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Poster session I

|A1|

Comparison of gene transcription and depressive-like symptoms following chronic morphine and dexamethasone administration in mice

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Summary of the objectives: The molecular mechanism underlying opiate withdrawal-induced depression remains unclear. One consistently reported dysfunction in depression is the dysregulation of the stress system, the hypothalamus-pituitary-adrenal axis (HPA) which is associated with alterations in glucocorticoid receptors (GRs) function. Hippocampus, a part of limbic system has important role in regulating the HPA stress response. Therefore, we utilized a mouse model of repeated morphine and GR agonist dexamethasone administration to examine the consequences of prolonged withdrawal-induced depressive-like behaviors and to compare and examine the underlying mechanism of action of these drugs.

Methodology: C57BL/6J mice were injected twice a day for 3 weeks with morphine (MOR, increasing doses, 20-100 mg/kg i.p.), once a day with dexamethasone (DEX, 4 mg/kg i.p.) or saline (SAL, 10 ml/kg i.p.). All groups of animals were left for 3 weeks to spontaneously withdraw without injection and depressive-like behaviors were evaluated. We used real-time PCR to map transcription in the hippocampus of mouse brain undergoing MOR and DEX treatment and followed by the 21 days of abstinence.

Key results: MOR- as well as DEX-abstinent animals exhibited a significant depression symptoms. Our analysis found that mRNA expression of GR (Nr3c1) was decreased in hippocampus of MOR- and DEX-treated as well as MOR- and DEX-withdrawn mice. ANOVA tests indicated significant differences between MOR and DEX-treated in immediate-early genes expression (c-Fos, Arc, Npas4 and Bdnf). Previous analysis revealed that MOR affects expression of CAMK family genes. We noticed that MOR and DEX treatment significantly modified levels of mRNA Camk1g, one of the characteristic gene of the structure of hippocampus.

Major conclusions: Acute administration of MOR and DEX initially affects glucocorticoid pathway in the hippocampus in a similar manner. In difference, the prolonged drugs administration and abstinence regulates the molecular pathways differentially. Thus, differential molecular mechanism appears to be involved in depression evoked by opioid withdrawal and chronic glucocorticoids in hippocampus.

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|A2|

Beta(1)-adrenergic receptor blockade during chronic restraint stress modulates the expression of apoptotic signaling-related genes in rat hippocampus

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Stress may impair the structure and activity of the hippocampus (HIP) and play a role in the pathology of stress related psychiatric disorders. The noradrenaline released during stress is known to stimulate the beta adrenergic receptors (beta-AR) highly expressed in HIP. Also, apoptotic and degenerative changes in the hippocampus were reported in animal stress models. It has been shown that in the control of apoptosis many intracellular pathways are implicated and the beta-AR as well. We aimed to evaluate the stress induced effects on expression of the apoptotic signaling-related genes in the HIP and to assess whether they can be affected by beta(1)-AR blockade.

Male Wistar rats underwent the chronic restraint stress procedure applied for 3 hours daily, for 14 days. During the last 7 days rats were treated with beta-AR blocker betaxolol (5 mg/kg p.o.) given immediately after daily stress. Next day after a completion of stress procedure, the rats were decapitated and their HIPs were dissected. Then, the real-time PCR reaction with TaqMan Low Density Arrays (TLDA) was used to study the pattern of gene expression and identification of particular genes under stress reaction.

Two-way ANOVA analysis showed that chronic restraint stress increases the expression of Ikbkg mRNA, and treatment with betaxolol enhances this stress effect. Moreover, clustering analysis revealed the existence of two groups of apoptotic-related genes – down- and up-regulated by chronic restraint stress. The former consists of Casp7, Ripk3, Tnfsf10, and the latter – Dffa, Bcl2l13, Daxx. Betaxolol augmented changes caused by stress in both groups.

The results indicate that chronic restraint stress induced changes in mRNA expression of several pro-apoptotic genes belonging to various pathways involved in a control of apoptotic process. Among them the stress increased Ikbkg gene encodes for regulatory subunit of the inhibitor of kappaB kinase complex which activates NF-kappaB. This transcription factor, critical during inflammation, is also an important regulator of synaptic plasticity. Furthermore, the enhancement by beta(1)-AR blockade of the stress-induced effects on genes' expression suggests that the regulation of these genes occurs via the protein kinase A dependent manner.

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|A3|

Group II metabotropic glutamate receptors activation reduce apoptotic processes evoked by hypoxia-ischemia in 7-day old rat pups

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Hypoxic-ischemic encephalopathy (HIE) results in permanent damage of central nervous system that may result in neonatal death or developmental disorders. 20-30% of infants with HIE die in the neonatal period, and 33-50% of survivors demonstrate permanent neurodevelopmental abnormalities and mental retardation. It was shown recently that activation of group II metabotropic glutamate receptors (mGluR2/3) in a short time after ischemic insult may results in neuroprotection but the exact mechanism of this effect is not clear. The aim of present study was to investigate whether mGluR2/3 activation after hypoxia-ischemia reduces brain damage and if the inhibition of apoptotic processes is one of the involved mechanisms.

We used an animal model of hypoxia-ischemia (H-I) on 7-day old rat pups. Animals were anesthetized and the left common carotid artery was isolated, double – ligated and then cut between the ligatures. After completion of the surgical procedure the pups were subjected to hypoxia (7.4% oxygen in nitrogen for 75 min at 35°C). Control pups were sham-operated (anaesthetized and left c.c.a. dissected, but not ligated). Animals were injected intraperitoneally with specific mGluR2 (LY 379268) or mGluR3 (NAAG) agonists 1 h or 6 h after H-I (5 mg/kg of body weight). The weight deficit of the ischemic brain hemisphere was measured and the expression of pro-apoptotic and anti-apoptotic factors (Bax, Bcl-2, HTR/OMI) was examined. The damage in the hippocampal CA1 region was examined by Cresyl violet (CV) staining.

Our results show that application of mGluR2/3 agonists after H-I results in neuroprotection. Both applied agonists decreased brain tissue weight loss in ischemic hemisphere at both times of application (from 40% in H-I to 15-20% in treated). Histological examination of the brain tissue showed that both mGluR2/3 antagonists applied 1 h or 6 h after H-I decreased the damage of neuronal cells and the disorganization of CA1 region of hippocampus. Our results show also that both mGluR2/3 antagonists applied 1 h or 6 h after HI significantly reduced number of TUNEL-positive cells in ipsilateral hemispheres observed after untreated HI. The activities of pro-apoptotic caspase-3 and -9 after HI insult increased significantly in comparison to control. The injection of either of mGluR2/3 agonists 1 h or 6 h after HI significantly reduced the activities of both caspases.

Agonist of mGluR2/3 applied 1 h or 6 h after H-I decreased expression of pro-apoptotic factors Bax and HTR/OMI and increased expression of anti-apoptotic Bcl-2 in the ischemic brain hemisphere compared to H-I.

Conclusions: The results show that activation of mGluR2 or mGluR3 in a short time after H-I insult triggered neuroprotective mechanisms and reduced apoptotic processes initiated by HI in developing brain.

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|A4|

Functional inhibitors of acid sphingomyelinase as a new therapeutic target for depression

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Background and aim: Dysregulation of the ceramide metabolism (e.g., by the acid sphingomyelinase) has been proposed as an important factor in the pathogenesis of depressive disorders. Moreover, some antidepressant drugs func-

tion as the acid sphingomyelinase inhibitors and decrease ceramide levels in the rat hippocampus. The aim of this study was to analyze effects of antidepressants having different mechanisms of action and chemical structures (imipramine, tianeptine, escitalopram) as well as the substance showing antidepressant activity in preclinical research (N-acetylcysteine) on several components of ceramide metabolism in the rat hippocampus and cerebellum.

Material and methods: Male Wistar rats received imipramine (IMI, 15 mg/kg), tianeptine (TIA, 10 mg/kg), escitalopram (ESC, 10 mg/kg), N-acetylcysteine (NAC, 100 mg/kg) or corresponding vehicles acutely or chronically (for 14 days). Twenty four hours after the last injection the animals were decapitated. Brain structures were analyzed using Western Blot.

Results: We found significant increases in the synthase ceramide 2 levels after acute administration of IMI or TIA in the hippocampus as well as after chronic administration of IMI in the cerebellum. Acute and chronic administration of IMI resulted in a significant increase in the level of the ceramide synthase 4 in the cerebellum and hippocampus, respectively. On the other hand, an acute administration of TIA and the chronic administration of NAC induced a significant decrease in the synthase ceramide 4 protein expression in the hippocampus. After chronic administration of IMI and ESC, a significant increase in the synthase ceramide 5 levels in the hippocampus was found; the increase in the latter enzyme was noted for the acute administration of NAC in the cerebellum. At the end, we report the increase in the hippocampal level of neutral sphingomyelinase after chronic administration of TIA and the decrease in the cerebellar alkaline sphingomyelinase after chronic treatment with IMI.

Conclusions: Our findings indicate that different antidepressant drugs alter the expression of number of enzymes in the ceramide metabolism what may further highlight the role of this sphingolipid in the pathophysiology of depression.

|A5|

The effect of acute normobaric hypoxia on circulating BDNF during exercise to volitional exhaustion in young sedentary man and elite endurance-trained athletes

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During hypoxia exercise performance involving large muscle groups is considerably lower as compared with normoxia. This phenomenon is attributed to both muscle fatigue and so-called central fatigue. Most recent data suggest that associated with central fatigue hypoxia-related reduction of central motor output precedes the development of peripheral muscle fatigue. It has been suggested that an essential gauge role in these phenomena is played by brain-derived neurotrophic factor (BDNF). If so, BDNF release should have been differed between sedentary and endurance trained subjects during exercise, because well-trained athletes are more resistant to exercise induced fatigue. To test this hypothesis 10 healthy young males and 10 elite cyclist performed incremental exercise to volitional exhaustion (EVE) at normoxic and hypoxic conditions. The normobaric hypoxic conditions were suited to 3000 m altitude (14.7% O_2). An impact of hypoxia on participants' organism was investigated by measurement of serum EPO that was increased during EVE in hypoxic conditions in both participants groups. In normoxic conditions an increase in serum BDNF during EVE was observed only in elite endurance trained cyclists whilst in hypoxic conditions elevated level in serum BDNF was seen in both groups. This finding suggests that exercise in hypoxic conditions could stand as a more potent and effective strategy for increasing circulating BDNF than exercise performed in normoxic conditions.

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|A6|

Endurance bout of exercise upregulates dopamine metabolism in nigrostriatal system of rats

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The physical activity status of the organism impacts the function of the nervous system. Among effects of enhanced physical activity, secretion of neurotransmitters, especially monoamines, have been linked to the exercise-induced neuronal adaptation/plasticity. Interplay between exercise and monoamines was initially derived from the "Central Fatigue Hypothesis", in which increased brain 5-HT release was found to be associated with central fatigue. Most recent data suggest a possible role of dopaminergic pathway located within nigrostratial system in the control of locomotion. We hypothesized this system can be also stimulated by a bout endurance exercise of moderate intensity. Rats were running on the treadmill (0° inclination) at 24 m/min to exhaustion. Rats were killed immediately after exercise and striatum and midbrain were quickly isolated on ice-cold glass Petri dish and estimation of TH mRNA and protein level, activity of MAOB and levels of DA as well as its metabolites concentrations in striatum and midbrain were performed. In the present study, the bout of endurance exercise of moderate intensity resulted in increased TH mRNA and protein level as well as MAOB activity in the striatum and midbrain. These results suggest that favorable effects of endurance exercise are related to the enhanced dopamine synthesis and metabolism in the nigrostriatal system.

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|A7|

PARP-1 and its role in transcription of mitochondrial respiratory complexes and enzymes of antioxidative defence under basal conditions and in Amyloid Beta toxicity

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Poly(ADP-ribose) polymerase-1 (PARP-1) is the major member of PARPs family mainly responsible for a post-translational modification of transcription factors, affecting expression of many nuclear and mitochondrial proteins. PARP-1 plays also an important role in DNA repair and in energy metabolism regulating cells survival and death. Overactivation of PARP-1 by excessive genotoxic stress may lead to cell death in Alzheimer's disease (AD). Our previous data indicated beneficial effects of PARP-1 inhibition in neurodegenerative disorders. The aim of this study was to investigate the role of PARP-1 in regulation of gene expression of enzymes responsible for function of mitochondrial respiratory chain complexes and for antioxidative defense in resting pheochromocytoma PC12 cells and in conditions of Amyloid Beta 1-42 (AB) toxicity.

The study was carried out using PC12 cells treated with PARP-1 inhibitor PJ34 (20 μM) or with AB oligomers (ABO, 1 μM) for 24 h. Quantitative RT-PCR as well as biochemical, immunochemical, spectrofluorometric and flow-cytometric methods were applied.

Our data indicate that pharmacological inhibition of PARP-1 in PC12 cells by PJ34 enhances transcription of enzymes responsible for mitochondrial metabolism. PJ34 increases the mRNA level of subunit of NADH:ubiquinone oxidoreductase (MT-ND1) of complex I and has similar stimulatory effect on expression of gene for subunit of succinate dehydrogenase (SDHA) of complex II. Additionally, PJ34 augments transcription of cytochrome b (MT-CYB) of complex III and subunit of cytochrome c oxidase (MT-CO1) of complex IV. These changes may enhance mitochondrial activity leading to higher production of free radicals. Analysis of enzymes involved in antioxidative defense indicates downregulation of mitochondrial superoxide dismutase (SOD2) and glutathione-disulfide reductase (GSR). ABO inhibit expression of SDHA, SOD2, and reduce

mitochondrial membrane potential (MMP) and cell viability. PJ34 has no protective effect on suppression of MMP and cell viability by ABO. Our data indicate that inhibition of PARP-1 enhances the expression of genes involved in mitochondrial function; however, has no protective effect on MMP and PC12 survival affected by ABO. These results show the complex role of PARP-1 in cell metabolism and cell fate and suggest dependence on cell type and experimental conditions.

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|A8|

Maternal immune activation alters the synaptic protein level and mTOR signaling pathway in rat offspring

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Autism spectrum disorders (ASDs) are complex neurodevelopmental brain diseases characterized by deficits in social interaction, language and stereotyped behaviours. Recent discoveries of single mutations in genes coding synaptic proteins in affected individuals suggested the synapse as a possible site of autism origin. Maternal immune activation (MIA) during pregnancy is a risk factor for autism in the offspring and is commonly used as animal model of ASD. Infections during pregnancy activate the mother's immune system and alter the fetal environment, and in consequence can affect synaptic function and behaviour in the offspring. However, the molecular link between infection-induced altered fetal development and risk for ASD are still unclear. In this study we investigated behavioural changes, synaptic protein expression as well as Akt-mTOR pathway protein level in the offspring of pregnant Wistar rats given an intraperitoneal (0.10 mg/kg) injection of lipopolysaccharide on gestational day 9.5. Our results indicated that communication (expressed by ultrasonic vocalizations, USVs) of rat pups born to MIA mothers compared to pups born to saline-injected mothers was impaired. Analysis of USV of 9-11-day-old animals showed a longer mean time vocalization in pups from MIA mothers with significantly lower frequency of USV. Moreover, the results showed no bedding preference in MIA offspring at post-natal day 15 compared to control rats, indicating the impairment of need being in proximity of the mother. Along with the behavioural changes MIA induced presynaptic protein alterations in adolescent rat offspring including decrease in the level of synaptobrevin and syntaxin-1, the key components of SNARE complex. However, the higher level of synapsin was observed in brain cortex and hippocampus. Together with presynaptic protein changes MIA induced reduction in PSD-95 in the cerebral cortex and hippocampus and down-regulation of SHANK 1-3. Moreover, alteration in the protein level of phospho-Akt, and 4E-BP1 was found in MIA subjects. It is possible that alterations of Akt-mTOR pathway result from aberrant synthesis of postsynaptic density proteins as supported by decreased level of SHANK and PSD-95. The altered synthesis of these proteins would generate changes in molecular and structural aspects of synaptic plasticity, contributing to ASD-like behaviours.

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|A9|

Upregulation of TNF-α and IL-6 in serum and cerebrospinal fluid of patients with ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder without effective cure. The involvement of inflammation in the pathogenesis of ALS, is increasingly recognized but still not fully understood. Therefore, the purpose of this study was to investigate the levels of inflammatory mediators such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-4 in serum and

cerebrospinal fluid (CSF) of ALS patients. The study was approved by the Local Ethic Committee of University of Warmia and Mazury in Olsztyn, Poland. The diagnosis of ALS and the evaluation of the revised ALS functional rating scale (ALSFRS-R) score were performed for each ALS patient by neurologist. Blood and CSF samples were drawn from 10 ALS patients and 10 patients with other non-inflammatory neurological disorders (NND) served as a control group. Enzyme-like immunosorbent assay (ELISA) was used to check serum and CSF levels of TNF-α, IL-6 and IL-4. Unpaired, independent 2-tailed Student *t*-test was used and the statistical data were expressed as the mean ± SEM, statistical significance was defined as a *p*-values below 0.05.

