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ORAL

|A1|

Synaptic Shank proteins alterations in rat offspring following maternal immune activation: implications for autism spectrum disorders

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Autism spectrum disorders (ASDs) are a heterogeneous group of neurodevelopmental brain diseases that are clinically defined by emotional and social deficits, impairments in social interaction and stereotyped behaviours such as repetitive movements or speech. The recent study suggested that alterations of ProSAP/Shank proteins located at the post-synaptic density and involved in synaptic formation, development, and function may play a crucial role in ASD pathomechanism. Mutations in the family of SHANK genes, SHANK1, SHANK2, and SHANK3, are strongly associated with ASD. However, the specific role of the various Shank proteins in the pathogenesis of ASD is still unclear. Prenatal maternal immune activation (MIA) is a risk factor for ASD and is commonly used as animal model of this disorders. In the present study pregnant dams of Wistar rats were injected intraperitoneally (i.p.) at gestational day 9.5 with 0.1mg/kg lipopolysaccharide (LPS), which mimics infections by gram-negative bacteria. We investigated the effect of MIA on the expression and protein level of ProSAP/Shank family proteins in male offspring of rats. Additionally, protein levels of postsynaptic organizing molecule PSD-95 associated with Shank, phosphorylated and total Akt, and brain-derived neurotrophic factor (BDNF) expression were measured in LPS-treated animals versus control subjects. Moreover, redox potential and pro-oxidative/pro-inflammatory proteins were analysed along with the autism-associated behaviour.

The data showed MIA-induced down-regulation of SHANK1, SHANK2, and SHANK3 in the cerebral cortex, without changes in other brain structures. Therefore, the further studies were carried out using only brain cortex. Western blotting revealed significant alteration of pre- and post-synaptic proteins level. Moreover, a statistically significant decrease in phosphorylated Akt (pAkt) and pAkt/Akt ratio in MIA subjects was indicated. In addition, the GSH/GSSG ratio used as an indicator of oxidative stress was reduced. Furthermore, the gene expression for interleukin-6 (IL-6), cyclooxygenase-2 (COX-2) as well as 12-lipoxygenase (LOX-12) were up-regulated. Along with the biochemical investigation behavioural tests were conducted to assess social communication, motions and anxiety, play behaviours as well as learning and memory. The results showed no bedding preference in prenatal LPS exposure animals at post-natal day 14 compared to control rats, indicating the impairment of need being in proximity of the mother. In conclusion, our findings indicate MIA-induced down-regulation of SHANK family and alteration of others synaptic proteins, reduced antioxidative capacity as well as activation of proinflammatory genes. These changes may disturb synaptic structure and function as well as social communication behaviour.

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|A2|

A search for mechanisms of Parkinson's disease (PD)-associated depression in an animal model

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Depression may appear in a preclinical period of PD. Alterations of serotonin (5-HT) transporter (SERT) and BDNF signaling have been suggested to be associated with major depression. However their role in PD associated depression is unknown.

The aim of the study was to examine if a partial dopaminergic lesion modeling pre-clinical phase of PD may induce 'depressive-like' behaviour of rats. Furthermore, a role of SERT and BDNF in this behaviour and antidepressant drug action were evaluated.

Rats were injected with 6-OHDA into the ventral part of the caudate-putamen (CP). Pramipexole [(PRA), a dopamine D3/D2 receptor agonist, the most effective compound in the treatment of depression in PD] or imipramine [(IMI) a classic tricyclic antidepressant] were administered for 2 weeks. 24 h later the behavioural tests were performed and rats were decapitated.

6-OHDA induced moderate DA depletion in CP, nucleus accumbens (NAC) and frontal cortex, loss of DA neurons in the substantia nigra, ventral tegmental area (VTA) and dorsal raphe (DR), decreases in binding to DA transporter (DAT) in CP and NAC. Contrariwise, 6-OHDA influenced neither the number of DR 5-HT neurons, nor forebrain 5-HT levels. The lesion prolonged the immobility in FST but did not influence motility of rats. PRA, but not IMI shortened the immobility in lesioned rats. PRA but not IMI increased levels of DA metabolites and its turnover.

