in vivo* microdialysis experiments, pharmacologically effective p.o. doses of MRZ-8676 were found to achieve free brain concentrations sufficient to completely block mGluR5, i.e. above 100 nM. This finding was further confirmed by the results of the *in vivo* mGluR5 receptor occupancy study showing ED50 of ca. 5 mg/kg after i.p. administration. MRZ-8676 strongly and dose-dependently reduced abnormal involuntary movements in the 6-hydroxydopamine (6-OHDA) rat model of LID starting at 25 mg/kg. No tolerance of the antidyskinetic effects was observed upon subchronic (6-day) treatment with 75 mg/kg p.o. MRZ-8676 produced moderate anxiolytic effect in two rodent anxiety models, the contextual fear conditioning (at 25 mg/kg) and the elevated plus maze (25 mg/kg). At the same dose, MRZ-8676 also attenuated reaction to pain in the first phase of formalin test, the rat model of persistent pain induction. MRZ-8676 did not produce any detrimental effects on motor performance of rats as investigated rotarod test up to 150 mg/kg p.o. In the open field short lasting increase in locomotor activity was observed (25–150 mg/kg). However, MRZ-8676 dose dependently impaired learning in aversive learning paradigm of the contextual fear conditioning test reaching significance at 75 mg/kg which is above minima effective dose in tests for dyskinesia, pain or anxiety. Summing up, MRZ-8676 has clear-cut antidyskinetic properties with a sufficient therapeutic window. Moreover, it has anxiolytic and analgesic properties. Receptor occupancy and microdialysis studies indicate that the behavioural effects of MRZ-8676 are associated with blockade of mGluR5 in the brain. Moreover, preclinical rational and status of clinical trials with mGluR5 NAMs in Parkinson´s disease, Fragile X and gastroesophageal reflux will be presented.

SI-L4

Metabotropic glutamate receptors as targets for psychotropic drugs

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Several studies have suggested that modulation of the glutamatergic system *via* metabotropic glutamate receptors (mGlu) could be a new, efficient way to achieve antipsychotic-like effects. Such an activity was shown for mGlu2/3 and mGlu5 receptor agonists/positive modulators, as well as for ACPT-I or LSP1-2111, a non-selective mGlu group III receptors orthosteric agonists. Herein, we report the pharmacological actions of Lu AF21934 and Lu AF32615, a novel, selective and brain-penetrant positive allosteric modulators (PAMs) of the mGlu4 receptor with proven anxiolytic, but not antidepressant-like activity, in several tests reflecting positive, negative and cognitive symptoms of schizophrenia in rodents. MK-801- and amphetamine-induced hyperactivities, as well as DOI-induced head twitches in mice were used as models for positive symptoms. Furthermore, the effect of Lu AF21934 on DOI-induced frequency of spontaneous excitatory postsynaptic currents (EPSCs) in slices from mouse brain frontal cortices was investigated. The MK-801 induced disruption of social interaction and of spatial delayed alternation in rats were used as models for negative and cognitive symptoms, respectively. Lu AF21934 (0.1, 0.5, 2 and 5 mg/kg) and Lu AF32615 (2, 5 and 10 mg/kg) dose-dependently inhibited both MK-801 and amphetamine-induced hyperactivities. Concomitantly, Lu AF 21935, an inactive enantiomer of Lu AF21934, was not effective. Moreover, the drugs antagonized DOI-induced head twitches in mice. DOI-induced increased frequency of spontaneous EPSCs was also decreased by Lu AF21395 and Lu AF32615. The MK-801-induced disruption in the social interaction test, measured as number of episodes and total time of episodes between two rats, was abolished by Lu AF21394 at a dose of 0.5 mg/kg and Lu AF32615 at a dose of 10 mg/kg. In the delayed spatial alternation test, the effective doses of Lu AF21934 were 1 and 2 mg/kg, and the AF32615 was active at a dose of 10 mg/kg. Altogether, we propose that mGlu4 receptor can be consider as promising target for the development of novel antipsychotic drugs, acting as a positive allosteric modulators of the receptor.

Session II
GLIA-COMMITED CELLS – THEIR PROPERTIES AND THERAPEUTIC POTENTIAL

SII-L1

Live imaging and monitoring of demyelination and remyelination

Zalc B

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We have generated a *Xenopus laevis* transgenic line allowing live imaging and conditional ablation of myelinating oligodendrocytes throughout the central nervous system. In these transgenic *pMBP-eGFP-NTR* tadpoles the myelin basic protein regulatory sequences, specific to mature oligodendrocytes, are used to drive expression of an eGFP (enhanced green fluorescent protein) reporter fused to the *E. coli* nitroreductase (NTR)

selection enzyme. This enzyme converts the innocuous pro-drug metronidazole (MTZ) to a cytotoxin. Using two-photon imaging *in vivo*, we show that *pMBP-eGFP-NTR* tadpoles display a graded demyelination response following exposure to MTZ, which depends on the exposure time to the pro-drug. We demonstrate that MTZ-induced cell death was restricted to oligodendrocytes, without detectable axonal damage, as shown by immunolabeling with oligodendrocytes or node of Ranvier markers and electron microscopy. After cessation of MTZ treatment, remyelination proceeded spontaneously, but was strongly accelerated by retinoic acid. Altogether, these features establish the *Xenopus pMBP-eGFP-NTR* line as a novel *in vivo* model for the study of demyelination/remyelination processes and for large-scale screens of therapeutic agents promoting myelin repair.

SII-L2

Neurotrophins in CNS regeneration: BDNF up-regulation recruits neurons and glia, and promotes recovery after spinal cord transection

Skup M

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The neurotrophins are a family of small proteins that were first identified as survival factors for sympathetic and sensory neurons and have since been shown to control survival, development and function of neurons and myelin formation in the central and peripheral nervous systems. Prosurvival and plasticity-promoting effects of mammalian neurotrophins: NGF, BDNF, NT-3 and NT-4 are mediated through activation of the tropomyosin-related kinase family of receptor tyrosine kinases (TrkA, TrkB, TrkC). The spinal cord of the adult rat is rich in BDNF protein which exceeds brain levels and is expressed in neurons occupying all spinal laminae (Skup et al. 2002, Macias et al. 2007). Locomotor exercise of the uninjured rats, an approach used to improve motor functions after injury, increased perikaryonal levels of BDNF mRNA within the majority of cells and of BDNF protein in processes surrounding large neurons of the lumbar motor nuclei. Exercise increased also staining intensity and number of TrkB receptor immunoreactive small cells of the spinal grey matter, which were identified as oligodendrocytes. When applied to the rats with complete spinal cord transection, exercise caused BDNF up-regulation in distinct populations of neurons in motor nuclei and increased motoneuron innervation (Skup et al. 2009, 2012). Data strongly suggested that the spinal network is under BDNF control, targeting neurons but also oligodendrocytes, recruited to neurotrophin signaling by the activated network. Multifaceted functions of BDNF make this molecule a promising one in attempts to stimulate neuronal regeneration and remyelination, but until recently treatments directed to increase the BDNF supply to injury-affected spinal networks only moderately improved locomotor functions. We therefore attempted to deliver BDNF *via* neurons transduced with adeno-associated virus serotype 1/2 (AAV1/2) expressing BDNF under the control of the synapsin promoter in the lumbo-sacral network below the complete transection. Based on functional, histological and biochemical assessments, I shall show that BDNF secreted from BDNF-expressing neurons in lumbar segments improves locomotor functions and alters excitability of the spinal network. Searching for the mechanisms of these changes we revealed that the increased segmental BDNF concentrations led to an increase in GAP-43 expression, GAD67 mRNA and protein expression and GABA levels, reducing post-lesion GABA deficits in the thoracic/lumbar segments. BDNF did not compensate the deficit of the potassium-chloride co-transporter KCC2, responsible for GABAA receptor-mediated hyperpolarizing inhibition. We conclude that sustained delivery of BDNF to the isolated spinal cord network causes neuronal rearrangements and increases inhibitory transmission under conditions of lesion-induced altered neuronal excitability, leading to locomotor improvement in paraplegic rats. Since glia affect excitability and remyelination we study astroglial/oligodendroglial responses to BDNF overexpression.

Support: Polish-German grant S007/P-N/2007/01, EMBO ASTF211.00-2007, NCN3975/(P)1/2010/39 & 3247/2010/39

SII-L3

Fate of glial progenitors is dictated by the local tissue microenvironment

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The oligodendrocyte progenitors (OPCs) are the abundant population of NG2-positive cells in the young and adult CNS. They are capable of myelinogenesis, but they are also among the first cells to react to CNS injuries. Over the last decade, these glia commited progenitors have been however the subject of intensive research in context of their assumed neural stem cell properties. In our studies we have addressed the question of the impact of the local tissue microenvironment on the OPC commitment and differentiation. Their susceptibility to external stimuli and assumed intrinsic neurogenic potential have been investigated in co-culture models with organotypic slices derived from two distinct CNS regions (hippocampus and spinal cord). The hippocampal slice culture exposed to oxygen glucose deprivation (OGD) was used to evaluate the cell differentiation in microenvironment conditioned by traumatized tissue. The results have shown that the local instructive clues not only trigger the neuronal commitment of oligodendrocyte progenitors, but also govern the oligodendroglial maturation. While the trophic factors secreted by hippocampal slices efficiently promoted neurogenesis, the observed effect was significantly abolished in co-cultures with the OGD-subjected tissue. The less pronounced susceptibility to adopting neuronal phenotype and the considerable slowdown of oligodendroglial differentiation was observed in the co-cultures with the spinal cord slices. Our findings indicate that OPCs actually

meet some of the neural stem cell criteria. The obtained results also suggest that the specificity of the instructive clue cocktail might module the fate choice of mobilized endogenous or transplanted cells, which is important while planning neurorepair strategies.
Supported by grant 0345/B/P01/2010/38

SII-L4
Experimental reconstruction of the injured spinal cord

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Tremendous efforts have been made to ameliorate and improve locomotor function after spinal cord injury (SCI) by the transplantation of various types of stem cells. In our study we compared the use of non-neurogenic stem cells – bone marrow stromal cells (MSCs), an immortalized stem cell line (SPCs) derived from human fetal spinal cord tissue or human induced pluripotent stem cell-derived neural precursors (iPS-NPs) – for the treatment of a balloon-induced spinal cord compression lesion. Suspensions of stem cells were implanted into the lesion one week after SCI, while the control groups were injected with saline. Locomotor and sensitivity tests were performed weekly for two months. Animals transplanted with any cell type displayed significant motor and sensory improvement compared to the controls. Morphometric evaluation showed that the white matter was spared in all grafted animals when compared to controls, while the gray matter was spared only in animals implanted with MSCs or iPS-NPs. Two months post-implantation (PI), all types of grafted cells survived in the lesion; however, MSCs, unlike iPS-NPs and SPCs, did not differentiate nor communicate with the host tissue. Compared to SPCs, which partially filled the lesion cavity, iPS-NPs interacted more with the host tissue. Besides differentiating into MAP2-, 5TH- and Dcx-positive neurons, iPS-NPs differentiated into CNPase^{+} oligodendrocytes. A few cells expressed ChAT, while others were DARPP32^{+}. SPCs expressed mainly GFAP; however, already at two months PI we found 25% of the cells to be positive for Nkx 6.1, and at four months PI the cells were positive for ChAT and Islet2, motor neuron-specific markers. qPCR revealed the increased expression of rat and human neurotrophin genes as well as human motor neuron-specific genes. Based on staining for GAP43, SPCs cells supported endogenous neurite sprouting and regeneration. Another important therapeutic goal is treating chronic SCI, possibly by a combination of stem cells and bridging scaffolds. Hydrogel bridges seeded with MSCs were implanted into SCI one month after injury. The implanted rats were behaviorally tested, then sacrificed 6 months PI and the spinal cord lesions histologically evaluated. The hydrogels adhered well to the surrounding tissue and completely filled the post-traumatic cavity. MSCs survived in the hydrogel, and neurofilaments, blood vessels and Schwann cells infiltrated the implant. Combined therapy also prevented tissue atrophy, while behavioral analysis showed an improvement in rats with combined treatment, compared with the control group. Our results demonstrate that the transplantation of neurogenic as well as non-neurogenic stem cells into the lesioned rat spinal cord improves functional outcome by providing trophic support to the spared axons in the injured tissue. Neurogenic stem cells have the ability to interact with the host tissue and differentiate into a more mature phenotype, such as motor neurons. Treatment of chronic spinal cord injury will require a combination of cell therapy and lesion bridging.
Supported by IAA500390902, GA CR: P304/12/1370, GA CR: P108/10/1560

POSTER PRESENTATIONS

P1
The valproic acid-induced effects on kynurenine pathway correlate with the changes in the central and peripheral concentration of amino acids

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Despite its widespread use, the mechanisms of valproic acid (VPA) action are not fully understood. In the current study, we have examined the peripheral and central effects of VPA administration on the metabolic pathway of tryptophan (TRP): concentration of its centrally active metabolites, kynurenine (KYN) and kynurenic acid (KYNA). Moreover, the role of a displacement of TRP from serum albumin binding sites, and changes in the peripheral and central concentration of amino acid including glutamate (GLU), GABA, alanine (ALA), glutamine (GLTM), glycine (GLY), aspartate (ASP), were also studied. We found that VPA administration produced a progressive and strong increase in the central concentration of KYNA, KYN and TRP. Simultaneously, TRP concentration in plasma declined while the peripheral increase of KYNA in plasma was weaker and occurred earlier than in the hippocampus. We also observed that administration of ibuprofen to rats, a prototypic drug used to study drug binding to serum albumin, strongly increased the amount of a free serum and hippocampal TRP concentration, to a degree similar to the effect of VPA. Moreover, we found that the most pronounced changes in the concentration of amino acids caused by administration of VPA include an increase of GLU and a decrease of ALA in the plasma as well as a decrease of ASP and an increase of GABA in the brain. The factor analysis revealed that the changes in the concentrations of TRP, determined both in the plasma and in the hippocampus grouped strongly with the changes in the plasma concentrations of GLU and the central concentration of ASP. Our results showed that administration of VPA strongly modifies the activity of the kynurenine pathway with significant changes in TRP, KYN and KYNA levels in the CNS. The reason for this may be a strong VPA-induced displacement of TRP from its binding sites to plasma albumin. It appears also that the changes in TRP evoked by VPA administration due to competition for transport into the brain, may result in a shift in the central

and peripheral balance between branched-chain (BCAA) and aromatic amino acids (AAA). This may lead to a decrease in BCAA transport to the brain, leading to a deficit of BCAAs as a donor of amino groups to the process of GLU resynthesis from pyruvate. Changes in the BCAA/AAA ratio, arising as a consequence of changes in the TRP level, could explain an observed increase in the plasma concentrations of GLU and a decrease in the ASP concentrations in the brain that occurred after administration of VPA. In sum, given the neuroprotective role of KYNA, the current study suggests that stimulation of the kynurenine pathway may also apply to the central and peripheral concentration of amino acids. The modification of the activity of the kynurenine pathway may at least in part contribute to the related antiepileptic and neuroprotective mechanisms of VPA action.

P2

Effect of VMAT2 inhibition on glutamate and adenosine in rat frontal cortex

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Our earlier studies showed that inhibition of VMAT2 caused depletion of dopamine in rat striatum accompanied with outflow of glutamate and production of hydroxyl radical. Inhibition of VMAT2 is observed in an early phase of Parkinson's Disease (PD) as evidenced by PET studies in PD patients and in non-human primates. Recently it is observed that many neurons also release a classical transmitter other than the one with which they are usually associated. It is shown that neurons releasing monoamines can also release the excitatory transmitter glutamate. All neurons contain glutamate for its role in protein synthesis and metabolism, but they also express VGLUTs required for excitotoxic glutamate release. Moreover, it is also shown that several catecholamine cells such as VTA dopamine neurons are able corelease glutamate. Disturbed function of both, VMAT 2 and VGLUT may start catecholamine neurons degeneration that occurs at the early pre-clinical stage of PD. Accumulation of cytosolic dopamine may be neurotoxic for neurons through the generation of free radicals. Similarly, glutamate released from neurons or glial cells *via* GLT-1 transporter or cystine-glutamate exchanger or purinergic P2X7 receptor may stimulate glutamate receptors on various cells, induce increase in intracellular calcium which leads to excitotoxicity and generation of free radicals. ATP is required for packing of dopamine or glutamate in neuronal and glial vesicles and disturbed vesicular function results in ATP metabolism to adenosine in the presence of 5'-nucleotidase. In our study we tried to understand the early changes in dopamine synapses and glial cell responses which may provide insights on PD pathology. We injected animals with reserpine to inhibit vesicular transport and measured veratridine-evoked (100 μM) dopamine, glutamate and adenosine release using microdialysis in frontal cortex of freely moving rats. Extracellular dopamine, adenosine and glutamate were assayed by HPLC with electrochemical, fluorescenece and VIS detection. Reserpine at a single dose of 2.5 mg/kg increased veratridine-evoked glutamate release to 200% and adenosine release to 5 000% of baseline 20 h after administration. Reserpine at a dose of 0.25 mg/kg given repeatedly for 14 days increased evoked-glutamate release to maximum 210% and adenosine to 1 400% of baseline. At the same time veratridine-induced DA release was also markedly increased as compared to control animals. Veratridine-evoked glutamate and adenosine release were increased by 150 and 600% of baseline, respectively in intact rats. Obtained results indicate that under conditions of damaged vesicular transport there is significant overflow of glutamate and adenosine as well as increase in dopamine release in the rat frontal cortex. Marked increase in extracellular adenosine release may lead to activation of adenosine A2A receptors located in glutamate terminals or glial cells causing damage through induction of oxidative stress by glutamate or dopamine. Corelease of neurotransmitters and neuromodulators from neuronal or glial cells with disturbed vesicular transport may underline cortical pathology observed in PD.