The concentrations of TNF-α and IL-6 in serum and CSF of ALS patients were greater than in NND patients. There was no statistical difference in serum IL-4 level between studied groups, however, in the ALS patients the amount of this cytokine was lower. In the CSF, the level of IL-4 was not detectable. Our study shows the upregulation of TNF-α and IL-6 in serum and cerebrospinal fluid of ALS patients, which suggests the importance of these inflammatory factors in the course of the disease.

|A10|

Tetrahydrocarbazoles stabilize elevated SOCE in a Huntington's disease model, MSNs from YAC128 mice overexpressing huntingtin-associated protein 1 isoform A

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Store-operated Ca^{2+} entry (SOCE) is one of the mechanisms that regulate Ca^{2+} homeostasis and is enhanced in Huntington's disease (HD). However, it is still unknown how mutated huntingtin affects SOCE and there is no effective treatment of HD. We previously showed that huntingtin associated protein 1 (HAP1) is up-regulated in the striatum of HD model, YAC128 mice. We also found that selected tetrahydrocarbazoles stabilize the ER Ca^{2+} release in cellular Alzheimer's disease model. The aim of this work was to investigate the role of HAP1 protein in SOCE dysregulation and check the effect of tetrahydrocarbazoles on the ER Ca^{2+} release and SOCE as well as cell death in YAC128 medium spiny neurons (MSNs). Single cell Ca^{2+} imaging, gene silencing and overexpression as well as cell death and mitochondrial membrane potential assays were used for this purpose. We observed that HAP1 isoform A overexpression decreases ionomycin induced ER Ca^{2+} release and enhances SOCE, whereas its silencing attenuates SOCE and decreases ER Ca^{2+} release induced by DHPG, an mGluR1/5 receptor agonist. In HD MSNs overexpressing HAP1A we found that certain tetrahydrocarbazoles have a stabilizing effect on elevated SOCE, however, no effect on ER Ca^{2+} release was observed. Moreover, we found that some of tetrahydrocarbazoles increase mitochondrial membrane potential, but they are not able to stabilize glutamate induced cell death in HD model. We conclude that HAP1A increases SOCE in HD MSNs by IP3R activation and tetrahydrocarbazoles exhibit stabilizing effect on the disturbed Ca^{2+} homeostasis in HD model.

|A11|

The involvement of Sp1 in transcriptional regulation of the glutamine transporter SN1 in cultured mouse cortical astrocytes treated with ammonia

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Ammonia neurotoxicity plays a key role in the pathogenesis of hepatic encephalopathy (HE). Astrocytes are the only compartment in the brain where ammonia detoxification triggers intracellular glutamine (Gln) accumulation. Gln efflux from astrocytes is mediated by a system N transporter, SN1 (SNAT3) that demonstrates the ability to change transport direction with changing extracellular pH (pHe) and transmembrane gradients of amino acids (Chaudhry *et al*., 1999; Broër *et al*., 2002). In addition, the gene coding SN1 (SNAT3) has a putative pH responsive element in the 3'-UTR (Solbu *et al*., 2005). Literature data suggest that during ammonia-induced metabolic acidosis in mouse kidney, SN1 is upregulated by interactions with the specificity protein 1 (Sp1) transcription factor (Balkrishna *et al*., 2013). In turn, Sp1 presents a tendency toward increase in cultured rat astrocytes treated with 5 mM ammonia (Bodega *et al*., 2006). However, the specific regulatory role of Sp1 in transcriptional regulation of SN1 in astrocytes treated with ammonia and its relation to ammonia-induced changes in pH was not analyzed so far. The latter hypothesis was tested in the present study.

Using real-time qPCR we measured SN1 and Sp1 mRNA level in cultured mouse cortical astrocytes treated with 5 mM ammonium chloride for 24 h and to mild (pH 6.8) acidosis. Simultaneously, the impact of ammonia and acid incubation on intracellular (pHi) and pHe was evaluated. Sp1 transcription factor silencing was performed using siRNA technology. pHi was measured using fluorescent probe 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetomethyl ester (BCECF-AM).

Sp1 mRNA level showed tendency toward increase (similarly to previous study on rat cortical astrocytes) in ammonia-treated astrocytes, whereas acidic media reduced Sp1 mRNA level. Ammonia did not alter SN1 mRNA level, but acidic media did. Neither treatment changed pHi, but pHe was elevated after ammonia treatment. Sp1 silencing in ammonia treated astrocytes decreased SN1 mRNA level while in acidic medium a return of SN1 mRNA level was observed.

In conclusion, our study demonstrates that SN1 mRNA expression is dependent on the presence of Sp1 transcription factor, suggesting that Sp1 is an enhancer of SN1 expression. Moreover, a decrease of pHe downregulates SN1 mRNA expression.

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|A12|

Is DCF test a suitable tool for the evaluation of tetrabromobisphenol A-induced oxidative stress in cultured neurons?

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Tetrabromobisphenol A (TBBPA) belongs to brominated flame retardants exhibiting cyto(neuro)toxic properties in cell culture models. The role of oxidative stress in TBBPA-induced cytotoxicity has been well established, mainly based on the results of DCF assay demonstrating enhanced ROS production in TBBPA-treated cells. However, according to recent reports, in cell-free solutions TBBPA chemically converts a parent compound DCFH-DA into fluorescent DCF, which casts doubt on earlier reports showing TBBPA-induced oxidative stress in cells. The aim of the present study was to asses reliability of DCF assay in evaluating TBBPA-induced oxidative stress in primary cultures of rat cerebellar granule cells (CGC). The experimental approach involved measurements in CGC and in cell-free solutions, using a standard plate reader, of the effects of 10 and 25 µM TBBPA on fluorescence of DCF. The level of oxidative stress in CGC challenged with TBBPA, which was also assessed by measuring GSH content and catalase activity, was modulated pharmacologically using NMDA and ryanodine receptor antagonists, 0.5 µM MK-801 and 200 µM ryanodine with 2.5 µM bastadin 12, respectively. They are known to have no intrinsic antiradical properties but inhibit TBBPA-induced calcium transients in CGC. The experiments on CGC demonstrated TBBPA-induced, concentration-dependent rise in DCF fluorescence which was accompanied by a concomitant decrease in GSH content and catalase activity. These phenomena were inhibited by NMDA and ryanodine receptor antagonists, which were by themselves ineffective in control CGC loaded with DCFH-DA. The results of experiments in cell-free system confirmed that TBBPA potentiates fluorescence of cell-free DCFH-DA solution, but this effect was not inhibited by NMDA and ryanodine receptor antagonists and was even enhanced by bastadin 12. Moreover TBBPA decreased fluorescence of rhodamine 123 solution, while in CGC loaded with this probe it enhanced fluorescence. Thus, the results of biological experiments in accordance indicate, that TBBPA induces oxidative stress in CGC, and DCF test in CGC reflects enhanced ROS production under these conditions. Instead, the effects observed in the cell-free DCFH-DA and rhodamine 123 solutions are irrelevant to data from the living cells. The presentation will show provisional explanation for these discrepancies.

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|A13|

Perinatal exposure to lead alters the synaptic structure and the expression of key synaptic protein in Wistar rats

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The heavy metal lead (Pb) is an abundantly existing environmental toxicant for the development of central nervous system. Prenatal lead exposure has negative impacts on many neurodevelopmental processes including synaptogenesis, apoptosis and causes abnormalities in learning, and cognitive functions in the offspring. However, up till now there is no exact mechanism explaining molecular events leading to synaptic endings impairments. The aim of the present study was to investigate the effect of perinatal exposure to low dose of Pb (Pb concentrations in whole blood below 10 µg/dl) on the synaptic structure and the synaptic proteins expression in the developing rat brain. Furthermore, the brain-derived neurotrophic factor (BDNF) level was analyzed. Lead (0.1% PbAc) was administrated to pregnant Wistar rats via drinking water (Pb-group) from the first day of gestation until weaning of the offspring. Pups were weaned at postnatal day 21 and then until postnatal day 28 received only drinking water. At the end of experiments, 28-day old pups were sacrificed and the ultrastructural changes as well as expression of presynaptic (VAMP1/2, Synaptophysin, Synaptotagmin-1, SNAP25, Syntaxin-1) and postsynaptic (PSD95) proteins, and BDNF level were analyzed in forebrain cortex, cerebellum and hippocampus. We showed that perinatal Pb exposure promotes pathological changes in synapses including nerve endings swelling, blurred and thickened synaptic cleft structure as well as enhanced density of synaptic vesicles clustering in the presynaptic area. Moreover, synaptic mitochondria were elongated, swollen or shrunken in Pb-group. Together with ultrastructural changes we observed lowering of the level of Synaptotagmin-1 in cerebellum, SNAP25 in hippocampus and Syntaxin-1 in cerebellum and hippocampus. In addition, in cerebellum Synaptophysin level was increased. The expression of PSD95 was significantly reduced in this structure and in forebrain cortex, but increased in hippocampus. These changes are accompanied by lower level of BDNF in all brain structures from Pb-group. In summary, we found that perinatal Pb exposure affected the synaptic structure and the key synaptic proteins expression, that could impair synaptic plasticity as well as the learning and memory processes in the offspring.

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|A14|

AMPA receptors are involved in STIM-dependent Store-Operated Calcium Entry in rat primary cortical neurons

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Store-Operated Calcium Entry (SOCE) is a process, which leads to refilling of endoplasmic reticulum (ER) with calcium ions (Ca^{2+}) after their release into the cytoplasm. The interaction between ER-located proteins (STIM1, STIM2) and plasma membrane (PM)-located Ca^{2+} channel-forming protein (ORAI1) mediates the formation of complexes and underlies SOCE in non-excitable cells. Recent studies have recognized the importance of SOCE also in neurons and found complex relationship between STIM proteins and neuronal Ca^{2+} channels, but its molecular mechanism in neurons requires more detailed investigation. Our previous data indicated that both STIMs are involved in Ca^{2+} homeostasis in neurons (Klejman *et al*., 2009), form complexes with endogenous ORAI1 (Gruszczynska-Biegala and Kuznicki, 2013) but play a distinct role in SOCE (Gruszczynska-Biegala *et al*., 2011). In contrast to non-excitable cells, Ca^{2+} influx in neurons is modulated mainly by voltage gated Ca^{2+} channels and ionotropic receptor-operated Ca^{2+} channels. Here we report, that the SOCE inhibitor ML-9 reduces AMPA-induced Ca^{2+} influx by 80%. To assess the role of AMPA receptors (AMPARs) in SOCE, they were inactivated in cortical neurons by their specific inhibitors. As estimated by FURA-2AM single-cell Ca^{2+} measurements

in the presence of CNQX or NBQX, thapsigargin-induced Ca^{2+} influx was decreased 2.2 or 3.7 times, respectively. These results suggest that during SOCE, calcium ions can enter neurons also through AMPA receptors. In addition, we found by co-immunoprecipitation assays, that when Ca^{2+} level is low in the neuronal ER, a physical association of endogenous STIM proteins with endogenous GluA1 or GluA2 subunits of AMPAR occurs. Taken together, these data suggest an involvement of AMPAR in SOCE and its link with STIM proteins.

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|A15|

The effect of one week of low-threshold stimulation of proprioceptive fibers on glutamatergic and cholinergic innervation of the ankle extensor α-motoneurons on early-phase after complete transection of the spinal cord

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Seven days of stimulation of low-threshold proprioceptive fibers in the tibial nerve in intact rats leads to synaptic plasticity in the Hoffmann-reflex (H) circuit involving two extensors operating at the ankle joint, i.e., soleus and lateral gastrocnemius (LG) α-motoneurons (MNs). We focused on the effects of stimulation on two types of excitatory inputs to α-motoneurons: the first input is formed by glutamatergic Ia sensory afferents contacting monosynaptically α-MNs; the second one is the cholinergic input originating from V0c – interneurons. We found that one week of continuous burst stimulation of proprioceptive input to LG α-motoneurons is effective in enrichment of their direct glutamatergic but also indirect cholinergic inputs.

Our recent aim is to clarify whether enhancement of signaling to ankle extensor α-MNs, via the same pattern of direct electrical stimulation of tibial nerve, will affect both Ia glutamatergic and cholinergic innervation of α-motoneurons of LG in rats subjected to complete transection of the spinal cord at low-thoracic segments. Tibial nerve was stimulated for 7 days with continuous bursts of three pulses delivered every 25 ms in 4 × 20 minutes sessions daily. Stimulation started on the second day after spinalization. Monitoring of H-reflexes and threshold M-responses recorded from the soleus muscle, allowed controlling strength of stimulation of Ia afferents. LG-MNs were identified with tracer injected intramuscularly. Glutamatergic Ia- and cholinergic C-terminals abutting on LG-MN perikarya were detected by immunofluorescence (IF) using input-specific anti-VGLUT1 and anti-VAChT antibodies, respectively.

The effect of spinalization on frequency of H-reflexes was inconsistent but frequency of complex responses after 2nd and 3rd stimuli in the burst increased after stimulation. Quantitative analysis of confocal images revealed that 7-days after spinalization the number of VGLUT1-IF terminals tended to decrease and stimulation of Ia fibers did not bring change. The number of VAChT-IF terminals did not change either after spinalization or stimulation. The volume of terminals tended to decrease after spinalization. To conclude, one week of continuous burst stimulation of proprioceptive Ia input to LG-MNs, which started early after spinalization, was not sufficient to bring substantial changes in innervation of LG-MNs.

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|A16|

Treatment with neurotensin-opioid hybrid peptide alleviates inflammation in murine model of contact sensitivity reaction and non-atopic asthma – preliminary results

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Background: Delayed type hypersensitivity (DTH) plays crucial role in pathophysiology of inflammatory disorders like contact sensitivity and non-atopic asthma. Immunization with low molecular compounds and local challenge with the cognate antigen elicit tissue swelling response and asthma like symptoms. The object of the study was

to investigate anti-inflammatory activity of PK20 (hybrid peptide) in experimental murine models of inflammation.

Methods: Non-atopic asthma and contact sensitivity response were induced in mice by skin sensitization with dinitrofluorobenzene (DNFB) followed by intratracheal challenge of dinitrobenzene sulfonic acid (DNS) and topical DNFB application on ears. After hapten challenge and eight hours later, mice were treated intraperitoneally with PK20, neurotensin or endomorphin-2 pharmacophores. In subsequent experiments the effect of neurotensin and opioid receptors blockade on PK20-induced anti-inflammatory activity was examined. Twenty four hours after hapten challenge, bronchoalveolar lavage fluid (BAL) was collected and total amount of inflammatory cells was counted using Burker-Turk chamber. Measurement of ear thickness was performed with engineer's micrometer. Ear swelling was calculated by subtracting swelling recorded for the vehicle-control ear from the swelling recorded for the DNFB challenged ear.

Results: Treatment with PK20 abolished the late phase of ear swelling and significantly reduced the total number of cells in BAL fluid in comparison to group treated with physiological saline. Blockade of neurotensin receptors was more effective in reducing hybrid anti-inflammatory activity than blockade of opioid receptors. These relations were more apparent in the ear thickness measurements than in the bronchoalveolar lavage studies. Application of endomorphin-2 and neurotensin pharmacophores diminished the ear edema and the number of inflammatory cells in BAL, however they were much less potent than PK20 treatment.

Conclusions: Hybrid peptide, PK20 reduces the signs of inflammation: ear swelling and infiltration of inflammatory cells into the lungs. Both neurotensin and opioid pathways seem to be involved in anti-inflammatory activity of tested compound. However, study with application of antagonists indicates stronger contribution of neurotensin component. The mechanism of PK20 action remains unclear and needs further investigation.

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|A17|

The dopaminergic innervation of the orbitofrontal prefrontal cortex (OFC) is significantly altered in the spontaneously hypertensive rats (SHR)

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The attention deficit hyperactivity disorder (ADHD) is associated with dysfunctions of the dopamine (DA) system and decreased dopamine activity in the prefrontal cortex (PFC). The orbitofrontal cortex (OFC), which is a part of PFC, may be critical for ADHD. This brain center is involved in response to reward and adjustment animal behavior when the rewarding properties of the reinforcement change. Moreover OFC is important during the developing of addiction and there are several lines of evidence that untreated ADHD is conducive to addiction and abuse. Thus, the question is whether OFC is altered in the individuals affected by ADHD and how the dopamine activity is altered. The aim of this study was to compare DA immunoreactivity in OFC of the spontaneously hypertensive rats (SHR, animal model of ADHD) and Wistar Kyoto rats (WKY, healthy controls).

Frozen brain sections from juvenile (4 weeks old) and adult (10 weeks old), male SHR and WKY rats were processed by single immuonofluorecence using thyrosine hydroxylase (TH; the rate-limiting enzyme of catecholamine biosynthesis) antibody. In the medial orbital cortex (MO), ventral orbital cortex (VO), lateral orbital cortex (LO) and dorsolateral orbital cortex (DLO) immunoreactive structures were carefully counted and the counts were compared between SHR and WKY rats.

The result show that the number of fibers expressing TH in LO, VO and DLO was lower, while in MO higher in the 4 weeks old SHR rats when compared to the WKY strain. In adult animals (10 weeks old) this pattern of TH distribution was reversed. In conclusion, these results suggest that the DA system is significantly altered in the orbitofrontal cortex of the SHR model of ADHD. As OFC mediates cognitive control of behavior, alterations in DA system in this region my lead to diversified symptoms of ADHD such as behaviors that are inappropriate for the context,

premature, poorly planned and often resulting in adverse consequences.

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|A18|

5-HTTLPR polymorphism and serotonin concentration among migraine patients

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Introduction: Migraine is a primary headache disorder that affects 11% of adults. There are two main, distinct clinically and probably also etiologically, subtypes of the disease: migraine with aura (MA) and migraine without aura (MO). The cortical spreading depression (CSD) is postulated to be involved in pathomechanism of migraine. In the consequence of CSD activation, many vasoactive factors are released, e.g. serotonin (5-HT). The correlation between 5-HTTLPR polymorphism of the serotonin transporter gene and level of 5-HT was found, but the possible impact on migraine remains unclear. It is known that the SS genotype of 5-HTTLPR is associated with lower reuptake of 5-HT, while the SL genotype is linked to impaired function of 5-HT transporter.

Aim of the study: The aim of the study was to analyze 5-HTTLPR polymorphism, 5-HT plasma concentration and clinical features of migraine.

Material and methods: The study included 96 migraine patients (MA: 43, MO: 53; mean age 39 ± 14) and 82 controls (mean age: 38 ± 14). The 5-HTTLPR polymorphism was determined by polymerase chain reaction (PCR) and visualized by electrophoresis. The high performance liquid chromatography with electrochemical detection (HPLC/EC) was used to determine 5-HT plasma level.

Results: Plasma concentration of 5-HT was higher in MA patients than in MO or control group ($p < 0.01$). The SL genotype of 5-HTTLPR polymorphism was more frequent and LL was less frequent in migraine than in controls ($p < 0.01$). The SS genotype was associated with higher 5-HT level only in MA. The correlation between duration of migraine and 5-HT concentration was also observed only in MA: the longer migraine history, the higher 5-HT level. Moreover, it was found, that the level of 5-HT changes with the length of a migraine attack, both in MA and MO.

Conclusion: Serotoninergic system may play more important role in pathogenesis of MA than MO.

|A19|

The changes in density of catecholaminergic fibres in the rostral prefrontal cortex spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY)

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Dysfunction of dopamine (DA) neuronal systems leads to several serious emotional disorders and it has been postulated that DA alterations may be key factor in the pathophysiology of attention deficit hyperactivity disorder (ADHD). Dopamine plays a key role in attentional, psychomotor, reinforcing and rewarding behaviors that are deficient in ADHD. The rostral prefrontal cortex (rPFC) is a part of dopamine neuronal systems and it has several features which may be critical for ADHD development. One of them is the fact that rPFC has been directly associated with attentional processes, visceromotor activity, decision making and goal directed behaviors. The second fact is that decreased DA release in rPFC impairs the actions of this area. The aim of this study was to compare DA immunoreactivity in the rostral prefrontal cortex of the spontaneously hypertensive rats (SHR, animal model of ADHD) and Wistar Kyoto rats (WKY, healthy controls). Juvenile (4 weeks old) and adult (10 weeks old), male SHR and WKY rats were used in the study. Frozen 10 µm-thick brain sections comprised rPFC were stained by standard single immunofluorecence using thyrosine hydroxylase (TH; the rate-limiting enzyme of catecholamine biosynthesis) antibody. Following parts of rPFC were analyzed: infralibic (IF), prelimbic (PrL) and cingulate cortex (Cg). In all these

areas immunoreactive structures were counted manually using test frames.