The lesion reduced the BDNF and trkB mRNAs in the hippocampus and amygdala. PRA increased BDNF mRNA but decreased trkB mRNA in VTA in lesioned rats. Furthermore, it reduced BDNF and/or trkB mRNA expression in NAC, amygdala and CP in these animals. The lesion lowered the [3H]citalopram binding to SERT in DR, and in all structures of 5-HT terminals. Similarly, PRA decreased the binding to SERT in the above regions.

These results indicate that a moderate DA lesion, which does not produce motor disturbances, may induce "depressive-like" symptoms which are reversed by DA agonist but not by a classic antidepressant. Mechanisms responsible for the above behaviour may be associated with decreased DA but not 5-HT transmission. Moreover, the increase of DA transmission and reduced BDNF signaling in NAC and amygdala induced by PRA may contribute to its antidepressant efficacy.

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|A3|

Towards treatment of amyloid toxicity in dementia

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The correlation between the total amount of fibrillar aggregates and cognitive decline depends not only on the quantity of the amyloid protein deposited but also on the number of synapses to be destroyed. Indeed, patient with Alzheimer's disease (AD) exhibiting similar level of clinical severity, higher levels of education are associated with more severe disease related changes of β-amyloid PET or CSF Aβ42 levels suggesting that amyloid toxicity may be the same in patients with matched clinical severity but gross differences in AD pathology. Pre-fibrillary Aβ species rather than elongated amyloid fibrils are likely to represent the primary pathogenic agents simply because the former provide on their surfaces more chemical groups than the latter, such as hydrophobic side chains and unbound hydrogen bonds that would not be accessible within amyloid. The origine of the toxicity of the oligomers may arise from inappropriate interaction in trans with the folding of cellular and extracellular structures including proteins, lipid membranes and nucleic acids. Therefore, prevention and treatment of protein misfolding disorders needs to address aggregation and misfolding in cis and trans by decreasing the concentration and disrupting the formation of these toxic species. This can be achieved, as we have shown firs, with Aβ-analogues that selectively bind to the native state of the peptide that suppress nucleation and proliferation of toxic pre-amyloid species, by antibodies to reduce the level of highly trans-aggregation prone species (such as Aβ oligomers), and by stimulating clearance by proteolytic degradation.

|A4|

Pathogenic microRNA (miRNA) signaling in Alzheimer's disease (AD) and age-related macular degeneration (AMD) target common genetic pathways leading to amyloidogenesis and inflammatory neurodegeneration

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Overview: Progressive amyloidogenesis and inflammation in the neocortex and retina are associated with aberrant innate-immune signaling and inflammatory degeneration in both Alzheimer's disease (AD) and age-related macular degeneration (AMD). Two pivotal players in this pathogenic system are the immune-repressor protein complement factor H (CFH) and the triggering receptor expressed in myeloid/microglial cells (TREM2), each found to be reduced in abundance in AD and AMD. Here we investigated the mechanism of CFH and TREM2 expression regulation in AD and AMD involving the up-regulated, NF-κB-sensitive miRNA-34a, miRNA-146a and miRNA-155, and the effects of specific anti-NFkB and anti-miRNA therapeutic strategies which may be useful in the clinical management of these disorders.

Methods: Aβ42-peptide- and/or TNFα-induced stress; AD/AMD tissues; bioinformatics; DNA array/RNA sequencing; human retinal/brain cells; LED-Northern dot blot analysis; micro-RNA array; NF-kB-inhibitors luciferase-reporter transfection; Western analysis.