P3

The evaluation of the role of mGluR7 in neuronal apoptotic processes by usage of AMN082, a selective mGluR7 agonist and mGluR7 knockout-derived cells

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Increasing body of evidence suggests a neuroprotective potential of metabotropic glutamatergic receptor group III (mGluR III) stimulation, however the role of particular subtypes of these receptors (mGluR4, mGluR7, mGluR8) in apoptotic processes is not fully recognized. Of special interest is the study on the role of mGluR7 which is widely expressed throughout the brain and recently developed selective positive allosteric modulator of this receptor, AMN082 (N,N=-dibenzhydrylethane-1,2-diamine dihydrochloride) enables investigation the biological role of mGluR7. In the present study, firstly we evaluated the possible neuroprotective effects of AMN082 (0.001–1 μM) on neurotoxicity induced by various apoptotic [stimuli staurosporine (St), doxorubicin (Dox) and low potassium (LP)] in 7 DIV cerebellar granule cells (CGC). The data showed that AMN082 (0.1–1 μM) partially attenuated the cell death induced by St and LP, but not by Dox. Next, we investigated the role of mGluR7 in neuronal cell death by testing the vulnerability of CGC from wild and mGluR7KO animals to toxic action of St, Dox and LP. No differences between groups under basal conditions have been found. However, after primary deprivation of CGC cells from potassium in culture medium and secondary application of proapoptotic stimuli we observed the higher vulnerability of mGluR7KO CGC to cell damaging effect of St and Dox but not LP. Further experiments performed on cortical glia cells demonstrated higher toxic action of St and Dox in mGluR7KO cells when compared to wild type one. Additionally, in mGluR7KO glia cells

we found higher basal and stimulated by St or Dox caspase-3 activity when compared to wild type one. The obtained data suggest that specific stimulation of mGluR7 by AMN082 could be protective against staurosporine and low-potassium induced neuronal ell death. Moreover, the presence of mGluR7 could be particularly important for survival of glia cells under harmful conditions.
The study was supported by statutory funds for Institute of Pharmacology PAS and grant No NN405611638 from the Ministry of Science and Higher Education, Warsaw, Poland

P4

Is the NMDA receptor involved in neurotoxicity of pvp-coated silver nanoparticles? Study on primary cultures of cerebellar granule cells

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Silver nanoparticles (NAg) possess antibacterial properties thus are widely used in many applications in medicine, life sciences and biotechnology. Nanoparticles can be found in vertebrate brain, but little is known about their neurotoxicity. The aim of this study was to investigate how NAg can contribute to neuronal cell death. In the study primary cultures of rat cerebellar granule cells (CGC) were used. We tested hypothesis concerning the role of glutamatergic NMDA receptors in NAg-evoked neurotoxicity. In our study changes in intracellular calcium (Ca^{2+}) homeostasis, uptake of $45Ca^{2+}$, reactive oxygen species (ROS) production, mitochondrial membrane potential and cells viability were investigated. We used commercially available 0.2% polyvinylpyrrolidone (PVP)-coated NAg <100 nm. To avoid sedimentation and agglomeration, before application to the CGC culture, NAg were sonicated with fetal calf serum. NAg were applied in concentration 2.5–75 µg/ml for 10, 30 min or 24 h, depending on experiment. As a pharmacological tool 0.5 µM MK801, a noncompetitive inhibitor of NMDA receptor, was used. After 10 min incubation in the presence of 25–75 µg/ml NAg dose dependent increase of $45Ca^{2+}$ concentration was observed in neurons. This increase was comparable to that evoked by 100 µM glutamate and was completely abolished by MK801. Using fluorescent intracellular calcium indicator fluo3 we observed increase in intracellular calcium level by 200% compared to control, which was partially diminished by MK801. ROS production was measured using fluorescent dye DCF. After 30 min incubation with 75 µg/ml NAg the increase by about 35% over control level was observed and application of MK801 reduced it significantly. Changes in mitochondrial membrane potential were determined using rhodamine (R123). We observed significant decrease in mitochondrial potential during 30 min incubation with different concentrations of NAg and also in this case administration of MK801 was protective. Cells viability was assessed after 24 h incubation with NAg µg/ml alone or together with MK801. Application of MK801 increased neuronal survival from 50% up to 80%. Our results show that excitotoxicity *via* activation of NMDA receptor, followed by calcium imbalance, destabilization of mitochondrial function and ROS production, seems to be important mechanism involved in neurotoxicity evoked by NAg in cultured neurons.
Supported by grant NN401619938

P5

An influence of compounds acting on glutamatergic and noradrenergic receptors on the tremor induced by harmaline; automatic measurements by force plate actimeters

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Harmaline, a derivative of beta-carboline is a well-known tremorgenic compound which induces the action and postural tremor in animals. Oscillation frequency of this symptom is equal to 10–12 Hz in rats. A synchronous activation of the olivo-cerebellar pathway and release of glutamate in the cerebellum has been suggested to be a primary cause of the harmaline-induced tremor. Subtype 4 of metabotropic glutamate receptors (mGluR4) is mainly an autoreceptor and its stimulation decreases glutamate release. MGluR4 receptors are abundant in the cerebellum and therefore their influence on the harmaline-induced tremor might be expected. However, mechanisms underlying this symptom are more complex and seem to involve also other neurotransmitter systems, especially the noradrenergic neurotransmission in the cerebellum. The aim of the present study was to examine an influence of an orthosteric agonist of mGluR4 – AF22898:8 on the tremor induced by harmaline in rats. An antagonist of beta-adrenoceptors – propranolol was used as a reference compound. Tremor of animals was measured automatically by actimeters where four force tranducers measured the force exerted by an animal on the floor. The Power Spectra analysis which uses a Fourier transform generated power spectra for examination of the tremor. The average power over three specific frequency bands AP1 (0–8 Hz), AP2 (9–15 Hz), AP3 (16–25 Hz), and tremor indices, which quantified the differences in power between the AP2 and AP1 (T1) and AP3 and AP1 (T2) were used to quantify the tremor intensity. Harmaline in doses of 7.5–25 mg/kg i.p. induced the generalized tremor which was dose-dependent and lasted longer than 2 h. Propranolol in a dose of 20 mg/kg i.p. diminished the tremor (decreased T1 and AP2) induced by harmaline (15 mg/kg i.p.). In contrast, AF22898:8 administered in doses of 2.5–20 mg/kg i.p. was ineffective. The present results indicate that the harmaline-induced tremor measured in the force plate actimeters consititute a good model for screening antitremorgenic compounds. However, in contrast to earlier expectations the agonist of mGluR4 had no influence on this symptom.

The study was supported by the grant of the National Science Centre nr N N401 570638, and partly by Statutory Funds of the Department of Neuro-Psychopharmacology, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

P6

Characterization of mice with genetically evoked selective degeneration of noradrenergic system and its possible influence on dopaminergic system

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Parkinson's Disease (PD) is characterized by an increased production of oxygen free radicals leading to alteration of the cellular constituents and subsequent dopaminergic cell loss within the region of substantia nigra (SN) and ventral tegmental area (VTA). However, it is well known that PD is not only associated with dopaminergic transmission. Involvement of extranigral structures in PD includes the noradrenergic system as well. *Post-mortem* studies of human brains revealed that neuronal loss associated with PD may proceed and is even greater in the region of locus ceruleus (LC) than SN/VTA. In PD animal models, the loss of noradrenaline made worse the dopamine nigrostriatal damage and, in opposite, an enhanced noradrenaline level may have a neuroprotective role. The aim of this study was to determine whether genetically evoked, selective loss of noradrenergic neurons may have any long-term, negative impact on the dopaminergic system. We applied the conditional inactivation of the gene encoding transcription factor TIF-IA (essential for the regulation of rRNA synthesis) by the Cre-loxP system to induce the progressive and selective loss of noradrenergic neurons which was achieved by expressing Cre recombinase under dopamine beta-hydroxylase (DBH) promoter. Resulting TIF-IADBHCre mice were born at expected rates, viable but showed clear signs of noradrenergic innervations failure e.g. ptosis, reduced locomotor activity, growth retardance and shorten life span. The animals were analyzed at 8 and 12 weeks of age. The selective loss of noradrenergic neurons was confirmed by immunofluorescent staining with the anti-tyrosine hydroxylase (TH) antibody. We observed approx. 90% reduction of TH positive cells in the LC of 8 weeks TIF-IADBHCre mice. The number of TH+ cells was not changed in the region of SN/VTA, neither in 8 nor 12 week old mutants. However, our preliminary data indicate that lack of the noradrenergic transmission may lead to enhanced expression of selected markers associated with neurodegeneration within the region of SN/VTA. Namely, we have found 1.4 fold up-regulation of mRNA encoding for glial fibrillary acidic protein (GFAP) as revealed by quantitative real-time PCR and increased level of oxidative stress shown by immunoblot detection of carbonyl groups by Western Blot in the SN/VTA of 12 weeks TIF-IADBHCre mice compared to control animals. If we provide additional evidences that selective noradrenergic degeneration affects functioning of dopaminergic neurons, TIF-IADBHCre mice may became a valuable, new model for study possible anti-PD treatment at early stages of the disease as dopaminergic neurons in these mice are not directly affected by the mutation. As for today, there are no experimental studies on a possible long-term negative impact of progressive noradrenergic degeneration on other neurotransmitter systems despite of clinically observed concomitant loss of SN/VTA and LC neurons in PD.

This study was supported by the grant no 2011/03/B/NZ7/05949 financed by National Science Centre and statutory funds of the Institute of Pharmacology, Polish Academy of Sciences

P7

Antidepressant-like effect of 1,2,3,4-tetrahydroisoquinoline in reserpine model of depression in rat – changes in noradrenaline and serotonin levels

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It is well known that monoamine neurotransmitters: noradrenaline (NA) and serotonin (5-HT) play a key role in central nervous system (CNS) in pathophysiology of depression. The alterations in their metabolism in the brain seem to be related to the therapeutic action of antidepressants. Abnormalities with monoaminergic storage and neurotransmission are associated with a number of neurological disorders as: e.g. depression. Reserpine, used as an antihypertensive, antipsychotic drug in a low doses is a potent inhibitor of the vesicular monoamine transporter 2 (VMAT2) and acts by depleting cells of their monoamines stores. It is known that patients who took reserpine chronically began to display symptoms similar to that seen in depression. The reserpine model of depression in rat based on universally accepted monoamine hypothesis of depression and offers good predictive validity in terms of monoamine-based antidepressant activity. The aim: the present study aimed to investigate the potential antidepressant properties of an endogenous amine 1,2,3,4-tetrahydroisoquinoline (TIQ) and its possible mechanisms of action. In behavioral study, the forced swim test (FST) was used to evaluate the effects of TIQ in reserpine model of depression in rat. Additionally, the motor function of rat was checked in locomotor activity test after investigated drugs administration. Further, the content of NA, 5-HT and their metabolites, as well as the rate of metabolism in different rat brain structures were determined by HPLC methodology with ED. The reserpine model of depression was induced by chronic (14 consecutive days) administration of reserpine in a low dose (0.2 mg/kg i.p.). Results: The results from both behavioral and neurochemical studies have shown depressive-like effect of reserpine after its chronic administration. In the behavioral tests, reserpine decreased the locomotor activity (about 30% vs. control group, $P<0.05$) measured in actometers (Opto-Varimex activity monitors,

Columbus Instruments, USA) linked on-line to a compatible IBM-PC. 14-days administration of reserpine induced also behavioral changes in FST: increase of immobility time with a simultaneous decrease of swimming activity (about 30% vs. control group). Depressive-like action of reserpine was also observed in neurochemical study by decline NA and 5-HT levels in the brain structures, mainly in the frontal cortex and striatum. TIQ (25 mg/kg i.p.) revealed antidepressant-like effect in FST and has the ability to reverse the pro-depressive effect of reserpine. In biochemical studies, TIQ completely antagonized reserpine-induced monoaminergic depression in rat brain structures. Conclusion: The obtained data indicate first, that chronically administered reserpine at a low dose leads to a good animal model of depression. Secondly, the antidepressant-like effect of TIQ based mainly on activation of monoaminergic system and antagonizing the effect of reserpine by inhibition of MAO-dependent oxidation of monoamines and increased their concentrations in the brain. Thus, in that light TIQ may be useful as a new safer and more effective compound in clinical practice for therapy of depression.

P8

An influence of lesions of the catecholaminergic cerebellar innervation on the harmaline-induced tremor in rats

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Recent studies have suggested a crucial role of the cerebellum in different forms of tremor. Abnormal synchronous activation of the glutamatergic olivo-cerebellar pathway and Purkinje cells results in the essential tremor in humans and the harmaline-induced tremor in animals. Moreover, an increased neuronal activity of the cerebellum has been found to contribute to the tremor in Parkinson's disease (PD). Since the cerebellum receives dopaminergic and noradrenergic pathways arising from regions affected in PD, the aim of the present study was to examine a contribution of the cerebellar catecholaminergic innervation to the harmaline-induced tremor in rats. Rats were bilaterally injected into the cerebellar vermis (lobules 8–10) with 6-hydroxydopamine (6-OHDA) (8 μg/0.5 μl) either alone or this treatment was preceded by desipramine (15 mg/kg i.p.). Harmaline was administered at a dose of 7.5 mg/kg i.p. on the 9th post-operative day. Tremor of forelimbs was measured as a number of episodes. After completion of behavioural experiments rats were killed by decapitation and the levels of monoamines and their metabolites were measured by HPLC in lobules 1–3, 4–7 and 8–10 of the cerebellum. 6-OHDA injected alone decreased the noradrenaline level by ca. 40–80% in the cerebellum and enhanced the harmaline-induced tremor. When 6-OHDA administration was preceded by desipramine, it decreased dopaminergic transmission in some regions of the cerebellum but induced its compensatory activation in others. Finally no influence of the latter treatment on the tremor induced by harmaline was observed. The present study indicates that the noradrenergic innervation of the cerebellum plays an inhibitory role in the harmaline-induced tremor.

The study was supported by the grant of the Ministry of Science and Higher Education No N N401 570638, and partly by Statutory Funds of the Department of Neuro-Psychopharmacology, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

P9

Elevated expression of α7 neuronal nicotinic acetylcholine receptor during the early stages of damage by oxidative stress in the aging rat brain

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Aging is accompanied by a high level of oxidized form of guanine, 8-oxo-2'deoxyguanosine (8-oxo-2'dG), and decreased level of 8-oxoguanine glycosylase 1 (OGG1) in the brain. The development and progression of neurodegenerative disorders are also characterized by dysfunction or loss of the brain nicotinic acetylcholine receptors (nAChRs). To study whether the differences in nAChRs expression in the rat brain occur due to aging or oxidative stress we analyzed RNA and protein levels of α7, α4 and β2 subunits by RQ-PCR and Western blot validation in three brain structures: cerebral grey matter (CGM), sub-cortical white matter (SCWM) and cerebellum (Ce) of twenty one female Wistar rats. The first group consisted of five 3.0–3.5-month-old females, which was assigned as a young control group. The remaining sixteen females aged of 18–24 month were divided into three following groups: (1) aged control group of 5 rats; (2) a vehicle group of 5 rats which received intraperitoneal injections of deionized water; (3) memantine-treated group of 6 rats. In each group, the selected brain areas have also been analyzed to determinate the levels of oxidative stress. In this study, age- and stress- dependent differential RNA and protein expression levels were approved only in OGG1 and α7 nAChR proteins. In all analyzed brain structures of young and old controls, the levels of oxidized form of guanine were similar. Stress relevant to water injection increased the level

of 8-oxo-2'dG in the cerebellum of old control rats (Ce, $P<0.05$). The old controls demonstrated an important reduction of OGG1 mRNA expression in CGM and Ce regions compared to young individuals (CGM $P=0.03$; Ce $P=0.2$). Western blot analysis has also revealed a reduction of OGG1 protein in the sub-cortical white matter of old individuals (SCWM, $P=0.03$). However, there was no important influence of water administration on OGG1 expression in all brain regions. In all analyzed brain structures, expression of α7 nAChR was down-regulated in old controls compared to young controls. However, this decrease was only significant in SCWM area (SCWM, $P<0.05$). Treatment with H_2O caused a significant increase in RNA and protein levels of α7 nAChR in SCWM as compared to this brain structure of the aged control rats (SCWM, $P<0.01$). Our results suggest that aging of the rat brain is mostly associated with decreased expression of OGG1 as well as with deficit of α7 nAChR in the sub-cortical white matter. Stress relevant to water injection increases the level of 8-oxo-2'dG in the aging rat brain, but clearly overcomes the α7 nAChR deficit. A significant increase of the α7 nAChR expression in the SCWM of H2O-treated rats suggests that these receptors play an important role in compensatory mechanisms facilitating the impaired cholinergic neurotransmission following oxidative stress in the aging rat brain.

P10

Paracrine interactions between non-activated N9 microglial cells in co-culture with SN56 cholinergic neuronal cells

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Microglial cells, through the proinflammatory mediators play an important role in host defense and tissue repair in CNS. They contribute to pathomechanisms of Alzheimer's and other neurodegenerative diseases. The aim of this work was to investigate modifying effects of non-activated migroglia on cholinergic neuronal SN56 cells subjected to common neuroprotective and/or neurotoxic signals. Chronic exposure to Zn or SNP caused loss of viability (30%), inhibition of pyruvate dehydrogenase (PDH) (40%), isocitrate dehydrogenase (60 and 50%) and aconitase activities as well as decrease of acetyl-CoA levels. These alterations in enzyme activities displayed strong direct correlation with depletion of acetyl-CoA ($r=0.86$, $P<0.0001$) and inverse correlation with cell viability ($r=0.87$, $P<0.0001$). Resveratrol, free radical scavenger, increased viability of Zn/SNP treated cholinergic cells but did not overcome suppresive effects of SNP and Zn on enzymes activities. Under same neurotoxic conditions, N9 microglial cells cultured on isoporated inserts and added to neuronal culture dishes, also overcame neurotoxic effect Zn and SNP maintaining control levels of acetyl-CoA, enzymes activites and high cell viability. These data sugest that in some specific, pathologic conditions, non-activated microglia may protect neuronal cholinergic neurons against neurotoxic insults by paracrine-like mechanism by protecting their energy metabolism. On the other hand resveratrol neuroprotection may depend on entirely different yet undefined mechanism.

Supported by GUMed MN-15, MNiSW NN401029937, IP2010035370, GUMed ST-57 projects

P11

N-acetylaspartate in zinc exposed cholinergic SN56 neuroblastoma cells

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Cholinergic neurons of brain septum were found to be highly susceptible to neurodegenerative conditions. The sources of this particular sensitivity remain unclear. There is suggestion that their low resistance to cytotoxic conditions might be due to comprehensive consumption of acetyl-CoA. In cholinergic neurons the acetyl-CoA, except of intramitochondrial utilization for energy and NAA synthesis, serves as a precursor of acetycholine in their cytoplasmic compartment. The later pathway, present only in cholinergic neuronal cells, can cause temporary shortages of acetyl-CoA under cytotoxic conditions. The aim of our study was to investigate how these conditions affect N-acetylaspartate (NAA) synthesis as another acetyl-CoA consuming pathway in cholinergic SN56 neuroblastoma cells. These cells are recognized *in vitro* model of brain cholinergic neurons. Neurodegenerative conditions were induced by chronic exposition SN56 cells to zinc, a known excitotoxic agent. NAA in cholinergic neuroblastoma cells was assayed by HPLC preceded by one-dimension solid phase/ion exchange extraction. Levels of NAA in nondifferentiated (NC) and differentiated (DC) cells were equal to 70 and 56 nmol/mg protein, whereas rates of its release were 21.6 and 20.5 nmol/h/mg protein. Levels of acetyl-CoA and activities of choline acetyltransferase in NC and DC were equal to 29.5 and 23.8 pmol/mg of protein and to 0.106 and 0.232 nmol/min/mg of protein, respectively. It indicates that 20% decrease of acetyl-CoA level in DC was caused by its increased utilization for acetylcholine synthesis. Zinc inhibited TCA cycle enzymes and pyruvate dehydrogenase activities at [IC50] values well below 0.10 mmol/L. Despite of that zinc concentrations up to 125 μM increased levels of acetyl-CoA and NAA both in DC and NC by 94 and 57% and by 27% and 22%, respectively. However, 0.175 mmol/L Zn resulted in impairment of 27 and 36% of NC and DC, as measured by lactate dehydrogenase release, respectively. In these conditions levels of acetyl-CoA in NC and DC were decreased by 68% and 45%, respectively. NAA levels were also suppressed by 63% and 51%, respectively. These data indicate the existence of significant, although differential interrelationships between rates of acetyl-CoA synthesis in mitochondria of cholinergic neurons and its utilization for NAA and acetylcholine synthesis. Increased acetylcholine synthesis may contribute to greater susceptibility of cholinergic neurons to cytotoxic conditions. On the other hand, NAA synthesis may not

be a factor decreasing availability of acetyl-CoA in neurons with high expression of cholinergic phenotype. Its alterations seem to be secondary to respective shifts in acetyl-CoA levels.
Supported by project IP2010035370 Ministry of Science and Higher Education

P12

Memantine up-regulates nicotinic acetylcholine receptors expression in the cortex and sub-cortical white matter of aging rat brain

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Memantine (MEM) is a potent open channel blocker of N-methyl-D-aspartate receptors (NMDARs), and primary has been developed for treatment of neuropathic pain, symptoms of dementia and AD. On the other hand, MEM is able to act as an open channel blocker on several other ligand gated ion channels, e.g., the α4β2 and α7 nicotinic acetylcholine receptors (nAChRs). The aged-related decline in the nAChRs expression could be associated with other senescence markers, such as increased oxidative stress leading to oxidative DNA changes (high level of 8-oxo-2'dG), accompanied with significant decrease in level of the OGG1 protein involved in DNA repair process. To study whether MEM treatment might influence on the α7 and α4 nAChRs expression in the aging rat brain tissues, we analyzed RNA and protein levels by RQ-PCR and Western blot validation in three brain structures: cerebral grey matter (CGM), sub-cortical white matter (SCWM) and cerebellum (Ce) of twenty one female Wistar rats. The animals were divided into following experimental groups: the first group consisted of five 3.0–3.5-month-old females, which was assigned as a young control group, and the remaining sixteen females aged of 18–24 month were divided into three following sub-groups: (1) aged control group of 5 rats; (2) a vehicle group of 5 rats which received intraperitoneal injections of deionized water (3) memantine-treated group of 6 rats. In each group, the selected brain areas have also been analyzed to determinate the levels of oxidative stress. In CGM and SCWM brain structures the level of 8-oxo-2'dG was significantly reduced in old rats after MEM administration (CGM P=0.05; SCWM P<0.05). Western blot analysis has also revealed a significant up-regulation of OGG1 level in CGM after MEM administration (CGM P=0.05). MEM specifically up-regulated mRNA level of cortical α4 subunit in the CGM region of aging rat brain (CGM, P<0.05). In the sub-cortical white matter an important increase of α7 mRNA level has been observed after MEM administration (SCWM P<0.05). The level of α7 nAChR protein was significantly up-regulated also in CGM and Ce regions of MEM treated rats (SCWM P=0.05; CGM P<0.05; Ce P<0.05). We demonstrated that processes related to aging, such as a decreases in OGG1 and nAChRs expression can be modified after memantine administration: (1) A significant increase in the CGM of α4 and α7 subunits, as well as up-regulated α7 level in the SCWM after MEM administration suggests that nAChRs play an important role in compensatory mechanisms facilitating the impaired cholinergic neurotransmission following treatment with MEM. (2) MEM significantly up-regulates cortical OGG1 protein expression and reduces the level of 8-oxo-2'dG in CGM. (3) A significant increase in both mRNA and protein levels of α7 nAChR along with reduction of 8-oxo-2'dG in SCWM, following treatment with MEM, suggests that the effect of MEM on cholinergic function may be associated with antioxidant mechanisms. Whether these protective effects of MEM are direct or are mechanistically remote from NMDARs antagonism, have to be evaluated in the further studies.