The result show that the densities of fibers containing TH in 4 weeks old SHR rats were lower in all analyzed areas of the rPFC in comparison with WKY strain. Moreover, in 10 weeks old animals the same differences were still observed in the infralibic cortex, while in the prelimbic and cingulated cortices they have been erased.

In conclusion, these results suggest that DA system in the rostral prefrontal cortex of the SHR model of ADHD is hypofunctional. As dopamine and rPFC play key roles in attentional processes, lowered DA expression in this brain region fits well with inattention observed in ADHD affected individuals.

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|A20|

TRP channels are engaged in memory consolidation and reconsolidation in passive avoidance task in one-day old chicks

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The stimulation of both ionotropic and metabotropic glutamate receptors and influx of calcium ions (Ca^{2+}) into neurons is a crucial step in intracellular cascade of memory formation. Recently the existence of additional mechanism involved in intracellular Ca^{2+} increase, triggered by internal signals like increase of Ca^{2+} within the cell and activation of G protein coupled receptors, was demonstrated. This mechanism involves transient receptor potential (TRP) channels. The aim of our study was to investigate the participation of TRP channels in intracellular mechanisms engaged in memory consolidation and reconsolidation.

The model of passive avoidance task in one day old chicks was used. Chicks were injected with non-specific TRP channels antagonist SKF96365 or with three different concentrations of 2-APB, the inhibitor of IP3 receptors, which in small concentrations (~10 μM) inhibits also TRP channels. The injections were made at different times before and after training, to find the most effective time. The injection of each antagonist immediately after training resulted in task amnesia when tested 24 h later. The injection of SKF96365 immediately after training resulted in constant amnesia that manifested 1.5 h after training, whereas amnesia after injection of 2-APB was observed as early as 30 min after training. The effect of application of TRP channels antagonist SKF96365 and 10 μM 2-APB on memorizing of the task in comparison with the effects of antagonists of mGluR1 and mGluR5, the receptors that trigger IP3 release, showed similarities when memory was tested 2 h and 24 h training. Application of SKF96365 or 10 μM 2-APB immediately after reminder given 2 h after initial training, resulted in transient amnesia of the task that manifested in a short time after reminder and lasted for as long as 6 h. Our results show that inhibition of both TRP channels and IP3 receptors has a strong impact on both memory formation and reconsolidation.

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|A21|

Overexpression of parkin protects PC12 cells against alpha-synuclein evoked mitochondria damage and cell death

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Parkin, an ubiquitin E3 ligase, that is responsible for the clearance of damaged proteins, is linked to rare familial forms of Parkinson's disease (PD) through loss-of-function mutations and to sporadic PD through posttranslational inactivation. However, the detailed mechanism underlying the neuroprotective function of parkin in dopaminergic neurons, especially its role in alpha-synuclein (ASN) evoked toxicity, is still unclear. Recent studies have focused on parkin's role in mitochondrial biogenesis and turnover, including mitochondrial fission/fusion as well as mitophagy. The aim of this study was to investigate the protective role of parkin in ASN-induced mitochondrial damage. Investigations were performed on PC12 control cells and PC12 cells with parkin overexpression treated with ASN oligomers as well as in cells with parkin knock-down. We demonstrated that exogenous ASN induced overproduction of free radicals including nitric oxide (NO), resulting in parkin S-nitrosylation and alteration of its activity. Concomitantly, in ASN treated cells the parkin protein level was

decreased, while no significant difference in mRNA level was found. Additionally, both NO stress, induced by ASN oligomers, as well as parkin knock-down triggered mitochondrial dysfunction, followed by significant decrease in mitochondrial membrane potential, overproduction of mitochondrial superoxide anion and depletion of cellular ATP level. Moreover, changes in the expression of proteins that regulate mitochondria biogenesis (peroxisome proliferator-activated receptor γ co-activator-1α, PGC-1α), fission (dynamin-related protein Drp1) and fusion processes (Opa1, Mitofusin-2, Mfn2) were observed. These events create a death-prone milieu that contributes to the loss of dopaminergic cells. Finally, we showed that parkin overexpression prevented mitochondrial superoxide production and attenuated ASN-evoked PC12 cell death, pointing to the importance of parkin in ASN-mediated toxicity. These findings may thus provide a molecular link between parkin dysfunction and ASN induced mitochondrial impairment in sporadic PD. We suggest that amelioration of parkin's function may be a novel therapeutic target to treat PD.

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|A22|

Protein kinase B signaling pathway in glioblastoma cells transfected with the liver-type glutaminase

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Glutamine (Gln) plays a crucial role in the metabolism of tumors of different origin, including gliomas. Deregulated expression/activity of glutaminase (GA, EC 3.5.1.2), an enzyme converting Gln to glutamate (Glu) and ammonia is a characteristic feature of many cancer cell lines and tumors. There are two coding genes for GA: GLS and GLS2. Mounting evidence suggests that proteins encoded by either of the genes play opposing role in tumorigenesis. In glioblastoma (GBM), the most aggressive brain tumor, GLS encoding kidney-type isoforms (KGA and GAC) is highly expressed, while expression of GLS2 coding for liver-type isoforms (LGA and GAB) is hardly detectable. Previous studies revealed that transfection of human glioblastoma T98G cell line with a sequence encoding GAB suppressed malignant phenotype of these cells and altered expression level of 85 genes (Szeliga *et al*., 2009). The cells transfected with GAB (herein referred to as TGAB) are more sensitive to alkylating agents used in GBM therapy (Szeliga *et al*., 2012) and to oxidative stress (Martin-Rufian *et al*., 2014). Activation of the PI3K/AKT pathway has been documented in GBM, but it is also observed in different cell types upon oxidative stress. Here we tested the hypothesis, that transfection with GAB modulates the PI3K/AKT pathway. Western blot analysis revealed a ~45% decrease in the level of phosphorylated AKT in TGAB cells as compared T98G cells and TpcDNA (T98G transfected with an empty vector). The level of the unphosphorylated AKT remained unchanged. No differences between TGAB and T98G or TpcDNA cells were observed in the level of PTEN, a negative regulator of AKT signaling pathway. In conclusion, our results suggest that transfection with GAB modulates AKT signaling pathway in T98G cells. A more detailed analysis of this phenomenon is currently conducted in our laboratory.

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|A23|

Circulating levels of miR-1, miR-29 and miR-30 are associated with BDNF level in serum of patients with Parkinson disease

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Numerous studies have shown that brain-derived neurotrophic factor (BDNF) regulates number of functions in the nigrostriatal system. It demonstates a neuroprotective effect against degeneration of dopaminergic neurons and improves both memory and motor activity. Reduced CNS concentration of BDNF in the elderly is accompanied with increased number of incidents of neurodegenerative diseases. Small non-coding RNAs of 21-24 nucleotides long

(miR-1, miR-29, miR-30-5a) play an essential role in the regulation of BDNF gene expression. The aim of the present study was to determine the concentration of BDNF and miR-1, miR-29, miR-30-5a in the serum of patients with idiopathic parkinsonism (iPD). The concentration of BDNF was determined by ELISA (R&D System, Minneapolis, MN, USA). Selected miRNAs were determined by array cart and real-time qPCR using specific primers TaqMan miRNA assay (Life Technology, Carlsbad, CA, USA) in the serum of patients with iPD and age-matched healthy subjects. The serum concentration of BDNF decreases with aging in healthy subjects. Moreover, the concentrations of BDNF, miR-1, miR-29 and miR-30 were statistically lower in the serum of patients with iPD compared with age matched healthy subjects. These results revealed that decreasing of miRNA-s levels was associated with reduction of BDNF level in serum of iPD patients. Our study provides a preliminary evidences that the regulation of BDNF level by miRNA may play a role in preventing of the neurodegenerative processes in nigrostriatal system.

|A24|

Role of nitric oxide synthase (NOS) in generation of oxidative/nitrosative stress in the cerebral cortex of rat with thioacetamide induced acute liver failure (ALF)

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Acute liver failure (ALF) is associated with deregulation of NMDA/cGMP/NO signaling and oxidative/nitrosative stress in the brain. However, the relative roles of the different NOS isoforms and the mechanisms underlying alterations in their activities during ALF are not fully clear. Here we investigated gene and protein expression of NOS isoforms, NOS activity, eNOS uncoupling and total NO production in cerebral cortex of rats with thioacetamide (TAA)-induced ALF. Sprague Dawley rats (250-280 g) received three i.p. injections of TAA (300 mg/kg) at 24 h intervals. The brain cortex expression of NOS isoforms (eNOS/iNOS/nNOS) was measured by Real-time PCR and Western Blot, NOS activity was tested by monitoring the conversion of radiolabeled arginine to citrulline. Reactive oxygen species (ROS) were quantified in the presence of NOS substrate L-arginine, using the carboxy-H2 DCFDA probe. NO was measured with the Griess procedure. The eNOS expression was decreased, whereas the eNOS dimmer/monomer ratio and nNOS/iNOS expression were elevated in TAA treated rats. While the total NOS activity was decreased, the iNOS activity was elevated and NO concentration tended to increase. ROS production was elevated by TAA. Unspecific NOS inhibitors L-NAME and LNNA attenuated ROS production in both control and TAA rats, but with higher efficiency in the latter case. Ca^{2+} chelation had almost the same effect as pharmacological NOS inhibition suggesting that Ca^{2+}-independent iNOS activity is not the main source of ROS. Incubation with high dose of tetrahydrobiopterin, which is critical for eNOS dimerization and subsequent NO production, also reduced ROS production indicating the eNOS uncoupling phenomenon in TAA cortex. The study points to eNOS downregulation due to lowered protein expression and uncoupling as a mechanism contributing to enhanced superoxide anion formation, and confirms the role of iNOS/nNOS in enhancing NO synthesis in ALF-affected brain.

|A25|

Toxicological research of 1-methyl-1,2,3,4-tetrahydroisoquinoline an exo/endogenous amine with antidepressant-like activity – *in vivo*, *in vitro* and *in silico* studies

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Tetrahydroisoquinolines, the most numerous naturally occurring alkaloids, include 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) which demonstrates significant neuroprotective activity observed in various neurotoxicity models and has structural similarity to dopamine (DA). It can interact with agonistic conformation of DA receptors. 1MeTIQ inhibits the formation of 3,4-dihydroxyphenylacetic acid (DOPAC) as well as production of free radicals and shifts DA catabolism toward the catechol-O-methyltransferase (COMT)-dependent O-methylation, and such mechanism of action seems to be important for its neu-

roprotective activity. It has been found that 1MeTIQ inhibits both monoamine oxidase A (MAO-A) and B (MAO-B) enzymes activity and increases neurotransmitters level in the brain. That is more, 1MeTIQ shows significant antidepressant-like effect in the FST and the reserpine model of depression in rats. Therefore, this compound might be effective for the depression therapy in a clinical setting but the success of this drug is determined not only by its good efficacy but also by an acceptable ADMET profile. ADMET prediction use in combination with in vivo and in vitro studies greatly simplifies the search for new, safer and effectively acting drugs. The aim of this study was to investigate the degree of histopathological changes in different rat tissues (liver, kidney, lung) after acute and chronic administration of 1MeTIQ. Additionally, prediction of its properties in terms of absorption, distribution, metabolism, elimination and toxicity in the human body was performed. The obtained data did not show extensive and significant toxic effects of tested substance in in vivo and in vitro studies in rats, and in silico ADMET prediction in the human body. These results can help to discover or model a new effective and safe antidepressant substance and have important significance in the treatment of depression in clinic. Additionally, the use in the treatment of depression substance existing endogenously, having neuroprotective, antioxidant and antidepressant-like effects in the central nervous system (CNS) might also be beneficial in controlling the adverse CNS inflammatory processes often accompanying depression.

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|A26|

Hyperbaric oxygen preconditioning-induced alterations in the expression of proteins, associated with progenitor cells and apoptosis, are modified by a proteasome inhibitor in the ischemic brain

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Nestin is a marker of central nervous system progenitor cells, pointing to the potential for cell repair and tissue remodeling after brain injuries. The p53, although have been named the guardian of the genome, may trigger apoptosis of injured cells after global brain ischemia, unfavorably for investigational therapeutic interventions.

Studies have found that loss of p53 may facilitate nestin expression in cerebral tissues. We evaluated the expression of nestin and p53 in the rat brain in a model of global cerebral ischemia induced by the occlusion of carotid arteries (two-vessel occlusion; 2VO) associated with hypotension. We hypothesized that hyperbaric oxygen preconditioning (HBO-PC) will reduce p53 expression in the ischemic brain in a proteasome-dependent fashion and will enhance the expression of nestin under these conditions. We also performed Klüver-Barrera stain, synaptophysin and NeuN immunohistochemistry on rat brain sections to evaluate neuronal, synaptic and myelin injuries after global ischemic insult and the effect of HBO-PC.

The adult Wistar rats were allocated into following groups: sham operation, 5 minute 2VO untreated or preconditioned with HBO-PC (2.5 ATA for 1 hour for 5 consecutive days) before ischemia as well as preconditioned in combination with proteasome inhibitor MG132 prior to each HBO-PC session. The brains were collected at days 1 and 7 for histological analysis.

At 7 days, the majority of nestin positive cells was found in the hippocampus and periventricularly in the brain, predominantly within HBO-PC group.

Synaptophysin immunoreactivity decreased at one week following global cerebral ischemia. With preconditioning, the sparing of synaptophysin positive structures in CA1 and cerebral cortex was observed 7 days after reperfusion. The level of p53 was elevated in the CA1 and cerebral cortex on day 1 after ischemia, while in surviving neurons after 7 days. Reduction of p53 expression was observed with the preconditioning. Klüver-Barrera and NeuN stains showed damage of myelin, CA1 and cortical neurons, reduced in preconditioned group. However, MG132 combined with HBO-PC abolished beneficial changes in the postischemic brain. Thus proteasome system may be involved in the mechanism of HBO-PC-induced reduction in the p53 levels and tissue repair after brain ischemia.

|A27|

Possible influence of kinin B1 receptor on the blood-brain barrier integrity during autoimmune encephalomyelitis in rats

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According to the previous studies, mammalian central nervous system presents all components of the kallikrein-kinin system. Kinins are vasoactive and pro-inflammatory peptides whose biological effects are mediated by two G-protein-coupled receptors: B1 and B2. As suggested, activation of B1R leads to the induction of inflammation by the release of pro-inflammatory cytokines and increased vascular permeability. Since inflammation and the blood-brain barrier (BBB) disruption are main components of multiple sclerosis (MS), there are reasons to investigate the role of B1 receptor in these processes in the animal model of the disease, which is experimental autoimmune encephalomyelitis (EAE). Female Lewis rats were immunized by injection of inoculum containing homogenate of guinea pig spinal cord. Animals were monitored daily for clinical signs and loss of weight and sacrificed in different stages of the disease. The second group was administered with DALBK (B1R antagonist) after immunization. The expression of B1R was analyzed by W-B; gene expression was quantified by RT-PCR. The level of cytokines was assessed using RayBio Rat Cytokine Antibody Array. Immunohistochemical studies were also performed on isolated fraction of microvessels towards protein markers of BBB tightness.

We noticed the increased level of B1R protein in the rat brain during the symptomatic phase of EAE. Administration of DALBK significantly improved the condition of animals by reducing the intensity of neurological symptoms and delaying the onset of the disease. Using a confocal microscope, we observed lowered immunoreactivity of pericytes receptor PDGFβ and tight junctions proteins (ZO-1, claudin 5) in microvessels' fraction obtained from EAE rats which increased after DALBK administration. Also preliminary analysis showed increased protein level of cytokines: IFN-γ, IL-1β, IL-6, TNF-α, VEGF in EAE animals, which tends to decrease after DALBK treatment in symptomatic phase of the disease. Results show that B1R-mediated proinflammatory effect of kinins may be involved in pathomechanisms operating during the pre-onset phase of EAE resulting in disturbances in BBB integrity through the influence on tight junctions proteins.

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|A28|

The catecholaminergic system of the dorsolateral prefrontal cortex is down regulated in the juvenile spontaneously hypertensive rats (SHR)

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The motor cortex (MC) is the dorsolateral region of the prefrontal cortex (PFC) involved in planning, control, and execution of voluntary movements. More recent findings also suggest that MC plays role in learning and cognition. The rat MC, like in other mammals, is commonly divided into primary (M1) and secondary (M2) motor cortices. Both M1 and M2 are involved in the execution of voluntary movements via their direct and parallel projections to the spinal cord. The MC as other parts of PFC is strongly innervated by dopaminergic fibers and dopamine (DA) and is essential for proper motor control. Any abnormalities in DA neurotransmission may lead to serious disorders affecting motor and/or emotional behaviors. The attention deficit hyperactivity disorder (ADHD) is characterized by abnormalities in motor and emotional behaviors and recent reports indicate dysfunction in DA neurotransmission may be one of the key factors in the pathophysiology of disease. The question is whether MC is engaged in ADHD and whether DA neurotransmission is altered in MC in affected individuals.

The aim of this study was to track changes in DA immunoreactivity in the motor cortex of the spontaneously hypertensive rats (SHR) – animal model of ADHD. Frozen brain sections from juvenile (4 weeks old) and adult (10 weeks old) male SHR and WKY (Wistar Kyoto) rats were processed by single immuonofluorecence using thyrosine hydroxylase (TH; the rate-limiting enzyme of catecholamine biosynthesis) antibody. In M1 and M2 immunoreactive structures were

counted manually and compared between SHR and WKY rats. The result show that the number of fibers exhibiting immunoreactivity for TH in both M1 and M2 was lower in the 4 weeks old SHR rats when compared to the WKY strain. Furthermore, in 10 weeks old SHR and WKY rats the number of TH expressing fibers was similar in the M2 while in the M1 this number was still significantly lower in SHR rats.

In conclusion, present results indicate that dopaminergic inhibition of the motor cortices is significantly lowered in ADHD affected individuals what may be one of the reasons of the motor hyperactivity observed in this syndrome.

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|A29|

Neuroprotection of raloxifene and bazedoxifene against hypoxia depends on developmental stage but not on caspase-3 related apoptosis

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Hypoxia occurs under different circumstances, such as stroke, obstructive sleep apnea, mountain sickness or cancer. The brain of newborn mammals has been considered less sensitive to oxygen supply than the adult one, but resistance of the immature brain to hypoxia has its limitations. Selective estrogen receptor modulators (SERMs) represent an alternative to estrogen devoid of its side-effects and acting as estrogen receptor agonists or antagonists in a tissue-specific manner. SERM representatives raloxifene and recently approved bazedoxifene are used in clinical practice against osteoporosis but their neuroprotective properties are only partially recognized. Our previous study has shown that raloxifene exerts neuroprotection against hypoxia-induced damage in mouse hippocampal cell cultures. However, the roles of apoptosis and the stages of neuronal development in neuroprotective capacity of SERMs have not been clarified. Furthermore, knowledge about neuroprotective potential of bazedoxifene is limited.

Therefore, the aim of the present study was to investigate neuroprotective potential of raloxifene and bazedoxifene in mouse neocortical cells at different stages of neuronal development with special concern on apoptosis-dependent effects.

Our experiments were performed on mouse primary neocortical cell cultures. On 2, 7 and 12 day *in vitro* (DIV) the cells were treated with raloxifene (0.01-10 μM) or bazedoxifene (0.01-5 μM) and subjected to 18 h hypoxia [5% CO_2/95% N_2]. Caspase-3 activity and lactate dehydrogenase (LDH) release were measured after 6 h of reoxygenation.

We have shown that 18 hours of hypoxia increased LDH release by 25, 91 and 61% at 2, 7 and 12 DIV, respectively. Hypoxia caused also about 35% enhancement of caspase-3 activity, but only at 7 and 12 DIV. Raloxifene and bazedoxifene (0.01-1 μM) inhibited the hypoxia-induced LDH release in all *in vitro* stages of neuronal development. Raloxifene and bazedoxifene did not change the caspase-3 activity but at concentrations higher than 1 μM evoked neurotoxic effect.

These data demonstrated strong neuroprotective capacity of raloxifene and bazedoxifene that revealed particularly at later developmental stages and did not involve caspase-3-dependent apoptosis. Our study may be utilized in searching for new SERM-based tools to protect developing brain against hypoxia.

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|A30|

Exposure of cultured mouse astrocytes to NMDA inhibits expression of mRNAs coding for astroglia-specific proteins by a calcium-dependent mechanism

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NMDA receptors are present in rodent astrocytes but their physiological role beyond generation of intracellular calcium signals has not been studied in much detail. This laboratory has previously shown that prolonged treatment of cultured rat astrocytes with glutamate or NMDA decreases the expression of the astroglia- specific inward rectifying potassium channel, Kir 4.1 (Obara-Michlewska *et al*., Neurochem Int, 2015). Here, expression of mRNAs

coding for Kir4.1, the water channel aquaporin-4 (AQP4) and glutamine synthetase (GS) in primary cultures of mouse cortical astrocytes exposed for 8-72 h to NMDA was analyzed using real-time PCR. The effect of NMDA on Kir4.1 was shown to be biphasic: a decrease after 8 h exposure was followed by an increase at 72 h. Expression of AQP4- and GS mRNA was found decreased at both 8 h and 72 h of incubation. The results showed that the NMDA-induced changes were abolished in cultures in which expression of the NR1 subunit of the NMDA receptor was blocked with NR1 siRNA. For all the three mRNAs, the decrease of expression at 8 h was observed when incubations were carried out in the presence, but not in the absence of Ca^{2+} ions in the medium, suggesting that the effects of NMDA receptor stimulation were ionotropic in nature. The ionotropic mechanism of astrocytic NMDA receptors function is also supported by observation that NMDA-induced Kir4.1 mRNA decrease is not prevented by ryanodine, the inhibitor of calcium release from sarcoplasmic reticulum.

The effects of NMDA occurred by a mechanism bypassing changes in subunit composition of the NMDA receptor, which appeared unchanged at the mRNA level. The NMDA-induced decrease of GS mRNA was accompanied by marked decrease of GS activity at 8 h. The results suggest that durable activation of astrocytic NMDA receptors may be relevant to astrocytic dyshomeostasis in neurological disorders associated with excessive glutamatergic neurotransmission.

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|A31|

Sequence analysis and structural modelling of the litaf/simple protein involved in the CMT1a disease

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Charcot-Marie-Tooth (CMT) disorders represent a heterogeneous group of diseases of the peripheral nervous system (various CMT forms) with a prevalence of 1 : 2500. CMT is characterized by a slowly progressive atrophy of distal muscles associated with distal sensory disturbances. Mutations in approximately 100 genes have been identified in peripheral neuropathies. This study is focused on patients with the CMT1A form. No biomarkers of CMT1A were identified, however, the Ile92Val (c.274A>G) sequence variant in the LITAF/SIMPLE gene correlates with an earlier age of onset of CMT1A [Neurogenetics 2015; 16: 27-32]. Analysis of sequences of the LITAF/SIMPLE gene in CMT1A patients, followed by a structural modeling of the LITAF/SIMPLE protein, were carried out. Secondary structure analysis, 3D-order analysis, identification of protein-RNA interaction sites as well as tertiary fold-recognition (FR) were carried out using the GeneSilico metaserver gateway [http://genesilico.pl/meta2/]. Also the MOE modelling environment was used. Significance of the Ile92Val substitution was analyzed in the proposed approach – it may affect the clinical course of the disease.

Summarizing, the present results show that: (i) molecular diagnostics of CMT1A confirms the clinical diagnosis, but its value is limited by current clinical technologies and procedures, (ii) the I92V LITAF sequence variant indicates that patients may be predispose to an earlier age of onset of the CMT1A disease, in particular, (iii) detection of the c.274A>G substitution in the LITAF gene provides an important prognostic information. Finally, the Ile92Val polymorphism is proposed as the genetic marker of the CMT1A disease.

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|A32|

Assessment of the therapeutic potential of hyperbaric oxygen combined with selected isothiourea derivative (ZKK-3) in malignant glioma treatment *in vitro*

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Objectives: Treatment of patients with high grade gliomas remains ineffective. It is considered to be related with hypoxia of neoplastic tissue. It can be assumed

that improvement of tissue oxygenation, achieved using high-pressured pure oxygen, can enhance the therapeutic potential of cytotoxic compounds. The aim of this study was to investigate if hyperbaric oxygen (HBO) administration will be beneficial for the previously documented anti-tumour properties of novel isothiourea derivative as well as oxygenation status of glioblastoma cells *in vitro*.

Methodology: Human glioblastoma T98G cell line (WHO grade IV) was cultured in medium supplemented with N,N'-dimethyl-S-(2,3,4,5,6-pentabromobenzyl)-isothiouronium bromide (ZKK-3). Cells were exposed to various oxygen conditions: normoxia, hypoxia, HBO, double hypoxia or hypoxia followed by HBO. It was investigated how HBO administration influence the proliferation and viability of glioma cells in comparison to normoxic and hypoxic conditions. Impact of HBO on the expression of HIF-1α protein was also examined. The proliferation of glioma cells was assessed 24 hours after ZKK-3 treatment using Multisizer 3 Beckman Coulter. The viability of T98G cell line was evaluated 24 and 48 hours post ZKK-3 administration by CellTiter 96®AQueous One Solution Cell Proliferation Assay (Promega). HIF-1α level in cell lysates was determined with ELISA test (HIF-1A ELISA Kit, Thermo Scientific).

Results: Administration of hyperbaric oxygen enhanced anti-proliferative properties of tested compound. Also the viability of neoplastic cells significantly decreased under the influence of ZKK-3/HBO treatment compared to normoxic conditions. Differences were more pronounced and observed for lower ZKK-3 concentrations when HBO administration was compared with hypoxia groups. Under low oxygen conditions expression of HIF-1α was markedly increased. On the other hand, exposure to HBO did not change protein content in cell lysates in relation to normoxia. Moreover, HBO was able to reduce HIF-1α level previously elevated as the result of existing hypoxia.

Conclusions: Hyperbaric oxygenation improves antitumour properties of selected pentabromobenzylisothiourea against malignant glioma cells *in vitro*. It is probably connected with reduction of hypoxia state which may be indicated by diminution of HIF-1α protein expression after HBO application.

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|A33|

Modulation of the oxidative stress in EAE rat brain by glutamate receptors antagonists

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Experimental autoimmune encephalomyelitis (EAE) is the main animal model for the investigation of pathomechanisms of multiple sclerosis (MS). So far the etiology of MS is unknown. Neurodegeneration in MS/EAE pathology is initiated by microglia activation and mediated by oxidative stress, excitotoxicity and inflammation. The elevation of glutamate in cerebro-spinal fluid, as well as changes in the expression of glutamate receptors (GluRs) and excitatory amino acids transporters (EAATs) were observed in brains of MS patients. In the present studies we tested whether NMDA glutamatergic receptor antagonists, amantadine and memantine, influence parameters of oxidative stress in different phases of EAE. Markers of oxidative stress such as lipid peroxidation (expressed by the level of malondialdehyde – the final product of polyunsaturated fatty acids peroxidation in cell membranes), the level of sulfhydryl groups (–SH), and expression of antioxidant enzymes were examined in the CNS. We noticed statistically significant reduction of both protein- and non-protein –SH groups level by about 40% and 20%, respectively. We also observed the increase in the level of lipid peroxidation by about 35% and changes in the expression of different forms of superoxide dismutase (SODs) during the course of EAE. Administration of NMDAR antagonists (amantadine and memantine) to EAE rats significantly improved analyzed parameters. We noted increase of both protein- and non-protein –SH groups level and decrease in the level of lipid peroxidation. Treatment with NMDAR antagonists changed also the expression of antioxidative enzymes – SODs. Obtained results indicate that the significant amount of ROS generated during EAE results from NMDAR-mediated processes. NMDAR antagonists (amantadine and memantine) are efficient to counter reactive oxygen species (ROS) in rat brains during EAE pathology being effective as antioxidants.

|A34|

Impact of triclosan on expression of NMDA receptor subunits in mouse neocortical neurons

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Triclosan (TCS) is an antimicrobial agent used extensively in personal care and sanitizing products such as soaps, toothpastes, and hair products. TCS has been incorporated into growing number of medical products as well as in household items such as plastic cutting boards, sport equipment, textiles and furniture. A number of studies has shown presence of TCS in different human tissues such as blood, adipose tissue, liver, brain and in breast milk or urine. N-methyl-D-aspartate receptors (NMDARs) are glutamate-gated ion channels widely expressed in the central nervous system and play key roles in excitatory synaptic transmission. NMDARs are heteromeric complexes incorporating different subunits within a repertoire of three subtypes: GluR1, GluR2 and GluR3. There are four different GluR2 subunits (A, B, C and D) of which the most important in neuroplasticity and excitotoxicity are GluN2A and GluN2B.

The aim of the present study was to investigate the impact of TCS on the expression of GluN1, GluN2A and GluN2B NMDA receptor subunits in *in vitro* cultured mouse neocortical neurons.

The cultures of the neocortical neurons were prepared from Swiss mouse embryos on 15/16 day of gestation. The cells were cultured in phenol red-free Neurobasal medium with B27 and glutamine. After 7 days of culture *in vitro*, neurons were exposed to 10 μM of TCS. After 3 and 6 h, mRNA expression of GluN1, GluN2A and GluN2B NMDA receptor subunits was studied. Additionally, protein expression of NMDARs was measured.

Our preliminary data demonstrated that in the presence of 10 μM of TCS, after 3 h mRNA expression of GluN1, and GluN2A NMDA receptor subunits decreased. Similar pattern was observed in respect to protein expression of the receptor subunits after 3 h treatment with TCS. After 6 h exposure to TCS mRNA expression of GluN1 subunit continued to decrease, but mRNA expression of GluN2B significantly increased, possibly because of secondary effects of TCS.

In summary, the presented study demonstrated that GluN1, GluN2A and GluN2B NMDA receptor subunits are involved in TCS mechanism of action. Since TCS interacts with NMDA receptor subunits, it can also affect the proper functioning and development of the nervous system.

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|A35|

The impact of tianeptine on the inflammatory status of brain in prenatally stressed rats

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Introduction: There is a robust evidence that dysregulation of immune system may be related to the pathophysiology of depressive disorder. The disturbances in the expression of pro-inflammatory cytokines in central nervous system are of particular interest. It has been shown that alterations in the environment during prenatal period, substantial for brain development, leads to long-lasting adverse effects. In the last years, stressful events during pregnancy have received an increased attention, because they influence brain homeostasis especially by affecting the brain immune system. The aim of this study was to examine the impact of prenatal stress procedure on the behavioral and biochemical changes in the IL-1b, IL-18 and TGF-b level in the frontal cortex and hippocampus of adult offspring rats. Moreover the impact of chronic treatment of an antidepressant tianeptine on the above mentioned parameters were tested.

Material and methods: Pregnant rats were subjected to stress sessions from 14th day of pregnancy until the delivery. At 3 months of age, control and prenatally stressed rats were tested for behavioral changes in forced swimming test. After behavioral verification, rats were chronically treated with tianeptine. Two weeks later the animals' behavior was tested again and levels of IL-1b, IL-18 and TGF-b in hippocampi and frontal cortices were determined by ELISA test.

Results: The obtained data showed that prenatal stress causes in adult offspring depression-like behavior. An increase in immobility and a decrease in swimming and

climbing behavior in the forced swim test were observed. Furthermore, the evaluation of the protein level showed increase in the IL-1b, IL-18 level and decrease in the TGF-b level in frontal cortices and hippocampi in adult rats offspring after prenatal stress procedure. Interestingly the chronic treatment of tianeptine not only normalized the behavioral disturbances but also the protein level of analyzed cytokines in both investigated brain structures.

Conclusions: Our study clearly demonstrated that prenatal stress procedure leads to persistent behavioral and biochemical disturbances in adult offspring rats. It also appears that the cytokines' systems can be an attractive target for antidepressant drug action.

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|A36|

Activation of the nucleus accumbens with nicotine and caffeine in the rat – results of immunocytochemical studies

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Nicotine and caffeine belong to the most frequently consumed psychostimulating substances all over the word. Their action is concerned with activation of the brain reward system, among which the nucleus accumbens (NAc) plays a critical role. The mechanism of activation of the NAc neurons is concerned with triggering of the signaling pathways related with the transcription factors like the cyclic AMP-response element binding protein (CREB) and DeltaFosB, as well as enzymes like extracellular signal-regulated kinase (ERK). In this study we compared the activation patterns of above-mentioned markers in the neurons of NAc of the rat after stimulation with nicotine and caffeine. Our results reveal apparent differences in the activation patterns of the studied markers in the NAc evoked by both psychostimulants. These results may be useful for explanation of some behavioral reactions elicited by each of the studied substances and for explanation of developing addiction.

|A37|

Neuropathic pain alters gene transcription in the nucleus accumbens

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Nucleus accumbens, which is an important component of the mesolimbic dopaminergic reward system, also plays a role in pain. However, the molecular mechanisms of this involvement remain unknown. In the present study we explored molecular pathways involved in the development of neuropathic pain. This may allow finding biomarkers for expression of neuropathic pain-like behavior and indicating brain regions involved in this process.

Neuropathic pain was induced by applying a Chronic sciatic nerve Constriction Injury (CCI) model in C57BL/6J mice. Two behavioral tests for neuropathic pain were used: the von Frey's test to measure mechanical allodynia and the cold plate test to assess thermal hyperalgesia.

Using qRT-PCR analysis, we found that nerve injury produced a significant increase in the expression of opioid propeptide prodynorphin and proenkephalin genes (PDYN, PENK), opioid kappa and delta receptors genes (KOR, DOR) and calcium/calmodulin-dependent protein kinase kinase 1 (CAMKK1) in the nucleus accumbens. Furthermore, we observed that neuropathic pain augmented the expression of stress – and inflammatory response genes coding for the glucocorticoid receptor (GR), FK506 binding protein 5 (FKBP5), and interleukins IL1 beta and IL6 in the nucleus accumbens. Moreover, elevated levels of Glial Fibrillary Acidic Protein (GFAP – astrocyte marker) but not C1q (microglia marker) mRNAs were detected.

Our results demonstrate that CCI produces lasting biochemical changes in a brain region implicated in mood regulation, reward learning and motor function. Taking into account the well-known roles of opioid systems in pain transmission and emotional processes, the observed changes in the expression of the opioid propeptides and receptors genes may contribute to changes in pain sensitivity and in affective response to nociceptive stimulation underlying the development of neuropathic pain. Furthermore, increased expression of GFAP, GR, FKBP5, IL-6 and IL-1beta genes suggests that cellular stress and inflammatory processes are involved in this type of pain not only on the level of the spinal cord but also in the brain.

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|A38|

Effects of chronic restraint stress and betaxolol treatment on p(Ser845)GluA1, GluA1, Arc and beta(1)adrenergic receptor levels in rat hippocampus

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Studies in humans and rodents have demonstrated that chronic stress adversely affects physiological functions and neuronal structure of the hippocampus (HP). In addition to corticosteroids, stress causes rapid release of noradrenaline in some brain areas, which can regulate neuronal function in HP by means of the adrenergic receptor. High level of noradrenaline results in stimulation of beta adrenergic receptors (beta-AR) leading to activation of protein kinase A (PKA) and phosphorylation at Ser845 of GluA1 subunit of AMPA receptors (AMPA-R). This augments synaptic insertion of AMPA-R. The opposite process – the down-regulation of AMPA-R at postsynaptic sites, depends on the activity-regulated cytoskeleton-associated (Arc) protein which enhances the basal rate of receptor endocytosis. We investigated the influence of stress procedure on the p(Ser845)GluA1, GluA1, Arc and beta(1)-AR levels in rat hippocampus and evaluated whether the blockade of beta(1)-AR with specific antagonist, betaxolol, during stress can modulate observed changes.

Male Wistar rats underwent the chronic restraint stress applied for 3 hours daily, for 14 days. During the last 7 days rats were treated with betaxolol (5 mg/kg p.o.) given immediately after daily stress. Next day after a completion of stress procedure the rats were decapitated, their hippocampi were dissected and the extracted proteins were assessed by standard Western blot analysis.

Chronic restraint stress increased phosphorylation of GluA1 at Ser845, with no changes in its total protein level. Betaxolol treatment did not change the stress-induced effect. Also there was no effect of stress and betaxolol treatment alone or in combination on the protein expression of beta(1)-AR and Arc.

The increased phosphorylation at Ser845 of GluA1 with no changes in total GluA1 and Arc protein levels suggests that prolonged stress augments the AMPA-R activity in hippocampal postsynaptic membranes. Phosphorylation at Ser845 of GluA1 results from PKA activation which depends on the beta(1)-AR activity. The lack of modulatory effect of beta(1)-AR blockade during stress on the level of p(Ser845)GluA1 together with the unchanged expression of beta(1)-AR observed in our study indicate that stress-induced change of AMPA-R activity in rat HP was not mediated by beta(1)-AR.