P13

Impact of Tetrabromobisphenol A on mouse hippocampal neurons: The involvement of peroxisome proliferator-activated receptor gamma (PPAR-γ)

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Intensive production of synthetic polymer-based materials such as polyvinyl chloride, phenolic and melamine plastics, polystyrene, polyethylene or fibre-forming polymers involves the use of compounds having an inhibitory effect on the ignition of the materials, which aims enhancing their safety of use. So far approx. 70 bromine compounds are used as flame-retarding substances. Several studies have suggested that some brominated flame retardants (BFRs) could potentially pose a risk to human health. Tetrabromobisphenol A (TBBPA) is the most widely used compound among BFRs. Products with both additive and chemically bonded forms of TBBPA have been shown to release it into the environment. Although studies indicate high metabolism of TBBPA in rats and humans owing to rapid conjugation with glucuronic acid and elimination in the bile, TBBPA has been detected in cow and human milk, human serum, human adipose tissue and umbilical cord serum. Due to its structural homology with bisphenol A, TBBPA is a candidate to be one of some endocrine disruptors. TBBPA was also shown to accumulate in different brain regions and to induce the behavioral alterations (Nakajima et al. 2009). Some *in vivo* studies suggest that exposure to TBBPA during the perinatal period may affect locomotor activity and/or memory and learning. However, only few studies have been undertaken to investigate the mechanism of TBBPA neurotoxic effects. Recently, it was demonstrated that TBBPA could act as the PPAR-γ ligand in NIH3T3-L1 cells (Riu et al. 2011). The aim of the present study was to investigate the effect of TBBPA on viability of cultured hippocampal mouse neurons. Additionally, the role of PPAR-γ in TBBPA-induced cytotoxicity of hippocampus neurons was studied. The cultures of hippocampal neurons were prepared from Swiss mouse embryos on 17/18

days of gestation. The cells were cultured in phenol red-free Neurobasal medium supplemented with glutamine and B27 onto poly-ornithine-coated plates. For experiment cells were exposed to TBBPA in a following concentrations: 1, 10, 50, 100 nM, and 1, 10, 50 and 100 μM. To study the involvement of PPAR-γ in mechanism of TBBPA action the specific agonist GW1929 and antagonist GW9662 were used. Cell cultures were exposed to experimental dose of TBBPA for 6 hours and after this time media were collected for measurement of LDH activity. Our study for the first time demonstrated that TBBPA in a wide range of concentrations stimulated, in a dose-dependent manner, the LDH activity in the cultured mouse hippocampal cells. Moreover, the cytotoxic effect of TBBPA was diminished by the addition of both PPAR-γ agonist and antagonist. The presented results suggest that neurotoxic effects of TBBPA are mediated by PPAR gamma. This study implicates this receptor as a novel toxicity target for TBBPA in neuronal cells.

This work was supported by the University of Agriculture in Krakow, Poland, DS No 3242/12

P14

Extracellular alpha-synuclein contributes to gsk-3beta-dependent Tau phosphorylation in PC12 dopaminergic cells: Its role in pathomechanism of Parkinson's disease

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alpha-Synuclein (ASN) play important role in pathogenesis of Parkinson's disease (PD) and other neurodegenerative disorders. Novel and most interesting data showed elevated tauopathy in PD and suggested relationship between ASN and Tau protein. However, the mechanism of ASN-evoked Tau protein modification is not fully elucidated. In this study, we investigated the role of glycogen synthase kinase-3β (Gsk-3β) and cyclin-dependent kinase 5 (Cdk5) in ASN-evoked Tau modification in dopaminergic PC12 cells. We used real-time quantitative PCR (qRT-PCR) analysis to assess Gsk-3β gene expression and Western blot technique to analyse protein phosphorylation. The presence of apoptotic cells was assessed by Hoechst 33258 fluorescent staining, and cell viability was determined by the 2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Our data showed that exogenously added ASN (10 μM) increases Tau phosphorylation on Ser396 and specific Gsk-3β inhibitor (SB-216763, 10 μM) opposite to Cdk5 inhibitor protects cells against Tau hyperphosphorylation. Western blot analysis showed that ASN affected Gsk-3β *via* increasing of protein level and activation of this enzyme. From immunochemical studies, was found that ASN treatment leads to significant increase in GSK-3β immunoreactivity by about 20%. GSK-3β activity evaluated by its phosphorylation status assay showed that ASN significantly increased the phosphorylation of this enzyme at Tyr216 with parallel decrease in phosphorylation at Ser9, indicative of stimulation of GSK-3β activity. ASN-induced apoptotic processes leads to decrease of PC12 cells viability, the apoptotic cells determined by phase contrast together with Hoechst 33258 fluorescent staining, indicated significantly increase of apoptosis in the presence of ASN. SB-216763 prevented ASN-induced cytotoxicity and enhanced PC12 cell viability. In conclusion, all these findings suggested that extracellular ASN is involved in Gsk-3β-dependent Tau modulation and its proapoptotic effect might be mediated at least in part by the Gsk-3β catalysed Tau hyperphosphorylation and impairment of cytoskeleton stability. GSK-3β inhibitors may offer promising tool against ASN-induced Tau modification and cytotoxicity in neurodegenerative disorders.

Supported by statutory theme 9

P15

6-OHDA-induced lesion of a8-a9 dopaminergic neurons increases the harmaline-induced tremor in rats

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Degeneration of dopaminergic nigrostriatal pathway is generally accepted to be a cause of Parkinson's disease (PD) motor symptoms such as akinesia, bradykinesia and tremor. Unfortunately the extent of the degeneration does not correlate with tremor occurrence and intensity, therefore cannot explain sufficiently its appearance. Mechanisms leading to induction of tremor are still not explained. Interestingly, image analysis studies have suggested contribution of an increased activity of the cerebellum to the PD tremor. The aim of the present study was to examine whether a selective, partial lesion of dopaminergic structures – the substantia nigra pars compacta (SNc, A9) and retrorubral field (RRF, A8) would influence the tremor behaviour induced by harmaline. Harmaline model of tremor induces an abnormal synchronous activation of the climbing glutamatergic olivo-cerebellar pathway and cerebellar Purkinje cells. 6-OHDA (8 mg /2 ml) was injected unilaterally into the region of the posterior part of the SNc and RRF to induce moderate size of degeneration, similar to early PD. Harmaline was administered in a dose of 7.5 mg/kg i.p. on the 8th day after the operation and tremor of forelimbs, head and trunk was measured. In precise behavioural studies we have found that the lesion of dopaminergic system increased intensity of the tremor induced by harmaline but did not influence its character. Stereological examination of the lesion extent revealed losses of dopaminergic (tyrosine hydroxylase-immunoreactive) neurons in the anterior (30%) and posterior (72%) SNc, as well as in RRF (72% on the average). Levels of dopamine and all its metabolites, as well as noradrenaline concentrations on ipsilateral to lesioned side were moderately decreased in the caudate-putamen, while, dopamine and DOPAC in the anterior cerebellum were increased. In the caudate-putamen, the ipsi/

contra ratio of dopamine level correlated negatively, while that of dopamine turnover positively with the tremor intensity. However, in the anterior cerebellum an inverse relationship was found. Moreover, this symptom correlated positively with serotonin level and negatively with the 5-HIAA/serotonin ratio on the contralateral side of the posterior cerebellum. The presented results indicate that modulation of dopaminergic and serotonergic transmissions by the dopaminergic system lesion, modelling early stages of PD, may influence cerebellar mechanisms triggering tremor.

The study was supported by the grant of the Ministry of Science and Higher Education No N_N401_570638 and by Statutory Funds of the Department of Neuropsychopharmacology, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

P16

The role of alpha-synuclein in regulation of Cyclin Dependent Kinase 5

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Alpha-Synuclein (ASN), a small cytosolic protein enriched in synaptic terminals, was implicated in the pathomechanism of several neurodegenerative disorders called alpha-synucleinopathies. ASN was shown to be a main component of characteristic intraneuronal protein aggregates called Lewy bodies (LB) and Lewy neurites (LN), observed i.a. in Parkinson's disease, dementia with LBs and in the LB variant of Alzheimer's disease. Recent studies demonstrated that ASN may exist also in the extracellular space. Low-molecular ASN aggregates distributed in the brain parenchyma likely may be more toxic than ASN in LB, however, the exact mechanism of cytotoxicity of extracellular ASN is not fully understood. Our previous studies demonstrated the significant impact of extracellular ASN on calcium homeostasis. ASN evoked deregulation of intracellular calcium concentration leading in consequence to enhancement of nitric oxide synthesis. Deregulation of calcium homeostasis affects other calcium-dependent enzymes, including Calpains. The aim of the present study was to investigate the involvement of Calpain-dependent activation of Cyclin Dependent Kinase 5 (Cdk5) in molecular mechanism of extracellular ASN cytotoxicity. The activation of Cdk5 is regulated by binding of regulatory subunits p35 and p39. Deregulation of calcium homeostasis may induce the Calpain-mediated breakdown of Cdk5/p35 into Cdk5/p25 leading to overactivation of Cdk5. In our studies we used rat Pheochromocytoma PC12 cells incubated with exogenous ASN (10 μM) in the presence of Calpain inhibitor Calpeptin (10 μM) and Cdk5 inhibitors Roscovitine (10 μM) and BML-259 (10 μM). Our results indicated that incubation of PC12 cells in the presence of extracellular ASN (10 μM) for 48 h evoked cell death, and Cdk5 inhibitors efficiently prevented ASN toxicity, indicating an important role of Cdk5 in molecular mechanism of ASN toxicity. The level of Cdk5 protein was unchanged, but phosphorylation of Cdk5 at Tyr15 was significantly increased, suggesting that the enzymatic activity of Cdk5 is increased in ASN-treated cells. The presence of p25 protein was observed, what suggests that Calpain-dependent proteolysis of p35 occurred in ASN-treated cells. Calpeptin, an inhibitor of Calpains, prevented ASN-induced cell death, confirming the important role of Calpain activation in mechanism of ASN toxicity. In summary, our results demonstrated that alteration of calcium homeostasis evoked by extracellular ASN induce Calpain-dependent overactivation of Cdk5. These molecular processes may be involved in ASN-evoked cell death *in vitro* and probably also in neurodegenerative disorders.

P17

Inhibition of serum amyloid A protein amyloid formation

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The acute-phase protein serum amyloid A (SAA) is present in the bloodstream at the concentration below 1 μM under physiological conditions, but its level increases significantly during the acute-phase response following infection or inflammatory condition. A consequence of the long-term elevated SAA concentration is deposition of normally soluble serum amyloid A in the form of insoluble fibrils, impairing tissue structure and function. These deposits cause development of a secondary type amyloidosis, called amyloid A protein (AA) amyloidosis, which results in a death of thousands of people per annum around the world. The ability of SAA to form amyloids seems to be connected with the N-terminal portion of the molecule. The capacity of the synthetic peptides derived from the N-terminal sequence of human or mice SAA to form fibrils *in vitro* proves that the most amyloidogenic region is embedded within the protein's first 15 amino acids. We decided therefore to use peptides consisting of 11–15 amino acids and the sequence derived from the N-terminus of the parent aggregating protein as a research tool for investigation of the molecular recognition and self-assembly mechanisms that promote the formation of SAA amyloid fibrils deposits. In this study, we tested the hypothesis that non-aggregating very short peptides derived from SAA sequence would interact with the analogous region in the protein molecule or its aggregation-prone N-terminal fragment, and block its assembly into oligomers and amyloid fibrils. We designed and synthesized a peptide with the sequence 1RSFFS5, derived from the human SAA primary structure, and then tested it as a potential inhibitor of the aggregation process of SAA protein. The hypothesis about the role of aromatic interactions in amyloid fibril formation led us to test another peptide: 17LVFF20, which is derived from the sequence of Aβ. We tested propensity of the N-terminal segment (1–15) of mice SAA for amyloid fibrils formation, incubating it either alone or together with the potential inhibitors. Thioflavin T (ThT) fluorescence test was used to detect amyloid fibrils

formation. These tests confirmed that the designed peptides are able to diminish propensity of the aggregation-prone SAA peptides to form amyloid fibrils. There are currently no effective medical treatment of diseases associated with the systemic amyloidosis. We believe that results of the presented project open up new possibilities in designing compounds that are able to prevent formation of amyloid deposits and could be a starting point for the design of peptidomimetic molecules more suitable as potential drugs.

The work was supported by grant NCN nr 2011/03/N/NZ5/01460 and grant BMN No 538-8440-1042-12

P18

New method of longitudinal spinal cord slice culture

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Organotypic slice cultures were established as a model that own properties of both cell culture and animal model. The most often used slice culture is derived from hippocampus but depending on the part of brain affected with pathology, researchers established cultures from cerebellum, midbrain or striatum. Above mentioned models allowed the investigation of disorders resulting from e.g. ischemia, trauma or toxic injury. Besides the brain injury, numerous studies were focused on spinal cord pathology connected with demyelination, inflammation or injury. Here, we describe the development of an *in vitro* model of longitudinal spinal cord slice culture. Compared to cell (neuron-oligodendrocyte) co-cultures, organotypic slices retain tissue organization as well as cell-cell contacts and therefore more closely mimic the environment *in vivo*. We demonstrate the applicability of this approach for xenograft transplantation of oligodendrocyte precursor cells derived from rat brain and mesenchymal stem cells derived from human umbilical cord. Stem cells fate after transplantation was observed in two paradigms: after cell transplantation on the top of spinal cord slice cultures (SCC) or co-cultivation of cell culture with SCC space separated for 24 h. We observed the different morphology and protein expression of stem cells derived from different sources. Moreover, the same stem cells co-cultured with slices derived from different part of brain (hippocampus or spinal cord) expressed other markers. The method of longitudinal spinal cord slices enables observation of long fibers trajectory, new connections and neurorepair mechanisms. Moreover, it provides a time-efficient and cost-effective adjunct to cell lines or *in vivo* transplantation models for study spinal cord pathology or experimental therapies. Furthermore, the approach can be readily used to assess the effect of pharmacological manipulations on myelin, providing a tool to better understand myelination and develop effective therapeutic strategies to treat myelin-related diseases.

Supported by National Science Centre grant: 05728/B/NZ4/2011/01

P19

Immunoexpression of the selected compounds of the SMN complex in spinal cord neurons in patients with sporadic amyotrophic lateral sclerosis (sALS)

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease leading to degeneration and loss of motoneurons in the spinal cord anterior horns. Although etiology of the disease is unknown there is a hypothesis assuming that survival motor neuron protein (SMN) may save motoneurons from degeneration not only in spinal muscular atrophy (SMA) but also in ALS. In animal models of ALS the neuroprotective role of SMN was observed but it is not known whether the phenomenon is present in humans. Therefore we decided to examine immunoexpression of SMN and functionally associated with it gemin 2, 3 and 4 in the anterior horn neurons of patients with sporadic form of ALS (sALS). Material and methods: The material was composed of 10 spinal cords of patients with sALS who died at the age of 52–87 years 1–8 years after the onset of the disease. On formalin-fixed and paraffin-embedded spinal cords immunohistochemistry was applied. The immunohistochemical reactions were performed with antibodies against SMN and gemin 2, 3 and 4 according to the avidin-biotin-peroxidase method. Results: In all the examined cases expression of SMN and gemin 3 in spinal cord neurons was found although intensity of the immune reactions was diverse. The immunolabel were the most intense in patients with acute course of sALS and gradually decreased with longevity of the disease. Not only motoneurons but also interneurons and sensory neurons revealed immunoexpression of SMN and gemin 3. The immune reaction to gemin 2 was negative. The immunoreactivity for gemin 4 was also negative or very weak. Conclusions: (1) In humans, expression of SMN and gemin 3 in neurons is present through the whole lifespan. (2) In sALS, expression of gemin 2 and 4 is abnormal: absent or diminished respectively. (3) Presence of all components of the SMN-gemin complex is probably necessary for its normal functioning. (4) Since the immunoreactivity for SMN, and gemin 2, 3 and 4 was similar in all the examined cases and 6 from the 10 cases were at the age of 65–87 years it seems that advanced age has no influence on expression of the investigated proteins.

This study was supported by the Ministry of Science and Higher Education grant NN 401 014640

P20

Immunoreactivities of some proteins forming SMN protein complex within spinal cord in rat model of fALS

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Introduction: Amyotrophic lateral sclerosis (ALS) is a major neurodegenerative disease to afflict the adult human population. ALS causes a progressive motoneuron degeneration within anterior horns of the spinal cord. Recent data indicate the presence of mutations in the SMN (Survival Motor Neuron) gene that cause a deficits in the level of the functional SMN protein and may be an exacerbating factor in the disease development of rat model of fALS. SMN forms the multiprotein complex with selected gemins (i.a. gemin 2, 3 and 4). It is known, that the complex is important for motoneuron development in ontogenesis as well as in the proper functioning of mature motoneuron. However, the level of the SMN and individual gemin expression during the life both in humans and rats still become uncovered. The aim of our study was to determine the immunoreactivities of SMN and gemins 2, 3 and 4 in rat model of fALS during all life span. Material and method: Male rats mutated in SOD-1 were subjected to experiments. Animals at age of 60 days (group 1), 90 days (group 2), 120 days (group 3) were asymptomatic. The last group involving symptomatic rats was created from animals older than 120 days. Rats were perfused in deep anaesthesia. The spinal cords were removed and processed in routine histological staining techniques as well as in immunohistochemical methods (to detect SMN and selected gemins proteins). Labelling sections of spinal cords were analyzed with light and fluorescent microscope. Result**:** SMN and all investigated gemins were present in spinal cord motoneurons in rats from all experimental groups. However, the level of staining was weaker in the paretic rats. In the opposition to other examined proteins the immunoreaction of gemin 2 was weaker starting from 90 day of life. Conclusion**:** The SMN protein complex is present in motoneurons within the spinal cord during all animal lifespan in the rat model of familiar ALS.