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|A39|

The apoptotic effects of chemical UV-filter benzophenone-3

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Introduction: Benzophenone-3 (BP-3) is one of the most widely used chemical UV filter that has been available as a sunscreen agent for over 40 years. BP-3 is used as an active ingredient at levels of up to 10% in Europe. Moreover, BP-3 is accepted by the FDA as an indirect food additive. Chemical filters are generally used in combination because no single active agent, used at levels currently permitted by legislation, would provide sufficient protection against UV. Recent epidemiological data demonstrated a strong correlation between prenatal exposures to BP-3 and abnormal innervation of peripheral tissues as observed in 3-4 year old children. However, knowledge about the effect of BP-3 on the central nervous system is limited.

Objectives: This study aimed at clarifying molecular mechanisms of BP-3 action on brain neuronal cells with particular focus on apoptosis which is strongly associated with brain development.

Methodology: Primary neuronal cell cultures, measurements of caspase-3, ROS formation, microarrays and Hoechst 33342 staining were performed as previously. Mouse primary neocortical cell cultures were exposed to BP-3 (1-100 μM) for 6-24 h.

Results: Our study demonstrated that BP-3 (25-100 μM) induced intrinsic apoptosis pathway in the mouse neocortical cell cultures. It was evidenced by 31% loss of the mitochondrial membrane potential and approx. 90-120% rise of

ROS formation and caspase-3 activity. These effects were accompanied by apoptotic fragmentation of cell nuclei as detected by Hoechst 33342 staining. Using specific inhibitors, we provided evidence that BP-3-induced apoptosis of embryonic neuronal cells not only is caspase-3-dependent, but also is a caspase-9-, GSK-3β- and p38/MAPK-mediated process. Furthermore, by the use of microarray analyses we demonstrated BP-3-induced upregulation of apoptosis related genes such as Bax, Bak, Bad.

Conclusion: Summing up, our study has demonstrated that BP-3 induces apoptosis in mouse neuronal cell cultures, that position this UV filter as a risk factor for neurodevelopmental abnormalities.

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|A40|

Cardiovascular and respiratory effects of endomorphin-2 injection into the femoral vein of anaesthetized rat

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Introduction: Endomorphin-2 (EM-2) is abundant in central and peripheral nervous system i.a. in the regions involved in pain transmission and respiratory and cardiovascular regulation. It presents high affinity and selectivity towards mu-opioid receptors. The sparse information on EM-2 action on respiratory system is focused primarily on the changes in the ventilation rather than in the shape of the respiratory pattern. The aim of this study was to determine the effects induced by intravenous challenge of endomorphin-2 on cardiovascular and respiratory variables.

Methods: Cardiovascular and respiratory parameters (blood pressure, heart rate, tidal volume, respiratory frequency and minute ventilation) were measured in 21 Wistar rats anaesthetized with 750 mg/kg of urethan and 150 mg/kg of a-chloralose. Animals breathed spontaneously room air via tracheal tube and were treated with an intravenous injection of endomorphin-2 (1 mg/kg) in the following scheme: in control conditions (n = 6); after bilateral dissection of cervical vagi nerves (n = 5); after blockade of opioid receptors with antagonist active in the periphery – naloxone methiodide (2 mg/kg) (n = 5); following blockade with blood-brain barrier penetrant – naloxone hydrochloride (2 mg/kg) (n = 5).

Results: Bolus injection of 1 mg/kg of endomorphin-2 into the femoral vein evoked an apnoea of mean duration of 5.9 ± 1.8 s. After the cessation of breathing tidal volume remained similar to the baseline value. Transiently diminished respiratory frequency failed to have an impact on the minute ventilation. EM-2 challenge caused marked hypotension and transiently slowed down the heart rate. All cardiovascular and respiratory effects of EM-2 were abolished by midcervical vagotomy as well as by blood-brain barrier penetrating (naloxone hydrochloride) and nonpenetrating (naloxone methiodide) opioid receptor antagonist.

|A41|

Diversity in regulation of cell cycle and apoptotic response between familial and sporadic Alzheimer's disease lymphocytes

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Alzheimer's disease (AD) was first described over 100 years ago. It is the most common cause of dementia with an estimated prevalence of 30 million people worldwide. The growing body of data have shown that AD is characterized by complex alterations in cellular processes that occur not only in neurons, but also in peripheral cells such as lymphocytes. Our previous studies have demonstrated that lymphocytes from sporadic form of AD (SAD) show G1 phase arrest and increased levels of p21 protein, the key regulator of apoptosis and G1/S cell cycle checkpoint. Since it is known that p21, besides controlling the G1/S checkpoint, can regulate apoptosis, we decided to investigate whether p21 levels play a role in the cellular response to an oxidative stress challenge like 2d-ribose (2dRib) treatment. We report here that cells from familial AD (FAD) are more resistant to 2dRib-induced cell death than control or SAD cells. Quantitative real-time PCR reactions and Immunoblotting experiments showed that p21 mRNA and protein levels

significantly increased in FAD cells in response to 2dRib. In addition, using cell fractionation and confocal microscopy imaging, we found a higher cytosolic accumulation of p21 in FAD cells. Transcriptional activation of p21 was shown to be dependent on p53, as it can be blocked by PFT-a and was correlated with phosphorylation of p53. Thus in human B-lymphocytes under oxidative stress evoked by 2dRib, 7 PS1 mutants seem to strongly exacerbate phosphorylation of p53 exhibiting gain of function effect over wtPS1. This activities of mutPS1 seem to represent a compensatory mechanism against acute oxidative stress, preventing depolarization of mitochondrial membrane and apoptosis in human FAD lymphoblasts. Altogether, our results showed that mechanism of apoptotic response to acute oxidative stress distinguishes cells from SAD and FAD patients. Thus, caution should be taken in extrapolating data obtained from cellular or animal models based in FAD mutations, as they may not be relevant in SAD. Consistently, therapeutic designs should take into account the possible effect variability in SAD versus FAD cells.

|A42|

Eplerenone affects kynurenic acid production in rat brain cortex *in vitro*

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Arterial hypertension is the predominant cause of cardiovascular disorders in humans. The most recommended antihypertensive drugs are inhibitors of renin-angiotensin-aldosterone system (RAAS), which showed great improvement in prolonging patients survival. RAAS plays an important role in blood pressure regulation both in the central nervous system and peripheral tissues. Eplerenone is a mineralocorticosteroid antagonist with diuretic and potassium sparing effect. In recent studies was shown that eplerenone reverses enhanced brain glutamatergic signaling in animal models with arterial hypertension.

Kynurenic acid (KYNA) is the endogenous antagonist of glutamate receptors and of α7 nicotinic receptors. Cerebral synthesis of KYNA from L-kynurenine (L-KYN) is catalyzed by kynurenine aminotransferases (KAT) localized mainly in astrocytes. KYNA has neuroprotective properties and its impaired production was implicated in various neurodegenerative diseases. Additionally, it was shown that KYNA plays a role in the regulation of blood pressure after local intracerebral administration.

The aim of this study was to evaluate the effect of eplerenone on the KYNA synthesis in rat brain cortex *in vitro*.

Experiments were conducted on male Wistar rats brain cortex. The effect of eplerenone on KYNA concentration and KATs activity was evaluated on cortical slices and cortical homogenates after 2 hours of incubation with L-KYN and different concentrations of tested drug. Eplerenone at the concentration of 0.01 mM and 0.1 mM didn't change the KYNA synthesis in the brain cortical slices. At the concentration of 0.5 mM eplerenone decreased KYNA production in the brain cortical slices to 90% of control. The activity of KAT I was decreased by eplerenone at the concentration of 0.01 mM, 0.05 mM, 0.1 mM and 0.5 mM to 92%, 71%, 69% and 61% ($p < 0.05$) of control value, respectively. The activity of KAT II was diminished by eplerenone at the concentration of 0.01 mM, 0.05 mM, 0.1 mM and 0.5 mM to 84%, 50% ($p < 0.001$), 49% ($p < 0.001$) and 45% ($p < 0.001$) of control, respectively.

Presented data suggest that eplerenone might have an influence on KYNA production in rat brain cortex, most probably by changing KAT's activity.

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|A43|

SNAP-25: potential link between genetic and environmental causes of autism

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Autism spectrum disorder (ASD) is a severe neurodevelopmental disability disrupting the behavior of patients. Its unclear etiology most likely involves both genetic and environmental factors. Many substances are suspected of causing ASD. SNAP-25 (synaptosomal associated protein, 25 kDa), which encodes a presynaptic protein involved in neurotransmitter release and the modulation of voltage-gated calcium channels, is a candidate gene that is implicated in neuropsychiatric disorders, including ASD. The aim of this *in vitro* study, which was performed on a primary culture of rat cerebellar granule cells (CGCs), was to test the hypothesis that suspected developmental neurotoxins can alter SNAP-25 mRNA and protein expression. The test substances included environmental toxins: tetrabromobisphenol-A (TBBPA), the organomercury com-

pound thimerosal (TH), and silver nanoparticles (NAg). Teratogens: valproic acid (VPA) and thalidomide (THAL), that cause behavioral changes similar to autism in rodents were also tested. The substances were administered to CGC cultures for 24 h at subtoxic concentrations. Obtained results demonstrated that SNAP-25 mRNA levels were increased by 49 and 66% by TBBPA and THAL, respectively, whereas VPA and NAg reduced levels to 48 and 64% of the control, respectively. In addition, the SNAP-25 protein content in CGCs was increased by 79% by TBBPA, 25% by THAL and 21% NAg, whereas VPA and TH reduced SNAP-25 level to 73 and 69% of the control, respectively. Thus, this study is the first to demonstrate the effect of different compounds that are suspected of causing autism on SNAP-25 expression; the results suggest that this protein may be a common target for not only inherited but also environmental modifications linked to ASD.

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|A44|

Expression regulation of opioid propeptides and dopamine receptors in the mesostriatal system by chronic intake of palatable foods

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Chronic (self-)administration of addictive drugs typically up-regulates the prodynorphin (PDYN) gene expression in the dorsal striatum or nucleus accumbens. Drug effects on transcription of other genes in the mesostriatal system, including those coding for proenkephalin (PENK) and dopamine eceptors, have also been characterized. The aim of our study was to determine if prolonged intake of highly palatable foods, which may produce addiction-like behaviours, affects the mesostriatal gene expression in the same manner as drugs of abuse.

Two models of chronic intake of palatable foods by C57BL/6 mice were used: 1) instrumental self-administration of cocoa-flavoured pellets (daily 1-h sessions for 20 days) and 2) diet-induced obesity (DIO) model, i.e. constant free-choice access to a chocolate-based diet for 10 weeks. mRNA levels of PDYN, PENK and the dopamine receptors D1 and D2 (D1R, D2R) were assessed in the mesostriatal system by *in situ* hybridization.

We demonstrated that the PDYN mRNA levels were down-regulated in the dorsal striatum and nucleus accumbens in the instrumental model, and in the central amygdala of DIO mice. The D2R transcript was down-regulated in the nucleus accumbens both in the instrumental self-administration and DIO model. Expression of PENK and D1R remained unchanged in both models.

In conclusion, neuroadaptations in the mesostriatal system produced by chronic intake of palatable foods seem distinct from those produced by drugs of abuse. Whereas tasty food self-administration fails to up-regulate the PDYN gene (unlike addictive substances), its characteristic effect seems to be down-regulation of the D2R in the nucleus accumbens, particularly in the shell (not shared by drug abuse models). This suggests that drug and food addictions involve different neuronal mechanisms.

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Poster session II

|B1|

Implementation of human induced pluripotent stem cells (hiPSC) to test DNT at the earliest stages of development

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Human induced pluripotent stem cells (hiPSC) hold similar properties as human embryonic stem cells (hESC), however are not ethically controversial and easy to obtain. They possess ability to form embryoid bodies (EBs). EBs are three-dimensional aggregates of pluripotent stem (PS) cells which can recapitulate the early development of human embryo, thus can serve as the alternative model for toxicity and drug testing of neurodevelopmental toxic compounds. Methylmercury chloride (MeHgCl) is a developmental neurotoxin with strong embryotoxic effect for human embryo. In mouse *in vitro* culture cells model it has been defined as weakly embryotoxic or not embryotoxic. Here we present that methylmercury chloride is strongly embryotoxic for hiPSCs that are cultured as 3D embryoid bodies' stage of development during neural commitment. Comparison of the MeHgCl effect on three hiPSC different stages of development: non-differentiated hiPS cells in monolayer, the embryoid bodies (3D culture) and hiPS cells neurally committed, showed that the susceptibility to tested compound is developmental stage dependent. The three-dimensional aggregates were more sensitive than hiPSC after direct induction of neuronal differentiation. We have also confirmed inhibition of the ability of hiPSC to form embryoid bodies after 24 h of MeHgCl exposition. The increase in free radicals and reduction of the mitochondrial membrane potential were accompanied by a reduced cell viability after MeHgCl treatment. The obtained results indicate that hiPSC can be considered as an in vitro model for testing human embryotoxicity and developmental neurotoxicity replacing mouse embryonic stem cell (EST) test.

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|B2|

Human breast milk as a new source of stem/progenitor cells

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Introduction: Human breast milk is a well-known rich source of nutrients and immunological factors for a newborn. Some studies indicate the presence of stem/progenitor cells in that source. The aim of this study is to confirm the presence of human breast stem cells (hBSC) in human breast milk and to evaluate pluripotent and regenerative potential of isolated cells.

Material and methods: Samples of breast milk were obtained in 0 to 4 day post-delivery from mothers after natural delivery or cesarean section in different gestational age. The isolation of cells was performed up to 4 hours since collection of the sample. Milk sample was diluted with equal volume of sterile phosphate buffered saline (PBS) and centrifuged. The fat layer and supernatant were removed, and obtained cell pellet was washed three times in PBS. Cells viability and concentration were estimated in a Burker chamber by Trypan Blue exclusion. Various types of media were tested: DMEM + 10% FBS, Essential 8 medium, CellGro medium, MammoCult. hBSC were seeded in low adherent plates or standard culture plates. The cells were characterized by immunofluorescence technique (IF) and reverse transcription polymerase chain reaction (RT-PCR).

Results: In our study we succeed in isolating stem-cell like population. Our results indicate that breastmilk cells from different donors displayed variable expression of pluripotency genes normally found in human embryonic stem cells (hESCs). These genes included the transcription factors (TFs) OCT4, SOX2, NANOG, known to constitute the core self-renewal circuitry of hESCs. The RT PCR data have also revealed the presence of pluripotency markers (OCT4, SOX2, NANOG) in isolated cells.

Conclusions: Stem/progenitor cells (SC) can be obtained from the human breast milk. The human breast milk can be a new noninvasive, needle free source of hBSC. The hBSCs can be considered to become a new, alternative source of SC and new standard for SC-based therapies.

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|B3|

Lowered oxygen concentration *in vitro* promotes and stabilizes mesenchymal ADRC phenotype and genotype for safe clinical application

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The number of mesenchymal stem cells (MSC)-based therapies is increasing year by year. Numerous independent studies confirmed therapeutic effectiveness of mesenchymal type of Adipose-Derived Regenerative Cells (ADRC) in various clinical settings. Nonetheless broad distribution and easy surgical accessibility of adipose tissue, as well as frequently observed heavy deterioration of patients' general conditions and needs of high number of autologous cells for mono- or multiple transplantations, force elaboration of safe and enhanced method of these cells' expansion in vitro. Previously, it was confirmed that oxygen concentration is one of the most important determinants of stem/progenitor cell metabolism, viability, proliferation and differentiation, leading to the enormous amplification of the MSC amount in culture.

The aim of this study was to characterize and confirm stability of ADRC phenotype and genotype during a long term cell culture expansion in 21% vs lowered to 5% oxygen concentrations. The ADRC were isolated by CellCelution System by commercial method performed in typical clinical surrounding. The MSC-like phenotype and ability to multi-lineage differentiation were assessed. Proliferation of cultures was controlled steadily by increased cell density and expressed as the population doubling time (PDT) and the cumulative population doublings (CPDs). The morphological control of ADRC phenotype stability and cell senescence were carried out during long term cultivation in both examined oxygen conditions by the use of typical immunocytochemical methods. The cells growing until 30th passage additionally underwent chromosome analysis – karyotyping.

Our results expressly indicate that the low oxygen "physioxic" culture conditions significantly favour ADRC long-term proliferation and well preservation of their phenotype and genome stability. The significant enhancement of the aforementioned low oxygen dependent ADRC properties in culture can positively influence their further therapeutic effectiveness.

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|B4|

Effects of sex hormones on apoptosis in cells with mutations responsible for Leber's hereditary optic neuropathy

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Leber's hereditary optic neuropathy (LHON) is the most common mitochondrial disease resulting in central vision loss due to optic nerve atrophy. Most cases are caused by a mutation in mitochondrial genes encoding one of three subunits of mitochondrial complex I (ND1, ND4, and ND6). LHON is considered as a disease of young men because of its early onset between 20 and 30 years and male preponderance (50% men and only 10% women with mutations predisposing to LHON are affected). The mtDNA usually is homoplasmic for the mutation in every cell. Changes in mitochondrial respiratory chain or ROS production can lead to induction of apoptosis. Most LHON mutations involve a decrease in Complex I activity and some data suggest that cells with these mutations are more prone to apoptosis. In females, estrogens are thought to modify the severity of mitochondrial dysfunction, including defective ATP synthesis, oxidative stress, and apoptosis (Giordano *et al*., 2011). However, no increase of LHON in post-menopausal women is observed. Estrada *et al*. (2006) reported that high concentrations of testosterone initiate an apoptotic pathway and induce neurotoxicity in neuroblastoma cells. So far not much data has been published regarding the role of testosterone in apoptosis in LHON affected men. The objective of this research is to determine the effect of sex hormones on cell death in LHON patient and

control cells. Human optic nerve cells are unavailable for studies, therefore a lymphoblast cell model was used. 4 cell lines from a unique Polish family harboring two LHON mutations m.11778G>A and m.3460G>A and healthy controls were enrolled into this study. The effect of testosterone on initiation of apoptosis was investigated via the canonical apoptotic pathway which involves activation of aspartate-specific proteases (caspases). Apoptosis was confirmed by PARP cleavage. Here we present for the first time the influence of the sex hormone testosterone on apoptosis in LHON. We did not observe any differences in level of apoptosis in the LHON patient and control cells. However, in the control cells apoptosis was activated via a caspase dependent pathway, while in LHON cells apoptosis was initiated by a different, caspase independent pathway.

|B5|

Effect of culture conditions on proliferation and differentiation of rat oligodendrocyte progenitor cells

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Background: Oligodendrocyte progenitor cells (OPCs) can give rise to myelinating oligodendrocytes but they are also involved in modifying neural microenvironment, e.g. in response to damaging conditions. It is crucial for studies of the biology of OPCs in vitro to culture them at physiological normoxia (2-5% O_2) and to have the possibility of tracing their paracrine activity in a defined, serum-free medium. The aim of our study was to evaluate growth of rat OPCs in vitro in different culture conditions which may influence cell proliferation and maturation.

Material and methods: Mixed glial cell cultures were established from brain hemispheres of 2-day-old Wistar rat pups and plated in a tissue culture flask coated with poly-L-lysine. The cells grew under standard conditions in DMEM containing 10% FBS for 12 days. OPCs were obtained from mixed glial cell cultures with a shaking method which uses differential adherent properties of glia. The isolated OPCs were then plated at densities between 1.2 and 6×10^4 cells/cm^2 and cultured under 5% or 21% O_2 either in serum-free medium or supplemented with 1% FBS. After 2 DIV or 5 DIV the cells were fixed and identified by immunolabeling with antibodies against NG2 for OPCs, GalC for immature oligodendrocytes, MBP and PLP for myelinating cells, and Ki67 for proliferating cells.

Results: The OPCs plated at higher density proliferated more intensely, but the process of their maturation was significantly slowed-down. The cells seeded sparsely had more complex morphology and were able to express myelin proteins even only after 2 days in culture. The 1% FBS addition to culture medium enhanced OPC differentiation at low oxygen level, but had no influence on the number of GalC- and MBP-positive cells at 21% O_2 level.

Conclusions: Although a low concentration of FBS in a culture medium appreciably promotes oligodendrocyte maturation, the OPC differentiation proceeds correctly through its typical stages in physiologically-relevant conditions created in vitro. Application of serum-free media and normoxic oxygen tension provides a good model to analyze active compounds OPC release during growth in well-defined conditions.

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|B6|

A histone deacetylase inhibitor, Sodium Butyrate, prevents an inflammatory response associated with neonatal hypoxia-ischemia

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Neonatal hypoxia-ischemia (HI) causes considerable mortality and long-term neurological deficits. Mounting evidence indicate that administering histone deacetylase inhibitors (HDACis) in adult animal stroke models provides neuroprotection, associated with reduced neuroinflammation. The aim of our study was to examine the effect of Sodium Butyrate (SB), a HDACi, on inflammatory processes after neonatal HI injury.

We used a model of HI induced in 7-day old rats. The left common carotid artery was ligated and animals were exposed to hypoxia (7.6% oxygen for 60 min). The hypoxic undamaged hemisphere and sham-operated rats served as control. SB was injected subcutaneously for 5 days starting immediately after HI. Microglial cells and astroglia were identified immunohistochemically with specific markers

(ED1 and GFAP, respectively). Additionally, we determined the expression of transcription factor NFkappaB and pro-inflammatory protein COX-2 using Western Blot, whereas the level of other pro-inflammatory factors (IL-1alpha, IL-1beta, IP-10, TNFa) were estimated by magnetic bead–based assay (Luminex).

HI markedly increased the density of microglia (ED1+) in the ipsilateral hemisphere which was further enhanced by SB application. A significant number of microglial cells after inhibitor treatment presented an anti-inflammatory M2 phenotype (ED1+/Arg1+). At the same time suppression of pro-inflammatory M1 cells (ED1+/IL-1B+) was observed. SB treatment also resulted in an over 2-fold elevation in the GFAP staining intensity in the ipsilateral injured hemisphere (HI+SB) however the amount of astroglial cells expressing IL-1β within the cortex of the damaged ipsilateral hemisphere was markedly reduced upon SB administration. Moreover, treatment with the inhibitor decreased the level of NFkappaB and pro-inflammatory factors (COX-2, IL-1beta and IP-10).

This study provides evidence that inhibition of HDACs by SB exhibits beneficial anti-inflammatory action.

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|B7|

Effect of myriocin and FTY720 on gene expression of sphingolipid enzymes in mouse model of Alzheimer's disease

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Background: Sphingolipids modulate neurotransmission, cell survival, and may modify amyloid beta (AB) toxicity in Alzheimer's disease (AD). Accumulation of pro-apoptotic ceramide (Cer) and loss of the protective sphingosine-1-phosphate (S1P) parallel the neurodegeneration and might modulate AB production. Deciphering the early AD mechanisms is of greatest importance: the long initial period of relatively asymptomatic neurodegeneration makes AD extremely difficult to diagnose before irreversible damage occurs.

Objectives: We examined the influence of sphingolipid-modulating compounds (myriocin and FTY720) on gene expression of enzymes metabolizing sphingosine/S1P, sphingomyelin, Cer/Cer-1-phosphate in streptozotocin (STZ) induced AD model.

Methodology: Intracerebroventricular administration of STZ was used to induce the neurodegenerative symptoms characteristic for the prevailing sporadic AD in C57Bl6/J mice. Animals were intraperitoneally treated for 14 days with myriocin (serine palmitoyltransferase inhibitor) and FTY720 (S1P receptor modulator). Gene expression levels in brain cortex and hippocampus were assessed using Real-time PCR.

Results: In the brain cortex, treatment with STZ and myriocin significantly elevated mRNA expression of sphingomyelin phosphodiesterases Smpd1 and Smpd2, and ceramide kinase, as compared to STZ+vehicle-treated controls. Thus, although myriocin inhibits de novo ceramide synthesis, elevated SMPDs should counteract the resulting drop in cortical Cer level while the kinase would increase ceramide phosphate. Administration of FTY720 together with STZ led to tendency towards reduction of cortical SMPDs. We also observed that hippocampal mRNAs of SMPDs and Cer kinase remained unchanged in STZ+myriocin and STZ+FTY720 treated animals. S1P phosphatase 1 (Sgpp1) was reduced in the hippocampus of FTY720 treated animals and showed a similar tendency in the cortex, probably facilitating the maintenance of S1P levels.

Conclusions: Our results show that myriocin and FTY720 exert significant effects on the expression of genes related to ceramide metabolism and S1P signaling. Myriocin tends to increase ceramide kinase expression while FTY720 appears to exert its influence in two ways, through the reduction of SMPDs, and Sgpp1. Changes in cellular sphingolipids may affect the metabolism of AB and its precursor which subsequently could impact cell survival and death.

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|B8|

Profile of surface glycans in subpopulations of mesenchymal stem cells

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The most common definition of mesenchymal stem cells says that mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into a variety of cell types, including osteoblasts, chondrocytes, myocytes and adipocytes. However, it is huge oversimplification, because only one-third of the MSC clones generated from bone marrow mononuclear cells are able to differentiate in trilineage potential suggesting that MSCs are constituted by a group of cells with different differentiation potential. Described variation relates to cells isolated from different sources and relate to the potential for differentiation. Results based on the analysis of real-time PCR and flow cytometric assay indicates that one of the most homogeneous fractions of MSCs is a sub-population of cells present on their surface marker CD271. It is characterized by high potential to differentiate into cells of the bone and cartilage, and is the most abundant source of stromal bone marrow. It is thought that a large variety of glycans closely located on the surface of the cell is important because of their specific interactions that control cell-cell adhesion, immune response or microbial pathogenesis. The structure of surface glycans reflects cellular changes, such as cell-cycle, differentiation, and various conditions of its physiological activity. All of these states are indicated on the cell surface with changes in the cell membrane which decorates glycoproteins and glycolipids. This is possible due to huge amount of – estimated at nearly 2000 – glycogen, or programming protein genes responsible for the synthesis of proteins responsible for the synthesis of glycan processing and transport structural. We performed a comparative analysis of surface glycans in the entire population MSCs and two sub-populations, possitive and negative for CD271 marker using a lectin panel specific for oligosaccharides present in human cells. Taking into account the role of glycans in different states of the cell, we need to be aware of the fact that they glycans will be extensively utilized and critically contribute to the use of stem cells for future therapeutic transplantation. In the successful cell therapy it is essential to establish standardized multi-step protocols to generate high quality and efficacy therapeutic biomaterial.

|B9|

Evaluation of the changes in the cell surface glycan profiles in the population of mesenchymal stem cells in response to the stimulation with pulsed electric field

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One of the major components of the cellular membrane that define cell-cell and cell extracellular matrix interactions is the cell glycome consisting of a large variety of complex glycans covalently attached to the membrane proteins and lipids. These glycoproteins and glycolipids are actively involved in, and often control cell-cell signaling, microbial infections, immune response, wound healing, and other events on the cellular and tissue levels. The dynamics of a glycomic profile reflects changes in the cellular activities such as division, differentiation, motility, secretory functions and malignant transformation. Glycoconjugate-targeting investigations would therefore be expected to streamline discovery of novel biological factors of potential clinical significance. With the aim of developing novel techniques to analysis of cell movement, we tested whether the system of deep brain stimulation contributes to creating changes in the cell surface glycan profiles. We performed a comparative analysis of surface glycans in mesenchymal stem cells population after pulsed electric field stimulation using a lectin panel specific for oligosaccharides present in human cells. On the basis of staining with fluorescein-labeled lectins, we have determined the presence of both terminal and internally linked α1-3/α1-6-Fuc, (a-1,3) and (a-1,6) linked mannose, sialic acid attached to terminal galactose in (a-2,6), alpha-linked mannose, terminal GlcNAc, and a-linked mannose. After electrical field stimulation changes were observed for all lectins. They concerned both signal intensity and

placement. Analysis of a panel of lectins, in order to evaluate changes in the profile of surface glycans revealed significant changes in the expression of cell surface glycans. We used glycans staining following hypothesis that application of different lectins highlighting different types of glycans in human cells. That would allow observed dynamics of cell surfaces reprogramming glycosylation in cell populations under the influence of the electric field of different values. Obtained results made possible to assess the impact of the electric field on the degree of cell migration and changes in cell phenotype and allowed choosing the right parameter of stimulation. We can assume that they can have a significant impact on the quality of the therapeutic preparation.

|B10|

Astrocyte-conditioned medium protecting cholinergic neurons against zinc-induced neurotoxicity

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Excessive accumulation of zinc in the brain is one of putative factors involved in pathomechanism of cholinergic encephalopathies. Our previous studies have shown that Zn-dependent losses of many enzymes of energy and acetyl-CoA metabolism, is much more harmful to cholinergic neurons than to the other cells, because they consume acetyl-CoA not only for energy production, but also for the acetylcholine synthesis. Astroglia are known to exert several neuroprotective functions through the supply of many metabolites into the neurons. On the other hand, abundant evidence shows that changes in astrocyte function may contribute to neurodegenerative diseases. Therefore, the aim of this work was to find out whether diffusible factors produced by astroglial cells can affect energy metabolism and survival of cholinergic neurons in neurotoxic conditions. SN56 neuroblastoma cells were used as experimental model of cholinergic neurons. To obtain astroglia-conditioning medium (ACM), C6 glioma cells were grown until confluence. After 36 hours the medium was collected as ACM and transferred to the neuronal cultures for the next 24 hours (with or without zinc). We reported that ACM protected cholinergic neurons against the zinc-induced damages. Zn in concentration 0.15 mmol/l brought about loss of neuronal extensions and intracellular connections as well as appearance of blebs and malformations of cell membranes. These alterations were reversed in part by ACM. In response to the same concentration of zinc, the number of non-viable trypan-blue positive cells was significantly reduced from 60 to 20% in the ACM-cultured cholinergic neurons. In the same conditions, the total number of cells increased three times. We also observed that ACM partially reversed the inhibitory effect of zinc on the energy metabolism of cholinergic neurons in the range of aconitase and isocitrate dehydrogenase activity and the total level of acetyl-CoA. These results indicate that diffusible factors released from astrocytes affect neurons energy metabolism and probably this way promote their survival.

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|B11|

Study of respiratory function in cybrid cells harbouring Leber's hereditary optic neuropathy mutations

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Leber's Hereditary Optic Neuropathy (LHON) is the most common mitochondrial disease. LHON is characterized by sudden, painless loss of vision associated with abnormalities of the optic nerve. The disease in over 90% of cases is caused by one of three mutations in the mitochondrial genome: 11778G>A, 3460G>A or 14484T>C. The co-occurrence of two pathogenic mutations responsible for LHON is extremely rare. Here we present for the first time the respiratory function analysis of cybrid cells harbouring a combination of the 11778G>A and the 3460G>A LHON mutations. Cybrid cell lines were constructed by fusion of enucleated fibroblasts, derived from skin biopsies of 3 LHON patients and 3 healthy controls, with ρ0 143B cells. The heteroplasmy level of each mutation was measured by the last cycle hot PCR-RFLP meth-

od. Cellular respiration was measured in four conditions (basal respiration and after addition of respiratory substrates, ATPase inhibitor and OXPHOS uncoupler) with the use of oxygraph (Oroboros-2k). Analysis showed that LHON cybrids had higher rates of respiration and respiratory capacity compared to control cells. Moreover, respiratory capacity increased with the increasing level of the 11778G>A mutation. These data suggest that LHON mutations could force cells to increase their respiratory capacity to overcome the mutation effect. Increased respiratory capacity could be due to mitochondrial biogenesis induction in response to energy stress caused by LHON mutations.

|B12|

Differentiating oligodendrocyte precursor cells produce interleukin-33 which shapes remyelinating lesion microenvironment

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Interleukin-33 (IL-33) plays an important role in maintaining organism homeostasis by several potential activities: as an alarmin, traditional cytokine or nuclear factor. Due to its multiple functions, IL-33 has been recently widely studied in pathophysiology of numerous disorders, including multiple sclerosis (MS). MS is an autoimmune disease characterized by central nervous system (CNS) demyelination. Remyelination is a physiological response to demyelination and is led by the oligodendrocyte precursor cells (OPCs) which differentiate within lesion and serve the axons with new myelin sheaths. However, remyelination often fails, which causes long-term neurological problems for the MS patients.

Using well described *in vivo* model of murine spinal cord demyelination and based on global gene expression profiling, we have selected the group of genes, which products could be potentially involve in the process of OPCs differentiation and one of them was interleukin-33 (IL-33). We hypothesize that IL-33 may play a role in activation of OPCs and remyelination outcome. Here, we provide evidence for the significant role of IL-33 in modeling the white matter damage environment and its impact on the axons remyelination. We demonstrate that IL-33 is highly produced in the intact CNS, by a population of oligodendrocyte lineage cells, but neither OPCs nor white matter mature oligodendrocytes. Moreover, using an *in vivo* model of chemically-induced murine spinal cord demyelination, we show that IL-33 is upregulated, produced and secreted by differentiating OPCs in response to the CNS white matter injury. Secreted IL33 alters the functional phenotype of microglia/microphages, which in consequence allows OPC for the complete differentiation. Our results clearly show, for the first time, that interleukin-33 is produced and secreted by activated OPCs, which shapes the lesion microenvironment and finally indirectly supports white matter regeneration.

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|B13|

Systemic inflammation at different developmental stages reduces numbers of calbindin-immunopositive neurons and their vulnerability to seizures evoked in adulthood

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Background: Calbindin-D28k is one of the major calcium-binding proteins in the brain. It regulates intracellular response against various stimuli and provides neuroprotection against calcium-mediated neurotoxicity. Previous studies have reported the vulnerability of calbindin-positive (CB+) neurons in the dentate granule cell layer of the hippocampal formation in epilepsy. According to previous studies, neuroinflammation may lead to an increase in seizure susceptibility and trigger epileptogenesis. However, emerging experimental evidence indicates that early age inflammation acting as a preconditioning factor may also have protective effects. The aim of this study was to examine long term effects of systemic inflammation induced at different postnatal developmental stages on the CB+ cell population within hippocampal formation in response to status epilepticus evoked in adulthood.

Methodology: Lipopolysaccharide (LPS) was injected intraperitoneally (2 mg/kg b.w.) to Wistar rats on postnatal day 6 or 30 (P06 and P30, respectively). When became two-month-old, the rats which survived inflammation were injected with pilocarpine to evoke status epilepti-

cus and sacrificed 3 days later. Brain sections were then processed for CB immunohistochemistry and CB+ neurons were counted bilaterally within CA1, CA2/3 and DG regions of the dorsal part of hippocampal formation.

Results: LPS injections alone on P06 or P30 caused significant decreases in numbers of CB+ cells but only within the DG area when compared to naïve animals. The epileptic seizures induced in both LPS-untreated controls and LPS-treated animals led to significant decreases of CB+ cell number (vs. naïve animals) but also in the DG. However, in rats injected with LPS on P30 the effect of seizures was significantly lower than in those injected on P06.

Conclusions: Transient inflammation induced during the brain development led to permanent decreases of CB+ neuronal population in the adult brain. The inflammation induced on P30 could also prevent large seizure-related reduction of the cells. This might result from long-term changes in nervous tissue reactivity – preconditioning.

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|B14|

Participation of interleukin 1-β in the axon elongation of dorsal root ganglia (DRG) neurons supplying the urinary bladder of the pig – an *in vitro* study

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Introduction: Pro-inflammatory cytokines such as interleukin 1-β (IL-1β) are considered to exert detrimental effects during brain trauma and in neurodegenerative disorders. In other way it was reported that IL-1β enhances neurite growth in brain slices. In this study, we analyzed the influence of this cytokine on the axon growth of DRG neurons supplying porcine urinary bladder.

Material and methods: Lumbar and sacral DRGs were collected from 8-10 weeks old pigs (n = 4) which received 2 weeks earlier multiple injections of the Fast Blue retrograde tracer in the wall of the urinary bladder. Cell cultures were prepared as described previously (Hausott *et al*., 2011). Neurons were re-suspended, plated at per glass coverslip coated with poly-D-lysine/laminin and cultivated in TNB medium at 37°C in 5% CO_2 and treated with IL-1β (50 ng/ml, experimental group). After 24 h in culture, neurons were fixed with 4% paraformaldehyde for 20 min, permeabilized with 0.01% Triton X-100 in PBS for 5 min and blocked with blocking buffer (10% goat serum in PBS) for 30 min. Cells were incubated with primary antibodies against neuron-specific β-III tubulin diluted in blocking buffer for 1h at room temperature and then incubated with secondary Alexa-488-conjugated antibody for 60 min at RT. Axon length analysis was performed using the WIS-NeuroMath (Rishal *et al*. 2012). The Mann-Whitney U test (GraphPad Software, Inc) was used to analyze the variability and the relative differences in the morphology of the neuronal cells.

Results: Morphological analysis revealed that addition the IL-1β to the culture of isolated DRG neurons supplying porcine urinary bladder did not significantly affect the number of axonal branch points and neurites as well as their length.