This study was supported by the Ministry of Science and Higher Education grant NN 401 014640

P21

Cerebral microvessels in rat subjected to EAE: Focus on pericytes and purinergic receptor P2X7

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Blood-brain barrier (BBB) is a structure that maintains central nervous system (CNS) homeostasis by isolating it from the normal blood flow. In physiological conditions BBB prevents CNS penetration by blood-derived molecules and is a barrier for the immune system. BBB is built by tight junctions between endothelial cells of microvessels, pericytes, and astroglial end-feets. Pericytes are very important part of BBB showing a great impact on properties of endothelial cells and BBB tightness. In pathological conditions (i.e. inflammation) the structure of BBB is loosened and cells of the immune system have a free access to the brain and the spinal cord. That is the main mechanism of pathogenesis in both multiple sclerosis (MS) and the rodent model of the disease – experimental autoimmune encephalomyelitis (EAE). Overactivation of purinergic receptor P2X7, is a possible mechanism leading to neurodegeneration observed during the course of MS/EAE. This receptor has two distinct functions: it participates in maturation and release of proinflammatory cytokines or can polymerase to create transmembrane pores which can drive cell to death by apoptosis or necrosis. Thus, we hypothesized that overactivation of this receptor on pericytes may lead to cell damage and/or loss of the protective function towards BBB. In this study we first analyzed status of BBB which was determined by expression of claudin 5 – a marker of BBB tightness – in correlation with the expression of P2X7R in microvessels' fractions and brain sections of rats subjected to EAE. Using immunoblots and confocal microscopic method we found negative correlation between P2X7R and claudin 5 expression which decreased significantly in all examined time points of the disease, reaching the minimum level (45% and 70% of control) at days 2 p.i. and 4 p.i., respectively. Additionally, we present the results of pericytes features and P2X7R expression in microvessels in early time after EAE induction. Condition of pericytes was visualized by immunofluorescent staining against PDGFRβ (a marker protein). Semiquantitative level of this protein was measured using Western blot analysis of brain homogenates and isolated microvessels fraction. The pattern of observed changes suggests contribution of pericyte-located P2X7R on BBB state and the involvement of this receptor into pathological mechanisms connected with development of EAE.

P22

Lactococcus lactis as a myelin antigen delivery system in Experimental Allergic Encephalomyelitis treatment

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Experimental Allergic Encephalomyelitis (EAE) is the animal model of Multiple Sclerosis (MS), human chronic and progressive autoimmunological disease that lead to neurodegeneration

in Central Nervous System (CNS). Although there are some hypothesis, like genetic, environmental or viral factors involvement, cause and patophysiology of MS remains still unknown, and that is the reason why there is no sufficient MS treatment so far. Most common MS therapy is use of immunosupressive drugs, but that is not very effective and costs number of health complications. Also new targeted therapies are burdened with the risk of side effects, which may be even lethal. Therefore, the efficient alternative treatment is urgently needed. The autoimmune base of the disease directed treatment searching into immunological mechanisms. Few years ago we proposed application of animal spinal cord hydrolysate for inducing oral tolerance, which effect lies in reducing of immunoresponse for previously fed antigen. We presented the effectiveness of this type of treatment in EAE rat model. The success of oral tolerance with mixture of peptides stimulates us to development bacteria that may express active peptide related to myelin fragment. As far as it is known, that the dose of fed antigen is crucial in evoking oral tolerance, the aim of our study, was to investigate which dose or doses of *Lactococcus lactis* expressing myelin peptides is sufficient for EAE treatment. We used autolising strain of *Lactococcus lactis*, producing one of three myelin peptides, which are considered to be crucial in MS developement: Myelin Basic Protein (MBP aa85-97), Proteolipid Protein (PLP aa139-151) or Myelin Oligodendrocyte Protein (MOG aa35-55). We mixed all three peptide variants, and made whole-cell extracts. For our experiments we used female Lewis rats (180–200 g), which were fed with ball-pointed needle with mixed bacteria extracts for 20 days. Doses of preparations ranged from 101 to 108 cells/rat/feeding suspended in 0.5 ml PBS. At the 10th day of feeding, EAE was evoked by hind paw injection of guinea pig spinal cord homogenate in Freund Adjuvant with *Mycobacterium tuberculosis*. During the whole experiment animals were weighted, and clinical symptomes were observed. The obtained results demonstrated, that the sufficient doses of *Lactococcus lactis* expressing myelin peptides, given orally to animals are 103 and 106 cells/rat/feeding. Further experiments including cytokine level measurement and microscopic observation of rats spinal cord are in progress.

P23

Alteration of sphingolipid biostat in experimental model of oxidative stress evoked by 1-methyl-4-phenylpyridinium (MPP+)

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Bioactive sphingolipids are important molecules that control wide spectrum of neuronal processes including neurotransmission, synaptic function, cells proliferation and death. Sphingosine kinases (SK1/2) are conserved enzymes that phosphorylate sphingosine to sphingosine-1-phosphate (S1P), which acts as a primary and secondary messenger. S1P binds to 5 receptors and plays essential role in neural signal transduction under physiological and various pathological conditions. Although growing evidence suggests important role of SK1/2 and S1P in neurodegenerative disorders including ischemia, inflammation and Alzheimer's Disease, till now disturbances of sphingolipids homeostasis in Parkinson's Disease (PD) remain unknown. Our study try to explain the role of SK1/2 and S1P in molecular mechanism of cell survival and death in model of oxidative stress evoked by neurotoxin 1-methyl-4-phenylpyridinium (MPP+), compound widely used in experimental model of PD. Our data presented that MPP+, comparable to SK inhibition evoked death of human *neuroblastoma* cells SH-SY5Y in time and concentration dependent manner. These changes are accompanied by increased free radicals concentration in these cells. Reduced level of SK1 protein was detected in SH-SY5Y cells after 24h exposure to MPP+ comparing to control. Moreover S1P pretreatment enhanced survival of these cells and protein level of SK1 comparing to MPP+ treated cells. Our data indicated that MPP+ evoked neuronal death is mediated by SK1/2 inhibition and altered sphingolipids signaling. These molecular events lead to caspase dependent apoptotic cells death and poly(ADP-ribose) polymerase-1 (PARP-1) degradation. All above results presented the alteration of sphingolipid biostat in experimental model of PD and suggested that S1P can offer novel, protective strategy.

Supported by NCN Grant 5870/B/PO1/2011/40

P24

The role of sphingosine kinases/sphingosine-1-phosphate in the regulation of alpha-synuclein and amyloid beta precursor protein secretion. Implications for neurodegenerative disorders

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Sphingolipid deregulation may be an important factor of age-related neuronal stress vulnerability. Current data suggests potential links between sphingosine kinases (SphK1&2), their product sphingosine-1-phosphate (S1P) and age-related protein conformation diseases. The aim of this study was to investigate a possible role of SphKs in alpha-synuclein (ASN) and amyloid beta (ABeta) precursor protein (APP) level and secretion. The studies were carried out using human SH-SY5Y neuroblastoma cell line stably transfected with the human gene for α-synuclein (ASNwt). Sphingosine kinase inhibitor (SKI) significantly increased ASN secretion in concentration-dependent manner. S1P also displayed similar influence. Neither compound exerted any significant effect on the ASN protein level. S1P may act *via* cell surface receptors or as an intracellular second messenger.

The similar effect of S1P and SphK inhibitors on ASN secretion may suggest that the regulation of its release is critically dependent on the varied (intra)cellular targets of SphKs and downstream signaling pathways. We have found that stable human ASNwt expression in SH-SY5Y cells caused a three-fold, significant increase of the cellular APP level. In ASN-transfected cells S1P enhanced APP secretion and reduced its intracellular level. This could be linked to the recently reported effect of S1P on secretase beta activity. Inhibition of SphKs significantly decreased APP secretion. In summary our data indicates that endogenous ASN regulates APP level in SH-SY5Y cells and that sphingolipids play a crucial role in the secretion of ASN and APP. These processes may have significant impact on neuronal survival and health.

P25

Ceramide and sphingomyelin levels are increased in brains of rats exposed to streptozotocin

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Insulin insufficiency and increased glucose levels are the major features of diabetes type 1 leading to cognitive dysfunctions and neurodegeneration. A reason why different brain structures are characterized by diverse response to increased glucose level is not known. Our previous study showed increased ceramide levels in the brains of rats with diabetes induced by streptozotocin (STZ) injection, which was abolished by myriocin, the inhibitor of serine palmitylotransferase. Ceramides may be important mediators of neuropathological changes and its elevation was found in many brain disorders. The main goal of our present study was to verify if hippocampus, prefrontal cortex and cerebellum response differently to hyperglycemia/hypoinsulinemia in terms of changes in sphingolipids concentrations. We attempted to identify potential source of ceramides by measuring the sphingomyelinase concentrations and by blocking the ceramide de novo synthesis pathway. We found that in cerebellum and hippocampus of hyperglycemic animals sphingolipids concentrations underwent subtle modifications while prefrontal cortex exhibited massive changes in ceramides and SMs content. Total ceramide levels was significantly elevated in prefrontal cortex of diabetic rats, which was reduced by myriocin, while rats exposed to STZ showed only small increase of total SM in this brain structure. The increased content of ceramides containing SAFAs (saturated fatty acids) in prefrontal cortex was diminished by myriocin. SAFA-contained SMs did not present changes. Elevation of MUFA- (monounsaturated fatty acids), and PUFA-ceramides (polyunsaturated fatty acids) in prefrontal cortex of STZ-treated rats was reduced by myriocin, similarly as MUFA-SMs augmentation. PUFA-ceramides and PUFA-SMs experienced only slight modifications. Both – ceramides and omega-6 – SMs increased dramatically and were downregulated by myriocin. We conclude that the prefrontal cortex may be particularly sensitive to hyperglycemic conditions and hypoinsulinemia. Moreover, *the novo* synthesis seems to be an important pathway of ceramide generation since usage of myriocin strongly reduced ceramide levels enhanced by STZ injection. Augmentation in ceramide content was correlated with enhancement of SMs production. These unexpected results may be explained by the incorporation of redundant ceramides into SMs, a mechanism by which the toxic level of ceramides is reduced in the brain.

Supported by grant 123-27575 P from the State Committee for Scientific Research, Warsaw, Poland

P26

Neurotensin NTS1 receptors mediate the cardio-respiratory effects of [Ile 9]PK20, a novel chimeric peptide

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Opioids with their large potency in pain relieve have certain undesirable effects like tolerance, dependence and respiratory depression. This is the reason for permanent efforts to create new analgesic compounds devoid of the adverse side effects. PK20 is a novel hybrid of opioid-neurotensin peptides synthetized from the C-terminal hexapeptide of neurotensin and endomorphine-2 pharmacophore. This chimeric compound shows clear central and peripheral antinociceptive activity in experimental animals, however nothing is known about the influence of PK20 on respiratory and cardiovascular parameters. The present study was designed to determine the cardiorespiratory effects exerted by an intravenous injection of [Ile9]PK20, analog of PK20 with substitution of tert-leucine by isoleucine9. We also attempted to evaluate whether the effects of the hybrid are mediated by the peripheral neural pathway like vagus nerve. Finally, the contribution of NTS1 neurotensin and opioid receptors in the [Ile9]PK20 cardiorespiratory pattern was tested. 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 [Ile9]PK20 at a dose of 100 μg/kg in the intact rats provoked an increase in tidal volume preceded by a prompt short-lived decrease. Immediately after the end of injection brief acceleration of the respiratory rhythm, was ensued by the slowing down of breathing. Changes in respiration were concomitant with a bi-phasic response of the blood pressure: immediate increase was followed by prolonged hypotension. Bilateral midcervical vagotomy eliminated both: tidal volume and respiratory rate responses. Blockade of NTS1 receptors with an intravenous dose of 500 μg/kg of SR 142948, significantly lessened post-[Ile9]PK20 cardiorespiratory effects. Naloxone hydrochloride – antagonist of opioid receptors – failed to block [Ile9]PK20-evoked responses. This study depicts that [Ile9]PK20 acting through neurotensin NTS1 receptors augments the tidal component of the breathing

pattern through the vagal pathway. This latter mediates also the respiratory timing response to the drug. Blood pressure effects evoked by an intravenous injection of [Ile9]PK20 occur besides the vagal pathway and might result from activation of the central and peripheral vascular NTS1 receptors. In summary the respiratory effects appeared not to be profound. However, considerable and extended hypotension evoked by [Ile9]PK20 sets the main disadvantage of an analgesic compound.

P27

The role of microglia cells activation in the effects of opioids

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Development of neuropathic pain is accompanied by many changes in immune and glial cells. These changes correspond to activation of immune and glial cells that have been shown to influence the opioid effectiveness and can be modulated by minocycline (a potent inhibitor of microglial activation). In earlier study we have demonstrated that function of opioidergic neurons may be modulated by the immune system. These changes have been shown to be responsible for the efficacy of opioids. The aim of our study was to examine the effect of the minocycline-triggered inhibition of microglia activation on the injury-induced changes and the efficacy of mu and delta opioid receptor ligands in a rat model of neuropathic pain (chronic constriction injury to the sciatic nerve). In cell culture studies, we examined the influence of opioids (morphine, DAMGO, DPDPE, deltorphin II) on activated primary cultured rat microglia by using MTT and/or NO assays. All experiments were performed according to the IASP recommendations and were approved by a local Bioethics Committee. On the spinal cord level the injury to the sciatic nerve induced an up-regulation of IL-1beta, IL-6 expression, CX3CR1 and C1q (marker of microglia, macrophage and leukocyte activation). Chronic administration of minocycline not only diminished neuropathic pain-related behavior and C1q-positive cell activation, but also attenuate the changes in proinflammatory factors like IL-1beta, IL-6 and CX3CR1 in the spinal cord and DRG. In *in vivo* experiments, the analgesic effects of mu-opioid (morphine and DAMGO), but not delta-opioid (DPDPE, deltorphin II) receptor ligands were lower in the rats under neuropathic pain. Moreover, the analgesic effects of morphine and DAMGO, but not DPDPE and deltorphin II were significantly potentiated by minocycline chronic administration. Our *in vitro* findings that non-stimulated microglia cells respond differently to opioids in comparisons with stimulated cells as measured by MTT and/or NO assays, corresponded well with the results of *in vivo* studies. Our study underlined that inhibition of microglial activation could differently influence analgesic effects of mu- but not delta-opioid ligands in injury-induced pathologies, which may influence the effect of various opioid drugs used in chronic pain therapy.

P28

Peripheral antinociceptive effect of biphalin in a mouse model of cancer pain

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It is generally accepted that classical opioids exert their antinociceptive effect mainly when binding to opioid receptors located in the central nervous system. However, a growing body of evidence points to the relevance of peripheral opioid receptors in periphery pathology, including cancer. A cancer is very often the cause of pain resulting from peripheral metastasis. The peripheral component of antinociception induced by a dimeric enkephalin analog – biphalin showing limited blood-brain-barrier permeability may prove important in cancer pain therapy. An additional advantage of biphalin is a possibility to treat pain symptoms with reduction of side-effects – a result of the central action of some other opioid analgesics, e.g. morphine. We examined the peripheral and central analgesic effect of 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 PBS-injected individuals. Biphalin produced stronger analgesia in the tumor-bearing paw than morphine upon a comparable central effect. Our results suggest that biphalin analgesia manifested in the periphery is linked to a less effective transport through the blood-brain barrier. We speculate that the centrally effective dose of biphalin equipotent to morphine simultaneously produces analgesia *via* peripheral opioid receptors. Thus, biphalin may become useful in cancer pain treatment as an alternative drug executing a local as well as a central analgesic response with limited undesirable side-effects

P29

The effects of ketogenic and calorie restricted diets on epileptic seizure in rats with mechanical brain injury

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Traumatic brain injury (TBI) is a major cause of mortality and morbidity in children and young adults. It initiates multiple cascades of events that lead to acute metabolic dysfunction and cellular energy crisis. TBI remains one of the most common and important causes of acquired epilepsy nowadays. The ketogenic diet (KD) is a specialized high-fat low-protein and low-carbohydrate diet which mimics the anticonvulsive effects of fasting, which were known to suppress seizures. KD is used primarily in children with seizures refractory to standard anticonvulsive drugs (AEDs). Many studies on the anticonvulsant effects of a KD have been performed. Unfortunately, the mechanism of action of the ketogenic diet remains unclear. Although the ketogenic diet is the best dietary ther-

apy for epilepsy, there are other possible approaches including overall restriction of caloric intake. Dietary restriction seems a promising alternative to classic ketogenic diet, possibly because it is associated with higher levels of ketone bodies, which are themselves neuroprotective. Caloric restriction (CR) is defined as a decrease in energy intake without lowering nutritional value. CR improves behavioral outcomes after ischemic brain injury in rats and could possibly act as a neuroprotective factor in global ischemia. It has been also shown that chronic administration of CR may provide protection in the event of TBI. The aim of this research was to study the changes in susceptibility to pilocarpine-induced epileptic seizures in rats with mechanical brain injury. In 30-day-old male Wistar rats (P30), mechanical brain injury was performed. Immediately after, the calorically unrestricted ketogenic diet (KD) and calorically restricted standard laboratory rat chow diet (CR) were introduced. In order to check how the ketogenic diet and caloric restriction alone influence the epileptic seizure susceptibility, two groups of 30-day-old rats were fed KD and CR untill postnatal day 60. At that time, seizures were induced by pilocarpine injection. During the following 6-h period, the animals were continuously observed and motor seizures intensity were rated on a 6-point scale. We have found that KD, both alone or administered to animals with history of experimental brain injury, significantly increases the maximum intensity of pilocarpine-induced seizures, compared to CR fed healthy and injured controls, respectively. Surprisingly, KD and CR seem to have opposite effects in healthy animals as well as animals with a history of experimental brain injury. We have found that KD increases the maximum intensity of pilocarpine-induced seizures, compared to both calorically restricted and unrestricted normal diets. CR, on the other hand, decreases the seizure-genic effect of pilocarpine. This results in a continuum in which calorically restricted animals exhibit the weakest, and KD-fed animals the strongest seizures. To our knowledge, the effects of calorically-restricted and ketogenic diets on pilocarpine-induced seizures have not been previously studied. In other well established models of epilepsy, KD either attenuates or has little effect on seizure intensity.

P30

Degeneration of astrocytes in the prefrontal cortex induces depressive-like behavior in rats: Behavioral, immunochemical and histological studies

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Postmortem studies of depressed patients showed that one of the most consistent findings is a decrease in the density of glial cells in human brain cortical regions, especially in the prefrontal and cingular areas. Furthermore, a decline in the number of astrocytes in the prefrontal cortex was found in rats after chronic unpredictable stress – one of the generally accepted animal models of depression. An important function of astrocytes in the brain tripartite synapse is the uptake of released glutamate. Hence the basic consequence of the loss of astrocytes is a reduction in glutamate uptake and an excess of glutamate in the synaptic cleft. The glutamatergic predominance in the excitatory-inhibitory balance is postulated to be involved in the pathogenesis of depression. Recently, depressive-like behavior have been demonstrated in rats after astrocytes ablation. Therefore in the present study we tried to ascertain whether astroglial degeneration in the prefrontal cortex was sufficient to induce a depressive-like behavior and could serve as an animal model of depression. Astrocytic toxin L- or D,L-alpha-aminoadipic acid (AAA), 100 µg/2 µl, was microinjected bilaterally into rat medial prefrontal cortex (PFC). The toxins were injected twice, on day 1 and 2; afterwords depressive-like behavior was assessed by a forced swim test on day 5 of the experiment. Some rats were additionally treated with the antidepressant imipramine (30 mg/kg, i.p.) 24, 5 and 1 h before the forced swim test. The rats' brains were taken out for an analysis on day eight. Histological verifications of the injection sites and immunohistochemical staining for the astrocytic marker glial fibrillary acidic protein (GFAP), were carried out. The GFAP positive cells were stereologically counted in the PFC. Also the level of GFAP expression was determined by the Western blot analysis in all the experimental groups. It was found that both L-AAA and DL-AAA induced a significant increase in immobility time in the forced swim test, without changing the overall locomotor activity, which indicates depressive-like effects of these compounds. The immunohistochemical and Western blot analyses showed a significant decrease in the number of GFAP-positive cells and GFAP level in the PFC of toxin-treated rats. The decrease amounted to ca. 50%. Both the behavioral and the GFAP changes were reversed or partially inhibited by imipramine injection. The obtained results suggest an important role of astrocytes in the PFC in mood regulation; moreover, they indicate that the degeneration of astrocytes in this structure may be used as an animal model of depression.