Conclusions: This is the first report specifying the share of IL-1β in the regeneration process of the porcine DRG neurons supplying the urinary bladder. A lack of changes in the total axon length, maximal distance of the longest axon and the number of the branch points suggest that this factor has not the capacity to promote regeneration of this specific subpopulation of DRG neurons.

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|B15|

Influence of nerve growth factor (NGF) on axon outgrowth by dorsal root ganglia (DRG) neurons supplying porcine urinary bladder – an *in vitro* study

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It is well know that nerve growth factor (NGF) increases initial rate of axonal regeneration of aged dorsal root ganglia neurons (DRG) and influences on the survival time of these neurons. However, there is a lack of data revealing the putative influence of NGF on the neurite outgrowth of urinary bladder-projecting DRG neurons. Therefore, the present study was aimed at determining the influence of NGF treatment on morphology of the porcine bladder-projecting sensory neurons.

Lumbar and sacral DRGs were harvested from 8-10 weeks old pigs (n = 4) which received multiple injections of the retrograde tracer Fast Blue in the urinary bladder wall 2 weeks prior to the collecting of DRG studied. Cell cultures were prepared as described previously (Hausott *et al*., 2011). Neurons were plated on glass coverslip coated with poly-L-Lysine/laminin and cultivated in TNB medium at 37°C in 5% CO_2 and treated with NGF (100 ng/ml, experimental group). After 24 h in culture, neurons were fixed with 4% paraformaldehyde for 20 min, permeabilized with 0.01% Triton X-100 in PBS for 5 min and blocked with blocking buffer (10% goat serum in PBS) for 30 min. Cells were incubated with primary antibodies against neuron-specific β-III tubulin diluted in blocking buffer for 1 h at room temperature and then incubated with secondary Alexa-488-conjugated antibody for 60 min at RT. Neurite length analysis was performed using the WIS-NeuroMath (Rishal *et al*., 2012). The Mann-Whitney *U* test (GraphPad Software, Inc) was used to analyze the variability in the relative differences in the morphology of the neuronal cells.

The result show the number of branch points was increased after NGF treatment, while the total axon length as well as maximal distance of the longest axon was similar to those observed in untreated control.

This is the first scientific paper concerning the influence of NGF on the porcine sensory DRG neurons supplying the urinary bladder during their regeneration. The rise in the number of axonal branch suggests contribution of this factor in the regeneration of axons from DRG neurons.

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|B16|

Immunological and Biological Properties of Glial Restricted Progenitors for Potential Application in ALS Therapy

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Introduction: The neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) constitute social problem related to civilization. First results of stem cell therapies in ALS seem to be promising. However, rejection of allogenic neural cells is still a common problem and there is an urgent need to define the mechanism of immune response and to develop effective immunosuppressive strategy. In this study, we evaluated immunogenic potential of glial restricted progenitor cells – the potential candidates for ALS therapy.

Material and methods: Glial Restricted Progenitor cell suspensions (GRP) derived from canine and murine fetuses and obtained human commercial cell line QSV40 were long time cultured in DMEM medium, enriched in growth supplements. The phenotypic characteristics of human, murine and canine GRPs were assessed via flow cytometry and immunofluorescence staining. Cells were evaluated for the presence of immunogenic markers: MHC-I and MHC-II and costimulatory molecules CD40, CD154, CD28 and CD80 as well as for the presence of markers specific for neural progenitors: A2B5, nestin, NG2, and for differentiated cells: β-III-tubulin, PSA-NCAM, GFAP, MBP. Differentiation *in vitro* of GRP cell into astrocytes was performed in culture with 15% FBS media.

Results: GRPs of all examined species express MHC-I but not MHC-II antigens. The presence of costimulatory molecules was not observed on surface of murine and human cells. GRPs preserve stable phenotype of A2B5,

NG2 and nestin in long term culture. Murine and canine GRPs were easily differentiated into astrocytes in medium supplemented with 15% FBS. Such phenomenon was not observed in human QSV40 cell line. Differentiation potential of murine and canine GRP was proven by GFAP expression.

Conclusions: GRPs are stable in long term in vitro culture. There is no expression of MHC-II and costimulatory molecules which might suggest low immunogenic potential of the cells. Not immortalized murine and canine GRPs show ability to differentiate into astrocytes. These biologic properties of GRP make them potential candidates for ALS therapy.

|B17|

Platinum nanoparticles as efficient inhibitors of cells proliferation in glioma

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Glioblastoma is one of the most frequent primary and aggressive (WHO grade IV) neoplastic malignant tumors of the central nervous system with a poor survival time. Despite developments in neurosurgery and treatment strategy, the chemotherapy is still inefficient and it is urgent to develop new strategies for tumour therapy. The objective of this study was to investigate the effect of platinum nanoparticles (NP-Pt) on the inhibition of glioma cell proliferation and compare the antiproliferative properties of NP-Pt with platinum based drugs – cisplatin against U87 and U118 glioma cell lines and tumour tissue. NP-Pt and cisplatin were incubated with U87 and U118 glioma cells or injected directly into glioma tumour tissue. Herein, we report a study on the antiproliferative properties of NP-Pt by examining the influence of nanoplatinum on the glioma cells morphology, the level of DNA synthesis, viability and cells migration ability of glioma cells, as well as protein expression of proliferating cell nuclear antigen (PCNA) at glioma tumour tissue. The obtained results showed that NP-Pt treatment of U87 and U118 glioma cells caused reduction of DNA synthesis and the migration of cancer cells. Moreover, the PCNA protein expression level was also decreased at tumour tissue after NP-Pt administration. The obtained results demonstrated the antiproliferative properties of NP-Pt. Consequently, NP-Pt can be considered as an effective inhibitor of glioblastoma tumour cell proliferation. However, the molecular mechanism of action as well as potential side effects need to be elucidated in *in vivo* follow-up research.

|B18|

Critical view on the selection criteria of optimal derivation method of Mesenchymal Stem Cells from Wharton's Jelly: phenotypic, functional and safety control

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Mesenchymal stem cells (MSC) exhibit enormous heterogeneity which can influence their regenerative properties, therapeutic effectiveness and finally their safety after transplantation. The source of stem cells and also the method of isolation, can significantly affect their properties. Human mesenchymal stem cells derived from bone marrow, adipose tissue or umbilical cord, have been used in many clinical trials. Recently Wharton's Jelly-derived MSC (WJ-MSC) gained special attention due to their low immunogenicity, strong immunomodulatory properties and ability to secrete adjuvant factors supporting regeneration. The aim of the study was to characterize and compare WJ-MSC isolated from umbilical cord stroma by mechanical and collagenase based enzymatic process and critical view on the selection criteria of optimal MSC derivation method.

Both obtained cell populations were characterized followed by ISCT criteria (expression of typical surface markers, mesodermal differentiation potential) and additionally a number of such features for MSC as proliferation and senescence rate, self-renewal capacity based on ability to create specific colonies (CFU-F) and neural differentiation potential. Cells were analyzed with flow cytometry, immunocytochemistry and quantitative RT-PCR techniques.

Despite comparable level of expression of typical for MSC markers, enzymatically isolated cells were less stable in culture with slower proliferation rate, low frequency of CFU-F formation ability, faster cell senescence and limited

mesodermal differentiation potential. Moreover a significantly higher expression of neuronal and glial markers: Nestin, βTubulinIII, GFAP, NF-200 as well as primitive marker α-SMA was observed in WJ-MSC obtained by mechanical tissue fragmentation. These unique properties of WJ-MSC for neural differentiation can lead to their promising future applications in the treatment of neurological disorders.

To guarantee the high quality of the isolated cells, we should focus on necessity of development of more sensitive and selective methods for prediction and control cells function during time of growth *in vitro*.

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|B19|

Measuring movement of stem cells placed in electric field – methods and problems

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One of the often-appearing problems in case of use of stem cells in regenerative medicine is evaluation of their movement when they are placed in electric field. That issue can be considered in two ways. On the one hand, desirable can be directed migration of stem cells. That is the case of the idea of delivering stem cells as close to their destination as it is possible, and then guiding their migration using electric field. On the other hand, desirable can be as well reduction of cells movement, i.e. keeping them in the place of delivery, also using electric field. In both cases, the key is selection of proper kind and strength of electric field. Research on this matter is now quite common. However, in order to study the influence of electric field on stem cells movement, necessary are reliable methods provided for precise determination of cells position and its changes over time. The aim of our study was to test whether different methods used for these purposes can influence on the evaluation of research results. We based our study on series of images obtained from digital microscope. Adult human bone marrow-derived mesenchymal stem cells were being placed in pulsed electric fields of different strengths and frequencies. In each case, images were acquired every 15 minutes, for duration of 3 hours. Then, locations of the individual cells, as well as parameters of their movement (i.e. velocity and linearity) were estimated using different methods. The results show that the influence of used method is not to be underestimated as it can have a significant impact on the evaluation of research results. Problems concerning different methods of evaluation of the migration of stem cells in electric field were listed as well, including their comparison in aspects of implementation and use.

|B20|

Magnetic resonance imaging in the evaluation of the changes in intervertebral discs under treatment – methods and problems

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Diseases of the intervertebral discs (discopathies) are one of the most common diseases of civilization. Discopathy is initiated through biochemical changes in the structure of discs. In this context, tissue engineering, through creating new possibilities of regeneration of degraded tissue, raises hope that there appear new and more effective methods of dealing with this problem. To evaluate the effectiveness of therapies most often are used

animal models in which in a certain way the process of degeneration of the intervertebral disc is initiated. Most commonly used method of determination of the degree of damage and possible subsequent regeneration of intervertebral discs is magnetic resonance imaging (MRI). Our study focused on the identification of differences that may arise in the assessment of the same cases based on images obtained in different cross-sections of the same MRI research. The issue of proper indication of area of interest has been raised as well, which is, as shown by clinical experience, especially problematic after a long period (i.e. several months) from the time of injury. Attempt was made to identify the most reliable methodology, which could be used to assess the degree of degeneration/regeneration of intervertebral discs. Images obtained in MRI study were analyzed to evaluate the process of regeneration of intervertebral discs after administration of mesenchymal stem cells isolated from bone marrow in porcine animal model, after the damage caused by the surgical laser vaporization. Evaluation was performed for the time of 4, 8 and 12 weeks from the time of damage. Based on our study, the following conclusions can be drawn. Firstly, due to the greater number of available measurement data, frontal plane can be indicated as more reliable than sagittal. Secondly, use of the one of the basic geometrical shapes as the discs' shape approximation can lead to significant errors in the evaluation of the results of the experiment – the borders of the intervertebral disc should be determined using more complex shapes, i.e. considering the anatomy.

|B21|

Influence on the cell body of neurons in central nerve system of cytokines by condition of demyelination

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To assess the relationship of morphological status of the neurons of the cerebrum, cerebellum and spinal cord and behaviour of mice with the "cuprizone" model of demyelination and remyelination after used of exogenous recombinant human leukemia inhibitory factor and interleukin 10. Adult mouse of line 129/Sv received daily for three weeks "cuprizone" with food. Control mice received normal food. After first week of cuprizone application mice were provided with exogenous recombinant human leukemia inhibitory factor and interleukin 10. The animals were evaluated for morphometric analysis which determined the proportion of neurons with unmodified, and with moderate and severe structural changes (staining of histological specimens of toluidine blue) and behavioral reactions (open field test). In morphological investigations we observed structurally modified neurons in the gray matter of the cerebrum, cerebellum and the spinal cord of all experimental groups of mice line 129/Sv. "Cuprizone" had a stronger psychological impact to most behavioral responses in mice. After use of exogenous recombinant human leukemia inhibitory factor and interleukin 10 we observed an increase in unmodified neurons in all parts of the central nerve system. The severity of violations of behavioral and pathological changes of neurons in the CNS after taking "cuprizone" and cytokines are harmonized and have clear differences. The experimental model using "cuprizone" aplication can be used as a model of the demyelination neurological degeneration and breaches of conduct.

|B22|

G1222A polymorphism of HCRTR1 gene and the concentration of hypocretin-1 in patients with migraine

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Hypocretin-1 is a hypothalamic neuropeptide with a known chemical structure and anatomical distribution. This peptide is a part of the hypocretin system which is linked with central pain modulation, probably by way of influence on trigeminovascular nociception and the cortical spreading depression. Recent genetic studies supports the position that hypocretins may play a role in the pathogenesis of primary headache disorders and the hypocretin receptor 1 (HCRTR1) gene could contribute to susceptibility to migraine without aura. The objective of this paper is to investigate the association between non-synonymous G1222A polymorphism of the HCRTR1 gene and

the concentration of hypocretin-1 in 96 migraine patients (11 males, 85 females) representing different subtypes of the disease: migraine with aura (MA: 43 patients) and migraine without aura (M0: 53 patients). A group of 82 healthy controls were subsumed under the study (10 males, 72 females). Polymorphism in exon 7 of HCRTR1 gene results in an amino acid substitution in the cytoplasmic region of the HCRT receptor and could modify its function. Polymorphism G1222A is associated with an increased risk of disease, probably due to the carriage of the A allele of HCRTR1 gene. The results of the study revealed significant difference in the frequencies of the GG, GA, and AA genotypes between patients with migraine as a whole and with M0 when compared with control subjects ($p < 0.05$, Fisher's exact test). The prevalence of genotypes did not reached statistically considerable difference in patients with MA. Interestingly enough, significant differences in the serum concentration of hypocretin-1 were observed among AA individuals. However, the research did not demonstrate diversity of the blood serum concentration of hypocretin-1 in migraineurs (648.1 ± 343.7 pg/ml) to compare with controls (670.3 ± 406.0 pg/ml; $p = 0.4610$) as well as when the patients were divided into migraine with and without aura groups ($p = 0.8655$). In connection with the above-mentioned facts it could be assumed that described genotypes did not seem to modify the serum concentration of hypocretin-1, except for AA genotype, which is associated with an increased migraine risk. The study revealed no relationship between hipocretin-1 and subtypes of migraine.

|B23|

Vasculo- and neuroprotective ability of mesenchymal stem cells derived from Wharton jelly in postischemic rat brains

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Mesenchymal stem cells derived from Wharton jelly (WJ-MSC) appear to be promising candidate for postischemic tissue repair. Unfortunately, moderate and inconsistent benefits of cellular clinical trials press for an urgent need to improve this therapy. The aim of our study was to evaluate the ability of WJ-MSC to differentiate into endothelial progenitor cells (WJ-EPC) and compare the influence of these both cell types on vascular network and proliferation of neural cells in ischemically injured rat brain. WJ-MSC were cultured in growth medium (MSCGM) or in endothelial differentiating medium (EGM-2). Cells were characterized by flow cytometry, immunocytochemistry and molecular methods on the basis of expression of endothelial and mesenchymal markers. Angiogenic activity of WJ-EPC was proved by DiI-Ac-LDL-uptake and Matrigel assay. Cells metabolic activities were determined by WST-1 reagent. Cytotoxic brain injury was conducted with the use of Na^+/K^+ pump inhibitor – ouabain (1 µl; 50 nmol). WJ-MSC and WJ-EPC were engrafted in 3D bioactive platelet lysate (PL) scaffolds into the striatum of normal and focally injured brains of adult Wistar rats. After 3, 7, 14 and 21 days brains were isolated and sliced with cryostat. Subsequently, immunohistochemical studies was performed with rat endothelial cell specific antibody (RECA-1), cell proliferation marker (Ki67), neural markers (Nestin, NF-200, β-tubulin III, GFAP) and microglial cell marker (ED1). WJ-MSC after 7 days in EGM-2 medium, acquired typical EPC cobblestone-like morphology, form capillary-like structures on Matrigel and took up DiI-Ac-LDL. Both cell types were positive for MSC and EC markers CD73, CD90, CD105, VEGFR2, VEGF, but only EPC culture expressed vWF and CD31 on significantly higher gene and protein levels. We showed that plateled lysate could be transplanted into the brain in rat models. Scaffolds with WJ-MSC and WJ-EPC exert vasculo- and neuroprotective properties after transplantation into striatum of focally injured rat brains which makes them a good candidate for cell therapy.

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|B24|

The influence of the dosage drug form on increasing the effectiveness of the therapy of neurodegenerative diseases

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Background: In recent years, the dynamic process of aging of the population is observed. It is estimated that

in 2025 the number of people aged over 60 will exceed the threshold of 1 billion, and the proportion of elderly will reach almost 14% of the world population. The number of people suffering from neurodegenerative diseases is also increasing. Effective treatment of these diseases is very important, and at the same time difficult. Currently, numerous studies focusing on finding effective, safe and simultaneously minimally invasive methods of drug therapy are conducted. The development of drug form technology allows to optimize the currently used treatment regimens and develop the new ones. The aim of the study was to analyze current data concerning drug forms used in the treatment of neurodegenerative disorders.

Material and methods: An analysis of the available literature of the past 16 years was conducted. We searched the following databases: PubMed, Medline, Embase, the Cochrane Library as well as the scientific medical journals.

Results: Recently, besides the traditional dosage forms like tablets and capsules, sustained release tablet, disintegrating tablets in the oral cavity, and transdermal patches were allowed in the treatment of Alzheimer's and Parkinson's diseases. Sustained release tablets contain increased amounts of the drug substance that provides the constant effective concentration of at least 8-12 h. The tablets disintegrating in the mouth are the safe drug for people with the swallowing problems thus convenient for elder patients. They disperse on the tongue, have a rapid onset of action of the active substance and minimize first-pass effect. The transdermal systems have the form of a patch, that allows administering the drug substance to the general circulation. Several nanoformulations containing the drug (e.g. nanoemulsions, lipid nanoparticles, polymeric micelles) which enable the targeted therapy, increase its efficacy and bioavailability of the drug are also tested. The nanoparticles have the ability to penetrate the blood-brain barrier and demonstrate biodegradability, stability and low toxicity.