This study was supported by Grant POIG.01.01.02-12-004/09Friday, November 23, 2012

Session III
RECENT ADVANCES IN BASIC AND TRANSLATIONAL CEREBRAL ISCHEMIA RESEARCH

SIII-L1

Clearing the fog: Changes in T2 relaxation time after stroke reflect clearing processes

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CT and MR imaging techniques are frequently used for the diagnosis and progress monitoring of ischemic stroke in clinical practice and research. After stroke, both methods are characterized by a transient pseudo-normalized imaging signal, the so-called fogging phenomenon. This study evaluates potential pathophysiological changes associated with fogging, as well as its influence on the correct determination of

the ischemic lesion in a rat stroke model. Male spontaneously hypertensive rats were subjected to permanent middle cerebral artery occlusion. Ischemic lesion volume, brain edema and grey scale value spread within the ischemic lesion were determined on T2-weighted MR sequences at days 1, 4, 8, 11 and 29 after stroke onset, and compared with immunohistochemistry for astrogliosis, microglia/macrophage infiltration and angiogenesis. All animals showed MR fogging at days 4, 8 and 11 after stroke. The transient normalization of T2 signals occurred independently from the development of infarct volumes, but coincided well with the spatio-temporal occurrence of necrosis, angiogenesis and microglia/macrophage infiltration. Our results suggest that the fogging effect reflects the clearance of necrotic tissue within the ischemic lesion and is thus not relevant for the determination of the lesion volume.

SIII-L2

Experimental approaches to enhance functional recovery following cerebral ischemia

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Stroke is a major cause of adult disability that poses an enormous healthcare burden. Effective pharmacotherapy for stroke remains an unmet need. Development of restorative therapies has been identified as a potential alternative in stroke. Emerging understanding of brain repair and plasticity mechanisms have revealed therapeutic targets including inhibition of axonal sprouting (e.g., Nogo, MAG), altered perilesional GABA and glutamate receptor signaling, endogenous neurogenesis and angiogenesis. The main advantage with restorative therapies is the delayed treatment after acute necrotic cell death, when patients are stable. In addition, restorative therapies can be combined with intensive rehabilitation and medication for poststroke complications to further facilitate recovery process. The problem with patient studies is, however, that many pharmaceutical companies have scaled down their stroke programs, because of failures with neuroprotective compounds. We should convince industry that restorative drugs target completely different mechanisms with extended therapeutic time window offering an attractive approach to help stroke patients.

SIII-L3

Transplantation of autologous bone marrow stromal cells (BMSC) for ischemic stroke – strategy and tactics for clinical application

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Objective: There is increasing evidence that the transplanted bone marrow stromal cells (BMSC) significantly promote functional recovery after central nervous system damage in the animal models of various kinds of CNS disorders, including cerebral infarct. However, there are several shortages of information when considering clinical application of BMSC transplantation for patients with neurological disorders. In this meeting, therefore, we discuss what we should clarify to establish cell transplantation therapy in clinical situation and describe our recent works for this purpose. Methods and Results: The BMSC have multiple abilities to differentiate into the neural cells and to promote neuronal survival and axon elongation, contributing to rebuild the neural circuits in the injured CNS. Using optical imaging and MRI techniques, the transplanted BMSC can non-invasively be tracked in the living animals for at least 8 weeks after transplantation. Clinical MR apparatus can visualize the tagged BMSC in the brain. FDG PET is quite valuable to monitor the recovery of brain metabolism after transplantation. The BMSC can be expanded using the animal protein-free culture medium within a clinically relevant period. G-CSF is useful to enhance their proliferation when the BMSC are obtained from the aged patients. There are optimal dose and timing of BMSC transplantation to yield significant therapeutic benefits. Conclusion: It is urgent issues to develop clinical imaging technique to track the transplanted cells in the CNS and evaluate the therapeutic significance of BMSC transplantation to establish it as a definite therapeutic strategy in clinical situation in very near future.

SIII-L4

Preclinical and clinical experience with non-invasive imaging and homing of stem cells

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Cell therapy is a promising strategy for the treatment of neurological diseases. Positive therapeutic effects have been obtained in animal models, and, recently, in a few clinical trials; however, the efficacy is still limited. Rather than intracerebral transplantation, body fluids, such as blood or CSF, are increasingly being used as a route of stem cell delivery to achieve a wider distribution of cells and to make the procedure less invasive. For circulating fluid-mediated cell transplantation, the improvement of cell homing to lesion sites is critical in advancing stem cell therapy. The optimization of cell delivery and targeting can be greatly accelerated with the use of non-invasive cellular imaging. Because of the high signal of iron oxide nanoparticles (SPIO) and the translational potential, MRI is the leading technology for *in vivo* cellular imaging. While MRI, because of its high spatial resolution, is unprecedented for the depiction of the location of transplanted cells, it does not provide information about cell viability. But, this can be complemented with reporter gene-based bioluminescent imaging (BLI) to image cell survival. MR imaging of SPIO-labeled human stem cells enables visualization of cell trafficking following intracarotid delivery. Transplantation of large, mesenchymal stem cells in a rat model of stroke resulted in early entrapment of cells in the ipsilateral hemisphere. The distribution of cells was dependent on the time from stroke induction to cell transplantation (1, 2, 3, and 7 days) and could be related to the evolution of blood sup-

ply to distinct compartments of this hemisphere over the first week after stroke. A massive outflow of cells from the brain was observed within the first day after transplantation. The transplantation of small, human glial restricted progenitors (GRPs) cells affected rat brain homing only if these cells were engineered to express VLA-4 integrin (VLA-4+), and the endothelium was activated by LPS to express VCAM-1, a receptor for VLA-4. The transplantation of VLA+ GRPs in a rat model of stroke affected the selective inflow of cells to the lesion and the persistence of the iron oxide signal for over a month. However, BLI revealed a gradual decrease of cell viability, with a loss of bioluminescence within one week after transplantation. The signal disappearance was thought to be the result of the rejection of human cells in non-immunosuppressed animals. The monitoring of cell fate post transplantation into the cerebral ventricles is also crucial, since the circulation of the CSF may affect the homing of transplanted cells. MR imaging of the intracerebroventricular (ICV) delivery of SPIO-labeled cells in a pediatric patient showed the feasibility of the procedure, with no adverse events and successful detection of SPIO-labeled cells. In this patient, in particular, cells transplanted to the frontal horn of the lateral ventricle were found in the occipital horn. Considering the patient position during surgery, such cell distribution could have resulted from cell sedimentation. The location of the cells was stable on follow-up MRIs, but a gradual disappearance of the SPIO signal was observed. ICV delivery in large animals (pig) revealed a more dispersed distribution of cells, which may be attributable to slit ventricles.

Session IV
INSIGHT INTO CNS EPIGENETIC REGULATION

SIV-L1
Genomic imprinting and the epigenetic regulation of adult neurogenesis

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Genomic imprinting is a normal process causing genes to be expressed from only one of the two parental chromosome homologues according to their parental origin. Imprinted genes function in a range of developmental processes. In recent years, data has emerged indicating discordance of imprinting between mouse and man, polymorphic imprinting between different individuals and tissue-specific imprinting within individuals. This suggests that imprinting might be an adaptable and dynamic process with the potential to act as a mechanism regulating gene dosage in different developmental contexts. Delta-like homologue 1 (Dlk1) is a paternally expressed imprinted gene that encodes both a transmembrane protein and a secreted isoform generated by alternative splicing, and is an atypical member of the Notch/Delta/Serrate family of developmental signalling molecules. Although widely expressed during embryonic development, only a few tissues including neurogenic regions of the brain retain Dlk1 expression in adults. Analysis of neurogenesis in the SVZ of Dlk1 mutant mice shows a reduction in the numbers of stem cells *in vivo* and an impairment of newborn neurons incorporated into the olfactory bulb as well as fewer primary neurospheres *in vitro* suggesting that normal levels of Dlk1 are necessary for the life-long maintenance of neural stem cells (NSCs). Within the SVZ, DLK1 is a niche factor secreted by astrocytes and that membrane-bound DLK1 is required in NSCs to respond to it. In contrast to the neighbouring Gtl2 gene, we observe specific absence of Dlk1 imprinting in the stem cell and astrocyte populations in the SVZ niche indicating that the mechanism conferring biallelic expression can override the imprint selectively at Dlk1 to control normal neurogenesis in the adult brain. This neurogenic requirement for both the maternally and paternally expressed alleles of the canonically imprinted Dlk1 gene supports the hypothesis that control of gene dosage by absence of imprinting is an important developmental process. We are testing the hypothesis that other imprinted genes important in neurogenesis may also modulate imprinting to control gene dosage.

SIV-L2
The role of microRNAs in neurological disorders

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microRNAs regulate all the cellular processes, and are strongly involved in differentiation of stem cells. Disturbances in the regulation of microRNAs expression and activity may deviate the stem cells fate, impairing their differentiation and contributing to diseases initiation or progression. In this talk the role of microRNAs in certain neurological conditions will be discussed.

SIV-L3
Epigenetic mechanisms of gene expression regulation in neurological diseases

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Neurological diseases, including intellectual disability (ID), can be caused by disturbances in epigenetic regulation of specific genes that encode proteins necessary for appropriate central nervous system functioning. The “epigenetically caused” diseases can be due to the imprinting defects formed during germinal cells development or gained throughout life as a somatic changes. They can also result from abnormal functioning of transcriptional machinery caused by mutations in genes coding for specific proteins. Two most classical examples of disease caused by imprinting defect in germinal cells are Prader-Willi and Angelman syndromes, both characterized by ID and developmental delay. Both these diseases are caused by

altered epigenetic regulation of genes localized on chromosome 15 (region q11–q13) that can be due to chromosome deletion or uniparental disomy. The other neurological disease that is related to abnormal epigenetic regulation is Fragile X syndrome characterized by ID and specific behavior. Almost all disease cases are due to the expansion of CGG repeat (>200) in the 5'UTR of FMR1 gene that leads to promoter methylation and lack of FMRP protein that is indispensable for neuron development and signaling. The example of neurological "epigenetic diseases" caused by altered transcriptional regulation is Rett syndrome caused by the mutation presence in MECP2 gene or its variant – Rett-like syndrome caused by the mutation in CDKL5 gene. Both these diseases are characterized by ID and childhood epilepsy. Herein, we present our experience from the research and diagnosis of above mentioned disorders in the context of neurological pathways altered by improper epigenetic regulation.

SIV-L4

Epigenetic modulation of neural stem cells: The influence on reprogramming and differentiation

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The differences between pluripotent and differentiated cells include stage specific chromatin structure and transcriptional hierarchy which are both regulated by and orchestrated with the epigenetic events. Such events include alterations in DNA methylation, histone modifications, polycomb gene group and noncoding RNA expression. In this lecture the overview will be given on chromatin dynamics and epigenetic modification status during neural stem cell development. Examples of regulatory machineries responsible for gene repression at each stage of neural stem cell development will be indicated. Neural stem cells are characterized by their ability to give rise to multiple neural lineages, including neurons, astrocytes, and oligodendrocytes. Previously we have obtained neural stem cells from human cord blood (HUCB-NSC) which has been investigated by our group for their ability to be reprogrammed or differentiated using combination of small molecules as epigenetic modulators. It was demonstrated that the influence of small chemicals: histone deacetylases (Trichostatin A -TSA) and methyltransferases (RG-108) on the expression of Oct4, Sox2, Rex1 and Nanog genes depended on developmental stages of HUCB-NSC. Incubation for 5 days in reprogramming conditions followed by short time culture (3 days) in ESCM (Embryonic Stem Cell Medium) on Matrigel resulted with only partial stimulation of the investigated pluripotency markers. Nevertheless, the differences in expression pattern between tested treatment conditions were observed. Cells grown under Serum Free culture conditions treated with a combination of epigenetic inhibitors as well as recombinant proteins after longer incubation in ESCM on Matrigel were able to gain full iPS morphology and showed continuous expression of pluripotent genes. None of the mentioned above factors were alone sufficient to reprogram NSC to stable pluripotency state. Additionally the mechanism of regulation DNMTs and HDACs genes (namely DNMT 3B and HDAC1) by methyltransferases and histone deacetylases inhibitors and their role in reprogramming and differentiation process of HUCB-NSC have been tested. The present study demonstated that small molecules such as TSA and RG-108 together with reprogramming proteins in lowered oxygen conditions can change epigenetic status of cells and activate and sustain pluripotent state in HUCB-NSC. In conclusion it is evident that the developmental stage of the cells and epigenetic modulation play an important role in the induction of pluripotency genes expression.

Sponsored by grant from Polish Ministry of Scientific Research and Higher Education Nr 5978/B/PO1/2010/38

POSTER PRESENTATIONS

P31

Phenotypes of cell pools populating the injury site in a rat model of surgical brain cortex lesion

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Because of their potential for self-renewal and the ability for generating many differentiated cell types, progenitor cells are a key player in regenerative and repair processes. In the central nervous system, pools of these cells have been identified in two regions: the subgranular zone of hippocampal gyrus dentatus and the subventricular zone. Neural stem cells that reside in these regions are subject to a specific neurogenesis-stimulating and -regulating environment called 'niche'. Our model of surgical brain injury (SBI) opens the avenues for studying the mechanisms of repair and reconstruction of brain cortex and enables demonstrating the presence of possible vascular niches in the peri-lesion zone. The present studies were aimed at characterizing of the immune phenotype of the cells that populate this region. The peri-lesion area of the brain cortex showed the presence of dying neurons and glial cells since the first post-lesion day. Simultaneously, activated microglial cells and astrocytes appeared, and part of the latter formed a scar on the surface of the damaged cortex. Another fractions of the cells that appeared following the SBI in both the lumen and the vicinity of blood vessels expressed either the macrophagal/monocytic marker CD14, or the marker of hematopoietic progenitor cells and small vessel endothelium CD34. Beginning on the first post-SBI day, the peri-lesion area showed also the presence and accumulation of a variety of cells with immature phenotypes.

These included immature endothelial cells building new blood vessels (angiogenesis) and cells with phenotypes of other brain parenchyma-forming cell subpopulations: (1) nestin-positive astroglial and non-glial cells, (2) cells expressing the marker of juvenile astrocytes vimentin-positive, and (3) cells showing doublecortin immunoreactivity (the marker of early differentiated neurons). These results clearly indicate that during the early post-SBI period the peri-lesion zone is being populated by a heterogenic pool of morphologically immature cells that most likely herald the advent of reconstruction and/or repair of the injured brain region.

Supported by the Polish Ministry of Science and Higher Education grant No N N404 522838

P32

Adjuvant stem cell-based therapy in acute retinal injury after sodium iodate administration in mice: Morphological and functional study

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Ophthalmic diseases, especially retinal degeneration belong to prominent causes of disability in developed countries. Thus, special attention has been focused on research aimed on establishing new protocols of efficient stem cell (SC)-based therapy of these disorders. The aim of this study was to determine and optimize a new strategy of SC-based therapy of selectively damaged retina after sodium iodate ($NaIO_3$) administration in C57BL/6J mice. First, we sought to assess the regenerative mechanisms triggered after acute chemical injury of retinal pigment epithelium and neurosensory retina induced by $NaIO_3$, in mice. The intravenous injection of $NaIO_3$ provides a useful model for the study of retinal degeneration since it mimics some retinal degenerative diseases in humans, e.g., gyrate atrophy, retinitis pigmentosa or age-related macular degeneration. We evaluated the kinetics of morphological and functional changes within mice retinas injured with $NaIO_3$ *via*: (1) morphological study; (2) evaluation of expression of selected neurotrophins (NTs) in injured retina; (3) visualization of proliferating and apoptotic cells; (4) electrophysiology. Our findings revealed that massive destruction of the tissue was associated with irreversible retinal dysfunction, whereas moderate retinal injury triggered regenerative mechanisms that restore bioelectrical function of the damaged retina. Next, we performed intravitreal transplantation of murine GFP+Lin- cells on the 1st day since $NaIO_3$ administration. We analyzed number and localization of intravitreally injected GFP+Lin- cells within recipients' retinas as well as the retinal functional changes (electroretinography). By employing stem/progenitor cell-based therapy we achieved noticeable improvement in retinal function, particularly in the case of only partial primary destruction of retinal tissue. Furthermore we investigated the neuroprotective effects of different NTs, administered intravitreally *via* genetically modified SCs into degenerating retinal tissue. The synthetic viral vectors based on lentivirus (LVs) backbone was used to deliver NT genes into mesenchymal stem cells isolated from bone marrow. Then, we conducted multipart analysis based on functional tests, e.g., electroretinography as well as histological, immunohistochemical, morphometric, and molecular biology studies. We found that specific, exogenously administered NTs, such as NT4/5 could effectively stimulate photoreceptor survival in the degenerating retinas. Our findings reveal that the proposed therapeutic strategy could be recommended as adjuvant therapy supporting endogenous regeneration of acute retinal damage. However, further more extensive studies are needed before the introduction of this kind of therapy into patients.

P33

Intracerebral transplantation of human umbilical cord blood-derived neural stem cells (HUCB-NSC) in rats after focal brain ischemia ameliorates tissue expression of endogeneous and exogenous neurotrophic factors

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There is a great interest in the possibility of repairing the nervous system by transplantation new cells that can replace those lost through damage in neurological disorders. Key functions such as the replacement of neural cells have been recently challenged by intrinsic bystander capacities of undifferentiated donor cells to restore these cells. A comprehensive knowledge how transplanted stem cells exert their therapeutic achievements is still lacking. Here we investigated the effects of HUCB-NSC infused into the damaged rat brain at 72 h post ischemia on endogenous neurogenesis. The goal of our studies was to examine the proliferation and migration of host progenitor cells, analyze the substantial matrix remodeling of tissue and the presence of neurotrophic factors in rat brain after focal ischemia followed by HUCB-NSC transplantation. Methods: 2×10^4 HUCB-NSC were transplanted into corpus callosum of naive or focally injured rat brain 3 days after ischemic insult. At 1, 3, 7 and 14 days rat brains were removed. Endogenous cell proliferation was determined by BrdU incorporation. Then immunocytochemical analysis of doublecortin (DCX) and PSA-NCAM (markers expressed by immature migratory neuroblasts), and in situ zymography of MMPs activity was performed. Additionally, total RNA was isolated from rat brain tissue and RT-PCR was performed using sets of primers of each of human and rat neurotrophic factor genes. Results: OUA-induced brain lesion resulted in increase of proliferating ($BrdU^+$) and migrating (DCX^+ and PSA-$NCAM^+$) cells in subventricular zone (SVZ) and sub-

granular zone (SGZ) regions in comparison to intact rats. This response has been potentiated by HUCB-NSC transplantation. At 7th day after HUCB-NSC infusion the intense migration of DCX^+ cells from SVZ towards ischemic boundary regions of the striatum was observed. Moreover, the activation of MMPs in cells was visible in SVZ. 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. In OUA-induced lesion rat brain tissue, the expression pattern of rat-origin neurotrophic factors mRNA was higher than in intact rats. HUCB-NSC transplantation into focal brain ischemic tissue significantly increased mRNA expression of several rat-origin growth factors, such as GDNF, CNTF responsible for regulation of proliferation and maturation of stem cells as well as IGF-1, HGF and presaposin functioning as anti-apoptotic mediators. The significant increment was observed 7 days after HUCB-NSC infusion. Using Real Time PCR method we were able to detect the presence of mRNA of BDNF, GDNF, NT3, IGF-1, HGF, semaphorin and presaposin of human-origin factors in the rat brain recipients of HUCB-NSC grafts. Conclusions: Transplantation of HUCB-NSC triggers early expansion of endogenous progenitor pool increasing fraction of proliferating cells in SVZ and SGZ of brain ischemic rats. 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 mechanism promoting recovery from ischemic injury remains to be clarified, although it is likely that it might be due to HUCB-NSC graft-induced release of neurotrophic factors by the host cells as well as the presence of human neural stem cells derived factors.

Supported by MMRC statutory fund

P34

Differentiation of neural stem cells derived from human cord blood (HUCB-NSC) under low oxygen conditions

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In the human body, stem cells are located in niches, which are extremely complex microenvironments (with specified oxygen conditions and cellular together with extracellular matrix components arranged as a 3D structure). The influence of signals from niches seems to play an important role in maintenance of stem cells pluripotency and in their differentiation. We have been investigating the influence of the different niche components on the proliferation and differentiation of neural stem cells into specific cell types as well as the molecular mechanisms underlying this cell responses. In this study we are investigating the influence of low oxygen tension conditions on proliferation and differentiation of Human Umbilical Cord Blood of Neural Stem Cell (HUCB-NSC). Human Neural Stem Cells (NSC) in their physiological niches are exposed to 2–8% oxygen level. For that purpose, HUCB-NSC, were cultivated in two oxygen tension conditions: 21% and 5% with or without the presence of differentiation factor dBcAMP (N6,2′-O-Dibutyryladenosine 3′,5′-cyclic monophosphate sodium salt). We compared the expression of the markers characteristic for proliferation (Ki67) as well as neuronal and astroglial lineage commitment (MAP2, GFAP, β-tubulin, NF200). The presence of tested markers was revealed on the protein (immunocytochemistry) and gene expression level (Real-Time PCR). Our data show, that the low oxygen tension promote HUCB-NSC differentation into neuronal lineage. We also observed that low concentration of oxygen increases cell proliferation.

Sponsored by grant from Polish Ministry of Scientific Research and Higher Education No 5978/B/PO1/2010/38

P35

The modulation of HIFs, pluripotency and differentiation genes expression by oxygen conditions in neural stem cells derived from human cord blood

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The oxygen tension is an important factor modulating cell fate and developmental decisions. There are evidences that HIFs (Hypoxia Inducible Factors) family is implicated in the regulation of pluripotency and differentiation genes. The goal of this study is to compare the influence of close to physiological oxygen conditions (5%) to atmospheric oxygen tension on differentiation process and pluripotent activity in HUCB-NSC. The expression of Hypoxia Inducible Factors, stemness and neural differentiation markers in NSC, cultured under 5% and 21% oxygen were checked on the transcriptional and translational level. We were looking at the interaction between HIFs (HIF1 alpha, HIF 2 alpha) and activity of neural differentiations genes (MAP2, GFAP, β-tubulin) as well as expression of pluripotency genes (Oct4, Sox2, Rex1 and Nanog). In order to demonstrate the impact of increased HIF1α and/or HIF2α level on cell differentiation we used DMOG (Sigma) which is of prolyl-4-hydroksylase inhibitor to increase HIF alpha levels. Our data show, that low oxygen conditions promote proliferation of HUCB-NSC at early stage of development and can activate Oct4 and Nanog genes in HUCB-NSC. The time of cultivation of the cells in low oxygen conditions and the developmental stage of the cells are the important factors for the induction of the expression of "pluripotency" genes. Hypoxia Inducible Factors HIF 1α and HIF 2α, but not HIF3α are expressed in HUCB-NSC at all stages of development. During neuronal differentiation of HUCB-NSC by using dBcAMP, 5% oxygen level act synergistically, promoting further differentiation (enhanced MAP2 expression). Application of prolyl hydroxylase inhibitor – DMOG resulted in increased expression of HIF1α but not HIF2α and increased the expression of MAP2 (only in 21% oxygen conditions) referring to variants without DMOG.

Sponsored by grant from Polish Ministry of Scientific Research and Higher Education No N N302 597838 and by NSC grant No 2011/01/B/NZ3/05401

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Impact of raloxifene and 3,3'-diindolylmethane on neuronal cells exposed to hypoxia

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During the neonatal period of life, hypoxia appears as a major risk factor which may result in complex cerebral dysfunctions like cerebral palsy or seizure disabilities. Natural neuroprotection against hypoxia-induced injury in females is considered to be due to the effects of circulating ovarian hormones, which are lost after ovariectomy or reproductive senescence. Although anti-hypoxic effects of estrogen have been documented, its clinical use has certain limitations. Selective estrogen receptor modulators (SERMs) and selective aryl hydrocarbon receptor modulators (SAhRs) may act as receptor agonists or antagonists in a tissue-specific manner, thus representing a novel approach for the treatment or the prevention of various types of neural degeneration and seizures. In this study we evaluated the mechanism of action of raloxifene and 3,3'-diindolylmethane (DIM) in response to hypoxia in mouse embryonic neuronal cells in primary cultures. Raloxifene is known to bind to estrogen receptors with SERM properties, whereas (DIM) exhibits properties of SAhRs. In our study, hypoxic conditions (5% CO_2/95% nitrogen) induced caspase-3 activity and lactate dehydrogenase (LDH) release in the hippocampal cell cultures. Raloxifene and DIM inhibited the hypoxia-induced LDH release by 10–51% and 9–61%, respectively. DIM inhibited also the hypoxia-induced caspase-3 activity by 2–18%, but raloxifene did not affect the hypoxia-induced apoptotic parameter. In our model of hypoxia, estrogen receptor alpha (ER alpha) antagonist MPP (0.01 μM) did not reverse raloxifene-mediated neuroprotection. However, a high-affinity estrogen receptor beta (ER beta) antagonist, PHTPP (0.01 μM), and G-protein coupled receptor 30 antagonist (GPR30), G-15 (0.01 μM), enhanced the neuroprotective effects of raloxifene, which point to neurotoxic potential of ER beta and GPR30 activation in hypoxia. Selective antagonist of aryl hydrocarbon receptors (AhR) alpha-naphthoflavone (1 μM) did not influence neuroprotective action of DIM, thus suggesting AhR-independent effect. These data demonstrated strong neuroprotective potential of raloxifene and DIM which may represent novel therapeutic tools for brain exposed to hypoxic insults.

This study was supported by the Polish National Center of Science grant No 2011/01/N/NZ3/04786

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Oxygen-glucose deprivation (OGD) promotes microglia activation and gliogenesis but not neurogenesis in organotypic hippocampal slice culture (OHC)

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Brain ischemia resembles other brain injuries in producing enhanced neurogenesis in neuroproliferative regions of the rodent brain, including subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus. Newly-generated neurons would be incorporated in the hippocampal local circuitry and involved in brain repair. Organotypic culture of hippocampal slices (OHC) provides an alternative model of hippocampus *in vivo*. Moreover, exposure of the organotypic slices to oxygen and glucose deprivation (OGD) mimics cerebral ischemia. The aim of the present study was to investigate whether deprivation of oxygen and glucose might stimulate cell proliferation and neurogenesis in organotypic hippocampal slice culture. Furthermore, we evaluate whether the activity of matrix metalloproteinases (MMPs) in the OHC parallels the rate of cell proliferation and/or further differentiation. Cell death in the organotypic hippocampal slices was determined with propidium iodide (IP) staining. Stem cells proliferation was detected by using DNA replication marker – 5-Bromo 2-Deoxyuridine (BrdU) followed by immunoreaction with specific antibodies. Newly generated BrdU(+) cells were identified by an analysis of neural, glial and microglial markers expression – NF-200 NeuN, GFAP, ED1, respectively. In order to check the activity and localization of metalloproteinases, MMP-2 and MMP-9, we conducted in situ zymography in conjunction with immunohistochemistry. Exposing rat OHC for 40 min OGD followed by 24h of reoxygenation induces cell death in CA1 area with only negligible damage in DG. At 1 week cell death appears all over the slice in control conditions as well as after OGD. The stimulation of cell proliferation was observed 7 days after OGD exclusively in CA1. At the same time the number of BrdU(+) cells in DG remained on the level characteristic for control cultures. The majority of BrdU positive cells presents expression of microglial specific stain (ED1) pronounced particularly in CA1 at 3 days after OGD. However, some BrdU labeled nuclei were encapsulated by GFAP positive processes especially in CA1 region of the hippocampus (3 and 7 days after OGD). We do not notice coexpression of BrdU-positive cells with NeuN(+) mature neurons. The study suggests that slice cultures do not show neurogenesis for chosen cultivation period. Activation of MMPs was localized mainly in microglial cells and may be associated with their proliferation *in situ*.

Supported by MSHE grant no 0154/B/P01/2010/38

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Hypobaric hypoxia and hyperbaric treatment prevents neuronal damage and affect antioxidative activity in neonatal hypoxia-ischemia rat model

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Hypoxic–ischemic encephalopathy (HIE) remains a serious condition that causes significant mortality and long-term morbidity. The aim of the study was to evaluate the effect of hyperbaric oxygen (HBO), hyperbaric air (HBA) and hypobaric

hypoxia (HH) on neonatal hypoxic–ischemic (HI) brain injury within a therapeutic window of 1–6 h. We used an experimental model of perinatal hypoxia–ischemia on 7-days old rats, where left (ipsilateral) common carotid artery ligation is followed by 75 min hypoxia. HBO, HBA (2.5 ATA) and HH (0.5 atm air) were applied at 1, 3 or 6 h after HI for 60 min. Treatment was repeated for 3 following days. Brain injury was assessed by comparing ipsilateral hemisphere and contralateral hemisphere weight. Based on the evaluation of weight ratio, HH, HBO and HBA treatment, regardless of time of treatment initiation, resulted in significant reduction of brain weight loss. We observed that HBO reduced brain damage by 58.1%, 57.6% and 54.9%, respectively to the time of treatment initiation (1, 3, 6 h after HI), HBA decreased the damage by 29.9%, 38.1% and 22.0% (respectively). HH also significantly lessened brain weight loss, from 38% after untreated hypoxia–ischemia to 12.9%, 23.1% and 23.8% after HH application respectively 1, 3 and 6 h after hypoxia–ischemia. Superoxide dismutase (SOD) activity and glutathione (GSH) concentration were also measured. HI caused decrease in GSH concentration and 6-fold increase in SOD activity in ipsilateral, but not contralateral hemisphere. HBO treatment applied 1 and 3 h after HI significantly increased GSH concentration and decreased SOD activity, the effect of HBA was less pronounced. HH treatment resulted in additional increase in SOD activity in both hemispheres. However, GSH concentration after HH returned to control values. HBO and HBA altered the expression of cytoplasmic SOD1, and these changes corresponded to changes in SOD activity, suggesting significant role of this protein in neuroprotecting properties of HBO. Our results suggest that HBO, HBA and HH may serve in attenuation of the effects of HI. Early treatment gives better results in brain protection. Our results suggest that HBO and HBA probably reduce synthesis of free oxygen radicals, which manifests in decreased SOD activity. HH however, seems to act on different mechanism, because it enhances SOD activity. It may be beneficial, as it helps to neutralize superoxide anion production, provided that this SOD activity increase is accompanied by activation of glutathione peroxidase (GPx) and catalase (CAT). This assumption needs further investigation.

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Hydrogen gas reduces brain injury in a global cerebral ischemia model

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Transient global cerebral ischemia-reperfusion injury can occur during acute severe hypotensive states and in cardiac arrest that is followed by resuscitation. This transient reduction in perfusion causes an insult to selective hippocampal neuronal populations *via* an apoptotic mechanism. Hydrogen gas has a neuroprotective effect and could be used as a pharmacologic agent of beneficial effect. As such we set out in this study to describe the effect of the inhalation of 2.9% hydrogen enriched air following an ischemia-reperfusion injury. A 2-vessel occlusion model was used to induce global cerebral ischemia for 6 minutes while maintaining a hypotensive state with a mean arterial pressure of 30 mm Hg through reversible exsanguinations in male Sprague-Dawley rats (280–330 g). The study included three groups: global ischemia without treatment (GI, n=6), global ischemia with hydrogen (GI + H_2, n=6) and sham surgery (Sham, n=6). Rats in the treatment group received 2.9% inhalational hydrogen for 1 hour starting 15 minutes following reperfusion. Neurobehavioral testing was performed on day one and T-maze testing prior to being euthanized on days 3 or 7. Treated rats demonstrated an improved outcomes in spontaneous alternations, seizure incidence and survivability. Quantitative Nissl histology and TUNEL of the CA-1 region of the hippocampus showed increased cell survival in the treatment group. We conclude that treatment with inhalational hydrogen following ischemia-reperfusion injury could be low cost method of decreasing the effects of neuronal cell death.

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Striking differences between mesenchymal stem cells derived from neonatal (Wharton jelly) and adult (bone marrow) human tissues

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Mesenchymal stem cells (MSC) exert unique ability to differentiate into various cells of mesodermal origin. These properties place MSC as a very promising source of cells for regenerative medicine and tissue engineering. Recently, it has been shown that experimental transplantation of MSC improves a variety of neurological dysfunctions. Bone marrow (BM) represents the mostly exploited source of human therapeutic stem cells but similar populations have been recently identified in many other tissues and organs. Among them umbilical cord Wharton jelly (WJ) has been recognized for its safety, accessibility and differentiation potential. This study compares human Wharton jelly-derived MSC (WJ-MSC) and human bone marrow-derived MSC (BM-MSC) in terms of cell phenotype, optimal growth and multilineage differentiation characteristics with special attention to neurogenic potential demonstrated by both type of cells. Materials and Methods: MSC were isolated from human Wharton jelly and human bone marrow then cultured *in vitro* in Lonza medium in defined conditions. Then both cell types were subjected to the specific induction media (Gibco) to analyze their potential to differentiate into osteo-, chondro- adipo- and myogenic lineages. Tran-

scriptional activity of genes characteristic for early and late stages of cell differentiation has been examined using RT-PCR. Concomitantly immunochemical analysis of certain gene-related proteins has been performed by immunocytochemical methods. Results: We have demonstrated that both isolated WJ-MSC and BM-MSC exhibited characteristic, mesenchymal cell specific phenotypes by expressing the panels of surface antigens (CD73, CD90, CD105, CD166) as well as typical for MSC multilineage differentiation markers. However, efficiency of these processes differs markedly between the cells derived from each of examined tissues. Thus, WJ-MSC appeared to be much less prone to adipogenic differentiation in comparison to BM-MSC. In contrast, WJ-MSC revealed higher proliferation and neural differentiations potential than BM-MSC. Consistently, only WJ-MSC-derived cells unveiled neural progenitor characteristics expressing panel of cellular markers typical for neural lineage differentiation, i.e. Nestin, NF200, GFAP. All together, these data allow us to hypothesize that the fetal origin of WJ tissue determines its distinguished neuro-mesenchymal characteristic. This is consisted with the data of Takashima et al. (2007) showing that neonatal MSC cultures contain substantially high number of cells being descendants of the earliest wave of developmentally discern neuroepithelial MSC lineage derived from cranial part of neural crest and clearly partitioned from MSC residing in adult bon marrow niche. Conclusions: The study demonstrated that WJ-MSC, similarly to BM-MSC, can be effectively expanded in culture up to 6–8 passages when maintaining cells in undifferentiated state expressing common MSC markers. In contrast, the both MSC lines differ markedly in their ability to lineage differentiation. The most striking difference was that only WJ-MSC can be induced to neural phenotypes. Consisted with this observation WJ-MSC seems to be more favorable than BM-MSC to cell replacement therapy of neurodegenerative diseases.

Supported by NSC grant No 2011/01/B/NZ3/0540

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mRNA transfection – a novel, clinically applicable tool for the robust induction of transient transgene expression in human mesenchymal stem cells

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Adult bone marrow-derived mesenchymal stem cells (hMSCs) display a spectrum of functional properties. Transplantation of these cells improves the clinical outcome in experimental models of cerebral ischemia and spinal cord injury. Therapeutic effects have been reported in stroke after the systemic delivery of MSCs. A minimally invasive, intra-arterial route is an attractive method for stem cell transplantation to the injured brain. However, MSCs lack the intrinsic mechanisms that enable homing of the cells to the area of infarction. Recent studies suggest that genetic manipulation can promote the forced expression of certain molecules responsible for adhesion and transendothelial migration of systemically delivered cells. It is anticipated that, for cell homing to the brain after intra-arterial delivery, the transient expression of integrins should be sufficient for diapedesis to occur. Since the capacity of MSCs to undergo functional transfection using pDNA is very low, we investigated an mRNA transfection method for the expression of transgenes in MSCs in order to overcome the limitations of the pDNA approach. Methods: Human mesenchymal stem cells (hMSC, PT-2501, Lonza) were thawed and cultured in medium MSCBM (PT-3238, Lonza) supplemented with 10% MCGS (PT-4106E, Lonza), L-glutamine (PT-4107E, Lonza), and gentamicin sulfate (GA-1000, PT-4504E, Lonza). Cells were maintained in a humidified atmosphere at 37°C and 5% CO_2 using 75 cm^2 flasks. For transfection experiments, hMSCs were transferred to 24-well plates and seeded at a density of 15 000 cells/well. For transgene induction experiments, pDNA-eGFP (BD Biosciences) at a dose of 0.5 and 1.0 µg/well, and mRNA-eGFP (StemGent) at doses of 0.12, 0.25, and 0.5 µg/well were used. The Lipofectamine® 2000 (Invitrogen), TransIT-2020 (Mirus), and Stemfect™ RNA Transfection Kit (StemGent) were used as transfection agents. After transfection, cells were maintained in culture conditions up to 21 days. Transfection efficiency was assessed by confocal microscopy using GFP fluorescent signal detection. Results: MSC pDNA-eGFP transfection results in a dramatically low efficiency, less than 1% of the cell population in each of the tested conditions. In contrast, mRNA-eGFP transfection resulted in an efficiency exceeding 95% in each of the tested conditions. This difference was highly statistically significant ($P<0.001$). Furthermore, cellular GFP level, and the persistence of transfection was dependent on the mRNA dose and the type of transfection agent. It was found that the dose of mRNA-eGFP 0.5 µg/well and the use of Lipofectamine was the most effective method with transgene expression up to three weeks. Conclusions: The mRNA transfection is a robust, clinically applicable tool for inducing the transient expression of transgenes in hMSCs, which are otherwise difficult to transfect by vectors that do not incorporate into the host genome. Using this method, application of engineered MSC could revolutionize regenerative medicine.

Supported by a National Centre for Research and Development grant No 101 in ERA-NET NEURON project: "MEMS-IRBI"

P42

Influence of various factors on DNA methylation in a group of elderly individuals

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In a group of 194 persons aged 54–86 years global DNA methylation was determined using Imprint DNA Quantification Kit

MDQ1 (Sigma-Aldrich). The association with several biochemical factors as serum glucose,serum creatinine and folic acid (determined by chemiluminescence) levels as well as with sex, age and cognitive status was estimated by multivariate stepwise regression analysis. The strongest association was stated with serum creatinine (P=0.0003), with serum folic acid (P=0.005) and with glucose (0.008) levels. The mechanisms and significance of these associations is discussed in view of human aging and age-connected diseases as atherosclerosis, diabetes and renal failure.