Conclusions: The development of nanotechnology of drug formulation increases the chances of an effective and safe treatment of neurodegenerative diseases.

|B25|

Exogenous cholesterol and non-sterol isoprenoids stimulate clusterin protein levels in PC-12 cells with APP overexpression

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Previously, we reported that PC-12 cells overexpressing wild type APP(-wt), or Swedish mutation APP(-saw) under control of GFP gene promoter have reduced viability escorted by enhanced autophagy. Present study was conducted to address the question whether mevalonate pathway affects the level of clusterin protein, the extracellular chaperone that protects cell viability. Clones stably expressing wild type APP (W), or Swedish mutation APP (S) under control of GFP gene promoter together with empty vector (G) transfected cells were differentiated into neuronal cells with NGF. APP overexpression was monitored by the presence of green fluorescence (FL) and Western blot (WB). FL confirmed stable transfection whereas WB showed sAPPalpha fragment present in APP(-wt) but not in APP(-sw) or empty vector transfected cells. Similarly, amyloid beta (Abeta) was found solely in APP(-sw) but not in APP(-wt) or empty vector transfected cells. Next, the cells were left untreated or treated with selected statins or non-sterol isoprenoids or both for 24 h. Atorvastatin [ATR, 50 microM] or simvastatin [SIM, 50 microM] used to inhibit mevalonate synthesis (HMG-CoA reductase inhibitors) diminished cell viability ($p < 0.05$) but did not affect clusterin protein levels. Interestingly, administration of non-sterol isoprenoids (geranyl-geraniol – GGOH, farnesol – FOH, mevalonate – MEV) and cholesterol PEG conjugate (Chol-PEG) in non-toxic concentrations strongly stimulated ($p < 0.05$) clusterin protein levels in PC-12 cells. The effects of Chol-PEG and non-sterol isoprenoids were more profound in APP(-wt) and APP(-sw) than in empty vector transfected cells. GGOH overridden statin-dependent cytotoxicity by re-establishing cell viability to observed in untreated cells but this effect does not seem to be dependent on clusterin as its level did not differ from other treatments. These results suggest important role played by mevalonate pathway in clusterin protein stimulation in APP overexpressing cells, although merely GGOH has direct effect on cell survival. Protein prenylation with GGOH

rather than FOH might play substantial role in neuronal cell viability.

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|B26|

Stemness or differentiation? The influence of small molecules and oxygen tension on neural stem cells fate decisions

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Small molecules can exert developmental context dependent activity: influence the maintenance of stem cells stemness or support their differentiation into specific cell types. Moreover, it was proven that oxygen tension may influence the cellular response to small molecules. In this study the effect of selected small molecules (TSA, RG108, PD0325901, SB431542) and the oxygen tension on developmental processes of neural stem cells derived from human umbilical cord blood (HUCB-NSC) was investigated. We have shown that 5% oxygen concentration promotes proliferation of HUCB-NSCs, but the presence of selected small molecules affect this correlation. HUCB-NSC cultured in 21% oxygen concentration with RG 108 revealed significantly higher proliferative potential.

All samples have been tested for the expression of genes typical for the pluripotency (OCT4, SOX2, NANOG, REX1) neural differentiation (B-TUBULIN III, MAP2, NF200) as well as related to the hypoxia (HIF1, 2, and 3 alpha) and epigenetic regulation (HDAC1, HDAC2, DNMT3A, DNMT3B). This expression was confirmed on the protein level by immunocytochemistry. In all tested experimental variants cells were negative for OCT4, SOX2 and REX1. Low NANOG expression was detected in two variants: HUCB-NSCs cultured in 5% oxygen concentration without addition of any small molecule and with TSA. Neural differentiation of HUCB-NSC was confirmed by the expression of neural markers in all tested samples, with two exceptions: there was no expression of MAP2 in cells cultured in 5% oxygen concentration with RG108 and NF200 in cells growing in 21% oxygen concentration with TSA. The expression of genes involved in regulation of epigenetic processes (HDAC1, HDAC2, DNMT3A, DNMT3B) was present in HUCB-NSCs in almost all experimental conditions, except cells growing in 21% oxygen with PD0325901 – for all tested genes and cells cultured in 5% oxygen concentration with RG108 – for DNMT3A, DNMT3B exclusively. The quantitative estimation of the expression of selected genes is presently under verification.

Our study based on changes of the expression of investigated genes revealed, that developmental response of HUCB-NSCs to small molecules may be modified by the level of oxygen tension in cell microenvironment.

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|B27|

Association between 1298A>C polymorphism within MTHFR gene and paediatric ischemic stroke – meta-analysis of 325 cases and 504 controls

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Background: It is observed that the level of homocysteine (HCys) plays important role in the functioning of endothelial cells. Elevated level of HCys is a risk factor for vascular diseases as well as brain atrophy. Several common polymorphisms within genes encoding enzymes involved in homocysteine metabolism (e.g. methylenetetrahydrofolate reductase; MTHFR) are suggested to be associated with development of arterial ischemic stroke (AIS), both in adults and children. Previous studies, including meta-analysis, have shown that 677C>T polymorphism within MTHFR gene is related to paediatric AIS. However, the role of other common MTHFR polymorphism, 1298A>C, is still uncertain. One studies show such relation, while others – do not. Most often, studies are performed on small number of patients due to the rarity of AIS. Considering that, we performed meta-analysis of available data addressing possible association between MTHFR 1298A>C polymorphism and AIS in children.

Material and methods: We searched Pubmed using "MTHFR polymorphism", "ischemic stroke", "paediatric", "1298A>C polymorphism", "children" as keywords. We included to a study 5 case-control studies. Due to the possibility of obtaining false positive or negative results, we did not include to the present meta-analysis studies with the number of patients lower than 40. A total number of 325 paediatric patients with arterial ischemic stroke and 504 controls were enrolled to the study. We conducted statistical analyses with the use of MedCalc software. Heterogeneity between the studies was evaluated using the Dersimonian and Laird's Q test. When heterogeneity between the studies was significant, the pooled odds ratio (OR) was analysed with a random effects model, otherwise, a fixed effects model was used. Results: In case of MTHFR AC+CC vs AA analysis, we observed significant heterogeneity between the analysed studies (Cochrane Q $p = 0.02$ and I2 = 65.19%), thus random effects model was used to estimate pooled OR. We observed that carrier-state of 1298C allele is not related to ischemic stroke in children ($p = 0.236$, OR = 1.37, 95% CI: 0.81-2.30).

Conclusions: The results based on a sizeable groups of paediatric patients suffering AIS and healthy controls demonstrated that 1298A>C polymorphism in MTHFR gene is not related to paediatric ischemic stroke.

|B28|

Neurochemical and neuropathological aspects of new psychoactive substances ("legal highs") use

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The presence of new psychoactive substances (NPS) use is a relatively new challenge for clinicians, forensic pathologists, lawyers and basic researchers. According to alerts of European Union Early Warning System (EU EWS), almost each week completely new substance (mainly synthetic cannabinoids, cathinones and opioids) appears on drugs of abuse market. These substances are often called "legal highs" due to the fact that after introduction on the narcotic market they are not illegal. The main current problem is lack of sufficient knowledge about its mechanism of action, psychoactive effects in humans and its acute, short- and long-term toxicity.

The aim of this work is: (1) to review the published and referenced in PubMed database results of basic studies on the mechanisms of action and toxicity of some of NPS, and (2) present own results of routine neurohistological examination (H&E stain) of the central nervous system (CNS) in fatal victims of NPS intake, examined post mortem in the Department of Forensic Medicine and Forensic Toxicology in Katowice on the prosecutor's order.

New designer drugs mimic the action of classical narcotics. In the last years the number of articles concerning mechanisms of action and toxicity of some of NPS is constantly growing, but still relatively small number of NPS was studied. There are many questions, which have to be answered. Our results of routine neurohistological examination of CNS in fatal victims of NPS intake indicate that there are no specific changes related with NPS intoxication. In all cases we found severe oedema and hyperaemia. Further studies on animal models (*in vitro*, *in vivo*) and on human post mortem material obtained during autopsy are strongly required for better understanding of NPS action, which is important for both, clinicians and forensic experts.

|B29|

Activation of NG2-positive glia after transient cerebral ischemia in the rat – immunocytochemical studies

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Reactive gliosis has been regarded as the specific and stimulus-dependent character of nervous tissue response to different pathological processes. Important issues for this reaction are cellular proliferation and de-differentiation of resident progenitors enabling their further development into various cell lineages. The source of differentiating cells and the intensity of their proliferation in different pathological circumstances are still the subject of discussion. A significant role is attributed to NG2-immunoreactive (NG2-ir) polidendrocytes (oligodendroglial progenitor cells), which constitute the most numerous population of progenitors in the developed brain. The aim of this study

was to assess the proliferation and differentiation capabilities of NG2 glia during reperfusion after 1 h transient cerebral ischemia in rats. An ischemia was evoked in adult male Wistar rats with subsequent 6 weeks of reperfusion in order to assess the reactive glial response. Transient cerebral ischemia triggers an intense proliferative reaction of polidendrocytes and astroglia. However, our study did not reveal the ability of changing the morphological features of glial lineage fate among the former and replenishing the population of reactive astrocytes.

|B30|

Alteration in gene expression of sphingolipid metabolism enzymes in PC12 cell line transfected with amyloid precursor protein

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Background: Sphingolipids are diverse class of lipids that play an important role in signal transduction, cell recognition, growth arrest and apoptosis. These lipids include sphingomyelin, ceramides, ceramide phosphate, sphingosine 1-phosphate (S1P). Disturbances in sphingolipid metabolism and alteration of the rheostat between ceramide and S1P have been suggested as important factors in pathogenesis/pathomechanism of Alzheimer's disease (AD). This study concentrate on the effect of endogenously liberated amyloid beta peptides (AB) on enzymes' gene expression involved in sphingolipid metabolism in pheochromocytoma (PC12) cells characterized by overproduction of amyloid precursor protein (APP).

Methodology: Rat PC12 cells transfected with human gene for wild type of APP (APPwt) and PC12 cells bearing double Swedish mutation (APPsw) were used in this study. Control PC12 cells were transfected with an empty vector. Biochemical and qPCR methods were applied. Following genes were analyzed: ceramide synthases: Cers2, Cers3; ceramidase: alkaline ceramidase Acer1; kinases: ceramide kinase Cerk1, sphingosine kinases: Sphk1, Sphk2.

Results: Our results demonstrated that endogenously liberated AB (APPwt and APPsw) significantly affected transcription of enzymes involved in the regulation of ceramide level in cells. Analysis of mRNA level for ceramide synthases and ceramidases in both cell lines demonstrated that Cers3 gene expression was upregulated in APPwt cells while expression of Cers2 remained unchanged. However, in APPsw cells significant reduction of Cers2 gene expression was observed. Moreover, the expression of Acer1 gene was decreased in APPwt and APPsw cells. Our study indicated downregulation in Cerk1 gene expression in APPsw cells. Additionally, significant reduction of the gene expression of Sphk2, was found in both APP transfected cells.

Conclusions: Our data indicated significant alterations in enzymes gene expression involved in regulation of balance between bioactive lipids: ceramide and S1P in cellular AD model. The observed changes of Cers, Acer1, Cerk1 and Sphk2 may lead to higher ceramide level, cells' degeneration and death. All these enzymes could be considered as a promising target(s) for cytoprotection against AB toxicity.

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|B31|

Poly(ADP-ribose) polymerase-1 as a crucial regulator of nuclear and mitochondrial Sirtuins transcription. Implications for cell physiology and pathology

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Background: Poly(ADP-ribose) polymerase-1 (PARP-1) is the oldest member of NAD+ dependent PARPs family. In the brain PARP-1 is responsible for more than 90% of protein poly(ADP-ribosylation). PARP-1 is involved in DNA repair, however its overactivation may lead to cell death. The interaction between PARPs and other NAD+ dependent enzymes – histone deacetylases class III - Sirtuins (SIRTs) is fundamental for cells metabolism, DNA repair, transcription in physiology and pathology.

Aim: This study was focused on the role of PARP-1 in regulation of SIRTs gene expression in PC12 cells under resting conditions and amyloid beta (AB) toxicity. The short term effect of AB42 was compared with long term

action of endogenously liberated AB in PC12 cells transfected with Amyloid Precursor Protein (APP).

Methodology: The experiments were carried out using PC12 cells treated with PARP-1 inhibitor PJ-34 (20uM) or with AB42 oligomers (ABO, 1uM) during 24 h in culture. Rat pheochromocytoma (PC12) cells transfected with human gene for wild type of APP (APPwt) and PC12 cells bearing double Swedish mutation (APPsw) were used in this study. Control PC12 cells were transfected with an empty vector. Biochemical and qPCR methods were applied.

Results: Our results demonstrated that pharmacological inhibition of PARP-1 activity by PJ-34 upregulated expression of both nuclear Sirtuins: Sirt1 and Sirt6 in resting conditions and in the presence of ABO. Inhibition of PARP-1 had no effect on cytosolic Sirt2 expression and its signalling. However, PJ-34 enhanced gene expression for mitochondrial Sirt4 which is mainly responsible for mono(ADP-ribosylation) and indicates very low deacetylase activity. ABOs upregulated mRNA level for both mitochondrial Sirtuins: Sirt4 and Sirt5 and PJ-34 decreased their expressions. Endogenously liberated AB peptides enhanced mRNA level exclusively for mitochondrial Sirt3 in APPsw cells suggesting its involvement in antioxidative defense.

Conclusions: Summarizing, these results indicate that PARP-1 is a very important regulator of Sirtuins transcription in control resting condition and in AB toxicity. Mutual dependence between PARPs and SIRTs can influence nuclear and mitochondrial protein functions and cells metabolism in physiology and in AB toxicity.

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|B32|

Breast-milk-derived cells – connection with postnatal development of nervous system?

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Introduction: Breast-feeding plays an important role for the development of the newborn. The breast milk among proteins, lipids, carbohydrates and other biologically active components also contains a heterogeneous population of cells that have the potential to differentiate into various mature cell types including neural cells [1]. The natural presence in breastmilk of stem cells with multilineage differentiation potential raises the question of the role of these cells during early infant development. It is proposed that the cells could develop the enteric nervous system – one of the main divisions of the nervous system, consists of a mesh-like system of neurons, that governs the function of the gastrointestinal system. Non-breast fed premature born infants show a significantly higher risk of developing diseases like infantile diarrhoea and necrotizing enterocolitis.

Material and methods: In aseptic procedures, mature breast milk (10-30 ml) samples were obtained from healthy breastfeeding women with early range of lactation stages. The isolation procedure was based on the protocols described by Hassiotou *et al*. [2] with modifications. The cells were cultured under standard conditions in basic culture medium based on low-glucose DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotic–antimycotic mixture.

Results: The aims of this study were to obtain the breast milk-derived cells cultured in vitro. The isolated breast-milk-derived cells were adherent to the plates. The presence of the cells with various origins was detected in human breast milk.

Conclusions: Human breast milk contains a heterogeneous population of cells that have the potential to provide a non invasive source of cells for proper growth and development of infants, but also for cell therapy and treating neonatal disorders. Because of its ethical, noninvasive and plentiful nature, breastmilk offers a novel resource of patient-specific progenitor cells for applications in regenerative medicine and also in treating neurodegenerative diseases.

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|B33|

Upconverting Nanoparticles: a new approach for biomedical applications. Cytotoxicity and uptake mechanism of the NaYF4:Yb3+,Er3+ nanoparticles.

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A new generation of multifunctional nanoparticles, defined as upconversion nanoparticles (UCNPs), are luminescent nanomaterials, which have ability to convert near infrared light (λ = 980 nm) to visible or UV light. The unique properties of the UCNPs are: low toxicity and low autofluorescence from biological samples. They are very useful candidates for in vitro cellular imaging and in vivo tissue imaging. The UCNPs have also potential applications in photodynamic therapy, drug delivery, biomolecules sensing and theranostics. To understand biological interactions between nanoparticles (NPs) and living cells, at the molecular level, we studied their cellular internalization process termed endocytosis. The endocytosis takes several forms: clathrin-mediated endocytosis (CME) and clathrin-independent pathways (CIE) including lipid-raft/caveolin-mediated endocytosis, micropinocytosis and phagocytosis. To examine which mechanism is involved in the NPs endocytosis we applied several inhibitors. Chlorpromazine was used to block clathrin-mediated endocytosis. Methyl-β-cyclodextrin (MβCD) and nystatin were evaluated as inhibitors of caveolae-mediated endocytosis; amiloride and cytochalasin D were used to inhibit macropinocytosis and phagocytosis, respectively. Sodium azide and low temperature (4°C), which are factors inhibiting almost all endocytic pathways were used to distinguish from the non-endocytic pathways. Influence of the β-NaYF4:Yb3+,Er3+ upconverting nanoparticles on cell viability and proliferation was evaluated by the following assays: MTT, PrestoBlue and RealTime MT-Glo Cell Viability Assay. Effects of endocytosis inhibitors on the internalization of β-NaYF4:Yb3+,Er3+ UCNPs by HeLa and HEK293 cells were evaluated using confocal microscopy and transmission electron microscopy (TEM). Pre-treatment of HeLa and HEK293 cells with the tested endocytosis inhibitors showed that more than one mechanism is engaged in the cellular uptake of the NPs. The NPs uptake was very fast. We found that the β-NaYF4:Yb3+,Er3+ nanoparticles entered cells as soon as 1 hour post transfection and colocalized with late endosomes and lysosomes. We did not observe ultrastructural changes and the level of apoptosis did not increase in comparison with the control group. The Energy-dispersive X-ray analysis (EDX) confirmed the presence of the UCNPs inside cells and their chemical content. The β-NaYF4:Yb3+,Er3+ UCNPs are promising agents in biomedical science because they revealed no apparent cytotoxicity and are very interesting for multi-functional optical imaging and therapy.

|B34|

Beneficial role of MMPs in neurogenesis after Sodium Butyrate treatment in a model of neonatal hypoxia-ischemia

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Matrix metalloproteinases (MMPs) are proteolytic enzymes that degrade extracellular matrix and carry out key functions during brain development but are also implicated in pathological functions within central nervous system. It was reported that histone deacetylase inhibitors (HDACis) provide neuroprotection against brain injury in adult stroke models, among others, by reduction of MMPs activity. Aim of our study was to investigate the influence of HDACi-Sodium Butyrate (SB) treatment on the activity of MMPs as well as its potential role in neurogenesis in the immature brain subjected to hypoxia-ischemia. We utilized a model of hypoxia-ischemia (HI) induced in 7-days old rats. After ligation of the left common carotid artery animals were exposed to hypoxia (7.6% oxygen for 60 min). Hypoxic undamaged hemisphere and sham-operated rats served as control. SB (300 mg/kg) was injected subcutaneously for 5 days starting immediately after HI. The activity of MMPs was examined using *in-situ* zymography. Hypoxia-ischemia resulted in elevation of MMPs activity mainly in hippocampus of the ipsilateral hemisphere

and SB treatment significantly decreased this activation. To investigate the role of MMPs in neurogenesis after SB treatment we used double immunohistochemical staining with specific markers. The administration of SB stimulates the generation of neuroblasts and progenitors of oligodendrocytes and elevates MMPs activity in these cells. These results suggest that SB treatment has beneficial effects by diminishing MMPs activity in acute phase of HI damage and by stimulating the proliferation of neural progenitors and MMPs activation in later stages after injury.

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