P43

In search for effective modulators of the proteasome activity

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Proteasome is a multi-activity enzyme involved in a ubiquitin-dependent turnover of cytoplasmic and nuclear proteins. It recognizes and digests short-lived regulatory proteins, influencing cellular processes as crucial as progression of the cell cycle, transcription, oncogenesis and flux of substrates through metabolic pathways. The enzyme is responsible also for the housekeeping chores, degrading misfolded or oxidatively damaged proteins. Defects in the proteasome action play a causal role in development of a number of diseases, among which are cerebral ischemia and neurodegenerative disorders such as Huntington's, Alzheimer's, and Parkinson's diseases. Being a multifunctional proteolytic machinery, the proteasome must act under a strict control to prevent massive degradation of all intracellular proteins, which would result in a cell death. One of the levels of such a control is the proteasome structure itself. The core particle called 20S proteasome is a barrel-like structure made up of four rings of seven subunits each. The outer (α) rings play predominantly a structural role forming a kind of a gated channel leading to the proteolytic chamber. The inner-β-rings harbor six active sites, concealed inside the cavity formed by the β subunits. So far, the only proteasome-targeting agents used in clinics are competitive inhibitors, directly blocking the enzyme's active sites. However, the multi-subunit barrel-like structure of the 20S proteasome encourages to test compounds which can target allosteric interactions between subunits and influence the gating mechanism, involved in the control of the substrates' uptake. Such modulators may provide a precise and substrate-specific regulation of the proteasome catalytic performance. Additionally, targeting the allosteric interactions may enable not only inhibition but also stimulation of the proteasome, which is crucial in managing disorders connected with the proteasome not sufficient activity, such as neurodegenerative diseases. A variety of protein ligands, interacting with the outer ring of the 20S proteasome and modulating its activity, is already known. They can serve as templates for design of putative small-molecule allosteric drugs. In an effort to find synthetic compounds able to enhance or suppress the performance of the proteasome active centers we utilize one of such protein ligands – HIV-1 Tat protein. The protein is known to inhibit the core proteasome and to interfere with the physiological PA28 activator in its binding to the 20S. G48RKKRRQRRRPS59 fragment of HIV-1 Tat (Tat1) occurred to be very efficient in the 20S proteasome inhibition. By single and multiple alanine substitutions we have recognized "hot spots" in the sequence of Tat1. NMR and molecular dynamics calculations allowed us to correlate these putative pharmacophores with the structural turns. By introduction of a non-peptide turn-inducing modification to the Tat1 sequence we have obtained the derivatives highly toxic for human cultured cancer cells HeLa.S3.

The work was supported by grants: NCN 2011/01/B/ST5/06616 and DS/8440-4-0172-2

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HIV-1 Tat-derived peptides as allosteric inhibitors of the proteasome activity

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The proteasome is a main protease of the ubiquitin-proteasome pathway, responsible for degradation of the majority of intracellular proteins in human cells. Since the proteasome regulates so many processes, abnormalities in its functioning play a causal role in a number of diseases, including muscular dystrophy, cardiovascular diseases and various cancers. The ubiquitin-proteasome pathway is involved also in disorders affecting central nervous system – cerebral ischemia/reperfusion injury and stroke. This implication in pathological condition makes the proteasome an important and very promising therapeutic target. The 26S proteasome, which is responsible for ATP-dependent proteolysis of ubiquitin-tagged proteins, consists of a barrel-like core particle – the 20S proteasome, and attached to it two regulatory particles 19S. The core particle is composed of four rings (αββα). The inner β-rings harbour active sites, which display distinct specificities and are responsible for cleavages of polypeptides after hydrophobic, acidic and basic residues (Marques et al. 2009). On the other hand, N-terminal residues of α subunits create a gate leading to the catalytic chamber. Because most of the already known proteasome inhibitors are competitive they are not selective enough and can block all active sites causing cell apoptosis. We believe that allosteric modulators may be an interesting alternative to active site inhibitors. The multi-subunit and multi-active sites structure of the proteasome creates an opportunity to selective allosteric regulation of its activity. We focus our searching on biomolecules which bind to the α-ring of the 20S proteasome and influence the enzyme's gating mechanism. HIV-1 Tat protein is one of the natural proteasome regulators competing with 11S activator for binding to the α-rings (Huang et al. 2002). We designed two peptides: G48RKKRRQRRRPS59 (Tat1) and R49KKRRQRR56Q66DPI69 (Tat2) based on a sequence of the basic domain of the protein (Jankowska et al. 2010). We found that both of them efficiently inhibit 20S proteasome. We tried to connect the biological activity of Tat peptides to their structure determined by means

of CD, FTIR, NMR and molecular dynamics simulation. Additionally, we synthesized alanine scan of Tat2 to determine the importance of individual amino acids. We exchanged not only single residues but also several adjacent amino acids at once and tested the influence of these changes on the proteasome activity. We also investigated the scan peptides' structure using FTIR.

The work was supported by grants: 538-8440-1043-12, DS/8440-4-0172-2 and NCN 2011/01/B/ST5/06616

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High-throughput assays for measuring oxidative stress and mitochondrial dysfunction in neuronal cell cultures

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Drugs of abuse may cause acute as well as chronic damage to the nervous system, and a common mechanism of neurotoxicity is to induce disturbances in mitochondrial function. The mitochondrion is also an important source for cytotoxic reactive oxygen species (ROS). If the mitochondrial membrane potential (MMP) becomes depolarized, it can increase the production of ROS. This project has evaluated whether the fluorophore JC-1, which measures the depolarization of MMP, and the fluorophore H2DCFDA that oxidizes and produce fluorescence in the presence of oxygen radicals, are useful tools to screen for drug-induced neurotoxicity. The studies have been performed in embryonal carcinoma (EC) P19 cells that are pluripotent and upon retinoic acid (RA)-treatment will differentiate in culture into neurons, astrocytes and oligodendrocytes. In order to determine the predictive validity of the model/methods, a number of compounds known to cause oxidative stress and mitochondrial dysfunction have been examined (hydrogen peroxide, ionomycin, sodium azide). Main techniques employed culturing, induction and differentiation of neuronal cells, pharmacological dose-response experiments, detection and quantification of fluorescence using microplate reader and fluorescence microscopy, microplate-based colorimetric methods for assessment of cell viability, pharmacological/toxicological data and statistical analyses using the GraphPad prism software.

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The relationship between mitochondria function and circadian clock in peripheral oscillators

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Circadian rhythms govern a wide variety of physical, behavioral and metabolic changes that follow a roughly 24-hour cycle, responding primarily to light and darkness in an organism's environment. These are controlled by the circadian clock mechanism, where rhythm-generating mechanism is encoded by a transcription-translation feedback loop. Numerous studies have pointed to a cyclic relationship wherein the rhythm impacts metabolic activity and metabolism feeds back to impinge upon the rhythm. Mitochondria play a pivotal role in regulating cellular energy and were shown to be strategically positioned at the intersection between circadian rhythm and cell metabolism. Nevertheless little is known about their function in controlling the circadian rhythm. In our study, we investigated the involvement of circadian clock in mitochondrial function as well as mitochondria-dependent regulation of circadian clock. The study was carried out in primary human fibroblasts, an already established model to investigate molecular clock mechanisms *in vitro*. We have found that mitochondria activity as well as network activities showed rhythmic changes within 24 hours. Circadian pattern was detected for mitochondrial ROS including superoxide anion production. A significant 24-hour oscillation was found for cellular redox state. Furthermore, mitochondrial ATP levels were rhythmic and the maximum of ATP production paralleled the peak of mitochondrial ROS level and the mitochondrial network formation. Circadian rhythm was also detected for calcium ions concentration. Increase of ATP synthesis as well as changes in calcium and ROS level activated AMP-dependent protein kinase (AMPK). We have found that in primary human fibroblasts AMPK protein level and activity fluctuate in an antiphase relationship with rhythmic ATP production. Summarizing, our data provide the evidence for circadian regulation of mitochondrial dynamics and suggest that changes of mitochondrial activity may directly influence cellular clock.

Supported by grants from Sciex 10. 258 to A.K. as well as Swiss National foundation (SNF No 310030_122572) and Synapsis Foundation to A.E.

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Can different expression of mitofusins be involved in pathomechanism of particular mitochondrial diseases?

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Mitochondrial homeostasis, resulting from fusion and fission processes together with mitophagy and mitogenesis, are widely studied nowadays. This is probably because we know more and more about the role of mitochondria in metabolic diseases (diabetes, hypertension), neurodegeneration (Parkinson's Disease, Alzheimer's Disease), but also in broad spectrum of inherited neurological syndromes (Charcot-Marie-Tooth). In our studies we aimed to examine the expression pattern of particular mitochondrial proteins, mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2), in mouse tissues. We aim to verify, whether potential differences in expression of those proteins can by implicated in pathomechanism of Charcot-Marie-Tooth type 2A neuromyopathy, related to mitofusion 2 gene mutations. Mitofusins are mitochondrial GTPases, implicated in fusion of outer mitochondrial membrane. In this process, mitofusins juxtapose two mitochondria by combining

homo- and heterodimers at the surface of two outer mitochondrial membranes. Although there is 63% homology between mitofusins, it is proved, that they show some different functions. As Mfn1 KO present more severe aberrations in mitochondrial network formation than Mfn2 deficient cells, Mfn1 is considered to have stronger fusion activity. It is also suspected, that it is Mfn1 that links fusion of outer and inner mitochondrial membranes. Nevertheless, Mfn2, but not Mfn1, is present at endoplasmic reticulum (ER). Mfn2 tethers ER to mitochondria facilitating calcium flux and (indirectly) autophagy. Moreover, Mfn2 seems to have some regulatory effect on cell cycle, beyond its fusion activity and its lower expression seems to correlate with insulin resistance and hyper proliferation in hypertension. So, the question is, how much these two proteins can replace each other while playing so different roles? Moreover, it is suggested that CMT2A predominantly affects peripheral nerves because mutated, malfunctioned Mfn2 is insufficiently compensated by Mfn1 due to its low expression particularly in this type of tissue. To discuss this issue, we have investigated the expression of Mfn1 and Mfn2, as well as protein content, in tissues, performing Real Time PCR and Western Blot studies. Preliminary data from Western blot analysis displayed equally high relative level of both mitofusins in nervous system (dorsal root ganglia, cerebral cortex, cerebellum, spinal cord) in comparison to peripheral organs (muscle, heart, liver, kidney, skin). Moreover, Mfn1 expression seems significantly lower in dorsal root ganglia, which are well established model of peripheral nervous system. This phenomenon was not observed for other tissues, even from central nervous system. So it seems quite possible, that axonal damage of peripheral nerves in CMT2A, may be observed due to the poor compensation of dysfunctional Mfn2 by fully functional Mfn1, which is not expressed at sufficient level.

The project was supported by NSC grant NN402474640

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Alzheimer's amyloid beta peptides disturb circadian oscillations of intracellular calcium concentration

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It is postulated that disturbances in calcium homeostasis play an important role in pathogenesis of Alzheimer's disease (AD). Changes of neuronal calcium concentration are responsible for the oxidative stress as well as altered metabolism and production of amyloid-beta peptides (Aβ). Aβ may further exacerbate calcium dysregulation, causing synaptic dysfunction, neurodegeneration and cognitive impairment. Recent data indicate that AD is associated with disturbances of circadian rhythm in the patients. However, till now nothing is known about the molecular mechanisms involved in AD-related circadian clock alterations. In our study we investigated the effect of Aβ peptides on the rhythmic oscillation of cytosolic and mitochondrial calcium levels. To investigate molecular clock mechanisms, the studies we carried out in human primary skin fibroblasts, a previously established experimental model. Our data showed circadian rhythm of calcium ions concentration in cytosol and mitochondria. Moreover we observed circadian oscillation of ROS formation and redox potential. Treatment with Aβ fibrils at the concentration of 0.5 μM disturbed cytosolic calcium oscillations and mitochondrial redox state. Studying mechanisms involved in this phenomenon indicated that Aβ did not affect ER calcium stores, but induced changes of calcium influx mediated by purinergic P2X7 receptor. The specific antagonist of P2X7 receptor Brillant Blue G abolished negative impact of Aβ and restored calcium circadian rhythm. Summarizing, our results indicate that Aβ may play a significant role in disturbances of circadian calcium oscillation, suggesting the importance of this phenomenon in AD-related changes in biological clock.

Supported by grants from Sciex 10. 258 to A.K. as well as Swiss National foundation (SNF No 310030_122572) and Synapsis Foundation to A.E.

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Endogenous STIM2 and endogenous ORAI1 form a calcium-sensitive and thapsigargin-insensitive complex in cortical neurons

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ER calcium sensors (STIM1, STIM2) and calcium channel-ORAI1 interaction is crucial for store-operated calcium entry (SOCE) in non-excitable cells, but in neurons their localization and dynamics are not clear. We showed earlier that in neurons STIM1 is involved in thapsigargin induced SOCE, while STIM2 is active after EGTA-driven depletion of extracellular Ca^{2+} (Klejman et al. 2009, Gruszczynska-Biegala et al. 2011). To confirm that this is not due to the overexpression of exogenous proteins we used Proximity Ligation Assay to analyze activities of endogenous proteins. Cortical neurons were cultured in 2 mM $CaCl_2$, 2 mM EGTA or 2 μM thapsigargin, fixed and incubated with primary antibodies anti-STIM2 and anti-ORAI1. The pairs of appropriate secondary antibodies with conjugated oligonucleotides were then added and Duolink II was performed to create the fluorescent products. We detected *in situ* the endogenous STIM2/ORAI1 complexes in somata and quantified in single neurons the number of hetero- and homo-complexes. The amount of hetero-complexes increased up to 10-fold in response to calcium depletion by EGTA. The number of STIM2/ORAI1 endogenous complexes correlated well with the number of overexpressed YFP-STIM2/ORAI1 complexes formed under the same conditions (Gruszczynska-Biegala et al. 2011). By co-immunoprecipitation we confirmed the in situ interaction between endogenous STIM2 and ORAI1 and that the interaction is increased after Ca^{2+} depletion in the medium. In conclusion, the present study provides a novel finding that endogenous STIM2 can

physically interact and form hetero-complexes with endogenous ORAI1 in TG-insensitive manner, suggesting that the proteins are key molecules that underlie the regulation of basal calcium levels in neurons and constitutive calcium entry.

This work was supported by funds from a Polish Ministerial grant (1900/B/P01/2010/39, JK) and from National Science Centre (2011/01/D/NZ3/02051, JGB)

P50

Modulation of the rat middle cerebral artery response to acidosis in hyponatremia by the opener of large-conductance calcium-dependent potassium channels (BKCa)

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Objectives: It is well known, that acidosis-induced dilation of the middle cerebral artery (MCA) in normonatremia depends on BKCa channel activation in smooth muscle cells of the arterial wall. Our studies on the effect of hyponatremia on the regulation of the rat MCA have demonstrated, that acute hyponatremia causes BKCa channel dysfunction manifested by its reduced sensitivity to agonists. The aim of our present experiments was, therefore, to study whether the response of MCA to acidosis is decreased during hyponatremia and if so, whether BKCa channel activator applied in subthreshold dose restores the response of MCA to lowering of extravascular pH. Method: MCAs were isolated from male Wistar rats brains and placed in the arteriograph chamber filled 3-(N-morpholino) propanesulfonic acid (MOPS) buffered saline solution containing 1% BSA. The vessels were perfused (100 µl/min) and set at a hydrostatic pressure of 80 mmHg. The MCA images were recorded using a microscope equipped with a camera coupled to a monitor. The measured parameter was the internal diameter of the vessel. Acute hyponatremia was induced in the chamber by decreasing Na+ concentration in the extra- and intravascular fluid from 144 mM to 120 mM. After equilibration of the MCA for 1 hour in normonatremic buffer, responses of this artery to BKCa channel activator (NS1619, 10-5M) in normo- and hyponatremia, to lowering of extravascular pH from 7.4 to 7.0 in normonatremia, in hyponatremia and in hyponatremia in the presence of NS1619 (10-5M) were studied. Results: NS1619 administration led to MCA dilation by 16 ± 1% (P<0.001) in normonatremia but had no effect on the vessel diameter in hyponatremia. Reducing pH from 7.4 to 7.0 resulted in the dilation of MCA by 18 ± 2% (P<0.001) in normonatremia whereas in hyponatremia constriction of the MCA by 4 ± 2% (P<0.01) in response to reduced pH was observed. The presence of BKCa channel activator restored the response of the MCA to acidosis during hyponatremia. Conclusion: These results confirm our previous findings that during acute hyponatremia, BKCa channels sensitivity in the wall of MCA is reduced. This explains lack of the dilation of this artery to extracellular acidosis in hyponatremia.

This study was financially supported by statutory activities of the Mossakowski MRC PAS

P51

Mutations in the methyl CpG binding protein 2 (MECP2) gene as a cause of the Rett syndrome

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The methyl CpG binding protein 2 (MECP2), protein that binds to methylated DNA sequences and represses the expression of specific genes, is essential for normal function of mature nerve cells. The protein is encoded by MECP2 gene and its mutations are responsible for approximately 90% of all Rett syndrome (RTT) cases. RTT is a neurodevelopmental disorder that affects mainly girls. Its characteristic features include arrested psychomotor development (6–18 months), congenital impairment, loss of speech, characteristic stereotypical movements, regression of gained skills and other neuropsychiatric abnormalities. Nineteen patients with primary clinical diagnosis of RTT were referred for molecular examination. The analysis of MECP2 gene included direct sequencing of exons 2–4 and deletion/duplication analysis using MLPA method. In nine patients we have found seven known point mutations, including three nonsense substitutions in four individuals (p.R168X, p.R255X, and p.R270X in 2 cases) and three missense changes leading to amino acids substitutions in the methyl-binding domain (p.R133C, p.K135E, p.T158M) or in the transcriptional repression domain (p.R306C in 2 cases). In three other patients, a partial deletion of MECP2 was found, including a deletion of exons 3 and 4 (encompassing 2 to 67 kb) and two different deletions of exon 4, encompassing 44 bp and 1 to 7.3 kb, respectively. Together, we were able to confirm the clinical diagnosis of Rett syndrome in 12 cases. The significant presence of large deletions encompassing entire exons suggests that the MLPA analysis should be performed as an important part of the molecular diagnosis in Rett syndrome.

P52

Analysis of mutation in SPR and HTRA2 genes in patients with Parkinson's disease

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Diagnosis of Parkinson's disease (PD) is often problematic because clinically it can be difficult to distinguish idiopathic PD from the other extrapyramidal disorders. It is known, that PD is caused either by environmental and genetic factors . Genetic mutations are the cause of familial form of PD and include genes PARK1-PARK18. The etiology of sporadic PD (SPD) is still not clear, but it is currently

assumed that genetic susceptibilities, may be involved. It is suggested, that in pathogenesis of the SPD beside SNCA and PARK2 genes, may be involved also SPR (sepiapterin reductase gene) [PARK3] and HTRA2 (HTRA serine peptidase 2 gene)[PARK13] genes. The HTRA2 gene, also known as Omi, was found to be associated with PD in German population. However, some studies have indicated that some variants of HTRA2 may not be related to PD. SPR gene, which is located in the PARK3 linkage region is inconsistently associated with a risk of PD but significance of mutations in this gene as well as HTRA2 in PD is still not clear. The aim of the study was the analysis of the frequency of T637A/G SPR as well G421T, G1195A and C1210T mutations in HTRA2 gene in Polish patients with PD and in control group. Peripheral blood was collected from 89 patients with PD clinical diagnosis (42F and 47M, the avr. age 62 ± 10.15 years), and from 113 healthy donors (79F and 7M, the avr. age 55.5 ± 9.54 years). Genomic DNA was isolated using standard protocols. Genotyping was performed by PCR/RFLP using specific primers and restriction enzymes (SsiI, MboII, MvaI, MspI) and sequencing. The SPR gene analysis detected T637A mutation in 3 (3%) PD patients compared to 2 (2%) persons in the control group. Moreover, mutations G421T and G1195A of HTRA2 gene have been identified in 3 (3%) [G421T – 1%, G1195A – 2%] patients with PD and none of controls. Analysis of C1210T HTRA2 mutation detected no mutated variant both in PD patients group and in control group. It was also observed that the stage of the disease was 1–2 in Hoehn and Yahr scale and response to L-dopa was good in patients with T637A SPR and G421T, G1195A HTRA2 mutations. It was also observed some tendency for depression manifestation in PD patients with T637A SPR mutation. It can be concluded, that mutations of SPR and HTRA2 genes probably may be one of the risk factor for manifestation of PD. Thus, the results of this study suggest that analysis of T637A SPR and G421T, G1195A HTRA2 genes mutations may be an additional diagnostic and prognostic factor in PD patients in the future.

P53

Polymorphisms of the PARK2 gene and Parkin protein levels in patients with Parkinson's disease

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Parkinson's disease (PD) is one of the most common degenerative diseases of the extrapyramidal system, the frequency of which increases with age. It is now believed that the causes of PD are environmental and genetic factors. Important genetic factors resulting in PD are mutations in the PARK2 gene, which may affect the level of Parkin. The aim of the study was conducted on 234 individuals of the Polish population: 89 patients diagnosed with PD, 32 patients diagnosed with Parkinson's syndrome and 113 individuals from the control group without neurological symptoms and characteristics of dementia. As a result of the methods of analysis demonstrated the following: G930C mutation of exon 8 in the PARK2 gene, which was analysed by performing PCR-RFLP. Detection of deletion of exon 2 using PCR. Whereas the evaluation of mutations within exon 11 in PARK2 gene was performed using HRM method and sequencing. Also performed to measure the concentration of Parkin's plasma in blood using ELISA method. The study results no presence of the deletion of exon 2 in the PARK2 gene in any individual study. At the same time, it was almost 3-times higher frequency of G930C mutation in exon 8 PARK2 in patients with PD and almost 6-times higher incidence of mutation G1281 A in exon 11 of PARK2 in PD patients compared with controls. At the same time, in the present study demonstrated that the presence of mutations in 8 and 11 exon of PARK2 gene does not appear to be associated with the generate of Parkin's plasma concentration. Genotype-fenotype study in the PARK2 gene can constitute intravital diagnostic tests in patients with PD, as well as in patients diagnosed with Parkinson's syndrome in the course of a degenerative disease.

P54

Hypoxic ventilatory response in the rats with 6-hydroxydopamine lesion of the medial forebrain bundle and peripheral dopamine receptor blockade

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The rat model of Parkinson's disease (PD) based on experimental impairment of nigrostriatal dopaminergic system by 6-hydroxydopamine (6-OHDA) has been elaborated to study mechanisms of respiratory disturbances associated with PD. Following striatal injection of 6-OHDA breathing with hypoxic mixture augments the hyperventilatory response to hypoxia suggesting an attenuation of the depressant effect of dopamine on ventilation. In the present study we ask whether injection of 6-OHDA into the medial forebrain bundle (MFB), that evokes more severe motor symptoms, elicits changes in the hypoxic ventilatory response and whether changes in ventilatory response to hypoxia following the unilateral dopaminergic denervation are transmitted by peripheral dopamine D2 receptors. The experiments were performed on adult rats. Ventilatory parameters: tidal volume, minute ventilation, and frequency of breathing were measured with the use of body plethysmograph method before and two weeks following unilateral, double injection of 6-OHDA into the MFB. Changes in the body weight and behavioral cylinder test were evaluated at the same time points and compared with the results obtained in sham operated rats. Effects of peripheral dopamine D2 receptor antagonist, domperidone (1 mg/kg i.p.) on ventilation during rest breathing and during 3 minutes exposure to hypoxia (8% O_2)were studied before and after 6-OHDA injection. Two weeks after 6-OHDA treatment the cylinder test showed limb use asymmetry. Body weight increased less than in animals without 6-OHDA injection. Following the MFB lesion the hyperventilatory response to hypoxia was augmented mainly by an increment of tidal volume. Before the MFB lesion the pretreat-

ment with domperidone enhanced resting ventilation and hypoxic hyperventilatory response. After 6-OHDA injection domperidone no longer altered both normoxic breathing and the hyperventilatory hypoxic response. The study shows that an impairment of dopaminergic system by MFB lesion causes comparable changes in breathing and ventilatory response to hypoxia as lesions in the other locations of the nigrostriatal pathways. In 6-OHDA model of Parkinson's disease changes in the hypoxic ventilatory response seem to be related to a reduction of peripheral D2 dopaminergic neurotransmission involved in the control of breathing.

P55

Impact of L-DOPA treatment of patients with Parkinson`s disease on mononuclear subsets and phagocytosis in the peripheral blood

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The question as to the role of immunological mechanisms in neuronal death of extrapyramidal cell systems in Parkinson`s disease is till now not fully resolved. One of the approaches includes an examination of circulating blood cells. In our studies consisting of 24 patients the peripheral blood was studied before and after medication with L-DOPA compounds. Patients with Parkinson`s disease demonstrated an increase of lymphocyte Cd95/CD3 as well as a considerable number of cells dead by apoptotic processes. After treatment with L-DOPA both the percentage of CD95/CD3, acknowledged as an antigen marker characteristic for apoptotic cells as well as the number of cells dead by apoptotic processes were decreased. These findings thus indicate that levodopa treatment in Parkinson`s disease has an impact on apoptotic processes in this instance, and this should be taken into consideration as a positive event in the pathomechanism effected by this treatment.

P56

The influence of imipramine and pramipexole on depressive-like behaviour in an animal model of a pre-clinical stage of Parkinson's disease

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Motor disturbances in Parkinson's disease (PD) results from the massive degeneration of dopaminergic neurons and terminals of the nigrostriatal pathway and a decrease in the dopamine (DA) level in the caudate nucleus and putamen. The clinical phase of PD is preceded by a preclinical period where depression is a frequent comorbid disturbance. Dysfunctions of monoaminergic systems could underlie depression in PD. Clinical trials suggest that a treatment with tricyclic antidepressant drugs can be effective in ameliorating depression in PD. Moreover, recent studies have suggested that the administration of pramipexole (the mixed dopamine D2/D3 receptor agonist) may reduce not only motor symptoms (akinesia, rigidity and tremor at rest) but also depression in PD. The aim of the study was to examine the influence of classic tricyclic antidepressant -imipramine and pramipexole on the 'depressive-like' behaviour of rats with moderate lesion of the nigrostriatal system. Male Wistar rats were injected bilaterally with 6-OHDA (3.75–15 μg/2.5 μl) into the ventral striatum (vSTR). Imipramine was injected i.p. at a dose of 10 mg/kg once a day and pramipexole s.c. at a dose of 1 mg/kg twice a day for 14 days. The locomotor activity in actometers and behaviour of rats in the forced swimming test (FS) were measured on the 15th day after the surgery. The lesion extent was analysed by HPLC and immunohistochemically. The lesion increased immobility and swimming and decreased climbing in FS, however, it did not influence the locomotor activity of rats. All the lesion-induced disturbances observed in FS were decreased by pramipexole. Imipramine increased only climbing, but had no influence on immobility in lesioned rats. Moreover, imipramine but not pramipexole reduced the locomotor activity in lesioned animals. After the administration of 6-OHDA levels of DA decreased (ca. 45%) in the dorsal striatum (dSTR), vSTR and frontal cortex (FCX). Pramipexole and imipramine injections had no influence on DA levels in lesioned rats. Levels of DA metabolites (DOPAC, HVA) were markedly increased in dSTR and vSTR after injections of pramipexole. Moreover, pramipexole significantly increased the turnover of DOPAC/DA and HVA/DA in dSTR and vSTR in sham-operated and lesioned rats. These results indicate that a relatively moderate dopaminergic lesion which does not produce any motor disturbances, may induce "depressive-like" symptoms which are reversed by dopamine agonist but not by a classic antidepressant. Acknowledgments Study supported by the
Project "Depression-Mechanisms-Therapy" (POIG.01.01.02-12-004/09-00), co-financed by EU from the European Regional Development Fund as a part of the Operational Programme "Innovative Economy 2007-2013"

P57

Sensitivity of neural stem cells derived from human umbilical cord blood (HUCB-NSC) to developmental neurotoxin – methylmercury chloride: Dependence on non-specific and receptor mediated interactions with biomolecules

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Human neural stem cells play an important role in *in vitro* developmental neurotoxicity testing. The purpose of this research was to investigate the sensitivity of neural stem cells derived from human umbilical cord blood (HUCB-NSC) to methylmercury chloride

(MeHgCl), and its dependence on the type of interaction on cell membrane/biomolecule interface. MeHgCl is well known neurotoxin with documented adverse influence on human central nervous system (CNS) development. Cells were cultured in 96-well plates covered with different adhesive substrates or on Petri dishes microcontact-printed with biofunctional domains. The following biomaterials were used: poly-L-lysine, the synthetic compound, which allows to create electrostatic interactions with cells, or fibronectin and vitronectin, proteins of extracellular matrix, which create receptor mediated interactions between cells and the adhesive substrate. After the incubation with different concentrations of the neurotoxin, the cell viability, ability to proliferate, and to differentiate into neural precursors of HUCB-NSCs was measured with Alamar Blue assay and immunfluorescence stainings. High concentration of MeHgCl (1 μM) significantly decreased viability of cells and their ability to proliferate. The response of cells to the toxic effect of MeHgCl was different depending on the type of adhesive substrate. Domains covered with fibronectin or vitronectin, decreased significantly HUCB-NSC sensitivity to the neurotoxin when compared to poly-L-lysine. Our results suggest that receptor mediated interactions on cell membrane/biomolecule interface may be protective in neural stem cells' response to certain neurotoxins.

Supported by MSHE grant No 5978/B/P01/38 and NN 302663940

P58

Alterations in brain development in rats treated with immunosuppresant drugs Sandimmune and Prograf and their vehicle components

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Cyclosporin A and tacrolimus are powerful immunosuppressants used as post-operation medication after allogenic transplantations. Unfortunately, the drugs Sandimmune (cyclosporin A) and Prograf (tacrolimus) exhibit negative side effects. These side effects may be linked not only to the active ingredients themselves, but also to the vehicle used for their delivery – Cremophor EL and/or ethanol. Sandimmune, Prograf, ethanol, Cremophor EL or Cremophor EL with ethanol (i.e. the complete vehicle) in a saline solution were administered to male Wistar rats either on 6th and 7th or 30th and 31st day postnatally. The functional changes in the nervous system elicited by these substances were assessed by observing the intensity of seizures induced by a single i.p. injection of pilocarpine at 60th postnatal day. Brain anatomy was also analyzed by comparing brain mass, lateral ventricle relative area, thickness of cerebral hemisphere wall, relative size of the hippocampus as well as total density of cresyl violet-stained neurons in the cerebral hemisphere walls between control and experimental animals. Our data point to a significant effect of all tested substances on central nervous system development. The greatest effects on seizure severity and brain structure were associated with the complete vehicle. Such effects (although less sever) were also observed for Cremophor EL and ethanol given separately.

P59

The role of interleukin 6 in stress response and anxiety-driven behavior in mice

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Interleukin 6 (IL-6) plays an important role in stress response and glucocorticoid action on the brain. It has been also shown that IL-6 plays a significant role in physiological and pathological brain development and is a crucial factor in the effects of prenatal immune challenge on physiological and behavioral abnormalities in adult offspring. We examined involvement of IL-6 in stress-induced changes in behavior and brain mechanisms in the adult mice. The PhenoRack system was used to non-invasively monitor mice home cage activity. Behavior of wild type mice C57BL/6J and IL-6 -/- knockout (IL-6 KO) mice were observed for 3 days using the PhenoRack system, which enables non-invasive monitoring of the mice home-cage activity (distance travelled, speeds and duration of movement, freezing). Mice were also subjected to standard behavioral tests. The open field test was used to establish the balance between exploratory behavior and anxiety evoked by the unknown, potentially dangerous situation. We measured the distance travelled in the open part of the arena, as well as time spent in it. We observed the sex-dependent effect of IL-6 on exploratory behavior. In all tested parameters IL-6 deficient females showed less anxiety than the wild type females. There was no difference in behavior in the open field between wild type and IL-6 deficient males. After finishing behavioral tests, the animals were killed with an overdose of pentobarbital and their brains were perfused transcardially with saline (0.9% NaCl) followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were cut on a cryostat into 40 μm sections and collected into 10 parallel series. Two of these series were stained with antibodies against glucocorticoid (GR) and mineralocorticoid (MR) receptors. One series from each brain was Nissl-stained for delineation of borders of the brain structures and evaluation of the number of cells in some of them. The number of hippocampal neurons and GR-immunopositive neurons in an area was estimated using the StereoInvestigator system (MicroBrightField Inc). Due to the crucial role of IL-6 in development of the hippocampus we evaluated the total number of cells in the CA1 field. We found that IL-6-deficient mice had significantly lower number of cells in the CA1 field and that almost all cells, unlike in wild-type mice, expressed the glucocorticoid receptor. Our preliminary results demonstrated that IL-6 is implicated in stress.

Supported by the Polish National Science Center grant No 1577

P60

Glutamate receptors antagonists attenuate neurological deficits and modulate neuroinflammation in Lewis rat subjected to experimental autoimmune encephalomyelitis

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Experimental autoimmune encephalomyelitis (EAE) is an animal model that mimics many aspects of multiple sclerosis (MS). Chronic or relapsing inflammation of the central nervous system results in the destruction of myelin sheath and cytokines play an important role in the pathogenesis of both MS and EAE. Myelin, oligodendrocytes and neurons are lost due to an inflammatory attack by leukocytes infiltrating the central nervous system (CNS) and releasing cytotoxic cytokines, anti CNS antibodies and large amounts of the excitatory neurotransmitter glutamate. Pharmacological studies have suggested that glutamate receptors mediate white matter injury in a variety of CNS diseases, including multiple sclerosis (MS). Memantine and amantadine are ionotropic glutamate receptors (iGluRs) antagonists. Memantine, a clinically applied drug with N-methyl-D-aspartate (NMDA) receptor antagonistic effects, dose-dependently ameliorates neurological deficits in Lewis rats subjected to experimental autoimmune encephalomyelitis (EAE). The aim of the present study was to investigate the effects of memantine and amantadine on the expression of proinflammatory cytokines such interleukin 1beta (IL-1β), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α) and various chemokines in the brain of EAE rats. Real-time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and Western Blot were used to analyze the cytokine profile. We noticed increased expression of array of cytokines in experimental group when compared to the control. Dramatic increase of IL-1β, IL-6, TNF-α, and chemokines concentration corresponding to the intensity of neurological symptoms and loss of weight was observed in EAE rats. Administration of iGluR antagonists at an advanced stage of unremitting EAE resulted in amelioration of the disease. Cytokine analysis revealed that memantine significantly decreased the expression of interleukins: IL-6 (65%), IL-1β (60%) and TNF-α (45%) whereas treatment with amantadine reduced only the expression of IL-6 (60%) and TNF-α (15%) when compared to EAE animals. These results show that antagonists of iGlu receptors modulate the course of the disease by reducing the expression of proinflammatory cytokines thereby confirming the involvement of glutamate receptors into pathological mechanisms operating during EAE.

This study was supported by grant nr NN401620038 from Polish Ministry of Science and Higher Education

P61

Ceramides and sphingosine-1-phosphate in molecular mechanisms of neuronal cell death

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Ceramides, bioactive members of the sphingolipids can be generated by *de novo* synthesis, sphingomyelin hydrolysis and by acylation of sphingosine. Ceramides are known to regulate several cellular processes, including differentiation, growth suppression, cell senescence and apoptosis. The ceramide levels increased in several pathological conditions such as brain ischemia, hypoglycemia, inflammation and in neurodegenerative disorders. Sphingosine, a metabolite of ceramide is phosphorylated by sphingosine kinases (Sphk type 1and 2) to sphingosine-1-phosphate (S1P). Sphingosine kinases are critical regulators of the sphingolipid biostat. The aim of this study was to investigate the role of ceramide and S1P in molecular mechanisms of neuronal cells death. The human *neuroblastoma* cell line (SH-SY5Y) was exposed to cell-permeable C2-ceramide. Ceramide decreased the viability of SH-SY5Y cells in concentration dependent manner. The intracellular free radical generation after ceramide treatment was about 3-fold higher comparing to control. Concomitantly our study indicated that ceramide induced poly(ADP-ribose) polymerase-1 (PARP-1) activation and decreased the level of apoptosis inducing factor (AIF) in mitochondria. Ceramide diminished the expression and level of anti-apoptotic Bcl-2 protein. PARP-1 inhibitor enhanced the level of Bcl-2 protein and cells survival keeping the level of AIF in mitochondria unchanged. The recent studies indicated that ERK1/2 are involved directly in regulation of PARP-1 activity. The specific inhibitor of these kinases protected cells against death evoked by ceramide in our experimental conditions. Moreover, our study indicated, that sphingosine-1-phosphate (S1P) increased Bcl-2 gene expression and SH-SY5Y cells survival after ceramide treatment. Summarizing, our data present that PARP-1 inhibitor and sphingosine-1-phosphate (S1P) through modulation of anti-apoptotic proteins protect mitochondria and neuronal cells against death evoked by ceramide.

Supported by statutory budget of MRC and NCN Grant 5870/PO1/2011/40

P62

Ifenprodil and NMDA ligands

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Depression is one of the most common affective disorders. According to the World Health Organization (WHO), it is currently

the fourth major global health problem (Kessler et al. 2003). The growing number of people suffering from depression has motivated the scientists to search for new antidepressant drugs. Since numerous studies revealed that NMDA receptor may be involved in the mechanism of action of the antidepressant agents, modulation of the NMDA receptor function by different ligands has been taken into consideration. There are some promising results demonstrating the antidepressant activity of the antagonists binding to the polyamine site of the NMDA receptor complex. Ifenprodil belongs to a family of the selective, atypical non-competitive antagonists of the NMDA receptors. It acts *via* inhibition of the polyamine binding site of the NR2B subunit (Williams 1993). An antidepressant-like effect of ifenprodil was observed in several behavioral studies, for example in the forced swimming test (Carter et al. 1990, Williams 1993, Layer et al. 1995, Scolnik 1999, Paoletti and Neyton 2007). It was shown that its antidepressant-like activity is increased by other antidepressant drugs (imipramine and fluoxetine). The aim of our work was to evaluate the antidepressant activity of the joint administration of ifenprodil and NMDA ligands in the mouse forced swimming test (FST). The experiments were carried out on male Albino Swiss mice. In order to avoid the risk of obtaining the false positive/negative effects in the FST test caused by a possible influence of the tested substances on the locomotor activity, the spontaneous locomotor activity was measured. The obtained results demonstrated that ifenprodil at the dose of 10 mg/kg enhances the antidepressant-like effect of the following NMDA receptor ligands: a competitive NMDA receptor antagonist – CGP 37849 (0.312 mg/kg), an antagonist at glycine site – L-701,324 (1 mg/kg) and a non-competitive antagonist at phencyclidyne – MK-801 (0.05 mg/kg). However, it did not potentiate the antidepressant activity of the inorganic modulators of the NMDA receptor complex, such as Zn^{2+} (2.5 mg/kg) and Mg^{2+} (10 mg/kg). Treatment with the tested agents did not influence the locomotor activity. In conclusion, our findings indicated that antidepressant-like activity of ifenprodil is connected with serotoninergic and glutamatergic system.