Results: A major ~4200 nucleotide (nt) human CFH mRNA included a 232 nt 3'UTR, containing recognition features for 27 miRNA-mRNA interactions. Similarly, multiple miRNA binding sites were found in the 299 nt TREM2 mRNA 3'UTR. Binding of miRNA-34a, miRNA-146a and/or miRNA-155 to the CFH- or TREM2-3'UTR dramatically decreased CFH or TREM2 expression. This deficit was in part reversed using NF-kB inhibitors or anti-miRNA pharmacological strategies.

Conclusions: Our perception on the mechanism and relevance of miRNA signaling in brain and retina continues to evolve. For the first time these data provide evidence for novel miRNA-mediated genetic switches in the CFH and TREM2 mRNA 3'UTRs that are differentially regulated in the brain and retina. These data further suggest that epigenetic mechanisms involving inducible miRNAs contribute to immune-inflammatory degeneration and amyloidogenesis characteristic of age-related disorders, and further support the use of novel transcription factor- and nucleic acid-based therapeutic strategies in the clinical management of AD and/or AMD.

Support: Research on miRNA in the Lukiw laboratory involving the innate-immune response in AD, AMD and in retinal disease, amyloidogenesis and neuroinflammation was supported through an unrestricted grant to the LSU Eye Center from Research to Prevent Blindness (RPB); the Louisiana Biotechnology Research Network (LBRN) and NIH grants NEI EY006311 and NIA AG038834.

|A5|

PPAR-gamma: therapeutic prospects in Parkinson's disease

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Skewed microglia activation with pro-inflammatory prevailing over anti-inflammatory phenotypes may contribute to neurotoxicity in Parkinson's disease (PD), via the production of cytokines and neurotoxic species. Targeting the microglia polarization process is a proposed strategy for neuroprotection. The peroxisome proliferator-activated receptor (PPAR)gamma is expressed in microglia and peripheral immune cells, where they are involved in macrophages polarization. PPARγ agonists mediate neuroprotection in PD models. We investigated the neuroprotective activity of PPARγ agonists in the chronic MPTP/probenecid (MPTPp) models of PD, and their action on microglia polarization via the evaluation of pro- and anti-inflammatory molecules. PPARγ agonists rosiglitazone and the novel compound MDG548, were neuroprotective in the MPTPp model and reduced microglia activation and iNOS produc-

tion in the substantia nigra compacta (SNc). Moreover, we found a gradual increase of pro-inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β, over anti-inflammatory molecules such as transforming growth factor (TGF)-β, IL-10 and CD206, within Iba-1-positive microglia, suggesting that a skewed polarization was associated with disease progression. Rosiglitazone and MDG548 administered during the full MPTPp treatment or for the last 10 days, reduced pro-inflammatory cytokines while increasing anti-inflammatory molecules as compared with the MPTPp treatment. Therefore, neuroprotective treatment with PPAR-γ agonists exerts an anti-inflammatory action via a modulation of microglia polarization correcting the imbalance between pro- over anti-inflammatory molecules, offering a novel immunomodulatory approach to neuroprotection.

|A6|

Regulated cell death pathways converging at mitochondria are promising therapeutic targets for neuroprotection

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Mitochondria play crucial roles in energy metabolism, regulation of free radical formation and calcium storage, thereby determining essential metabolic functions and cell survival, in particular in neurons. Under physiological conditions, mitochondria are highly dynamic organelles that undergo constant fission and fusion and these dynamic morphological changes are essential for mitochondrial functions. Consistent with the critical role of mitochondrial dynamics in neurons, impairments of mitochondrial fission and fusion are associated with a wide array of inherited or acquired neurodegenerative diseases. In order to elucidate impaired mitochondrial dynamics in different paradigms neuronal cell death, including oxytosis, ferroptosis and necroptosis, our studies focus on the regulation of mitochondrial integrity in neuronal cells in vitro and after cerebral ischemia in vivo. In neuronal HT22 cells, glutamate and erastin induce pronounced production of ROS followed by mitochondrial fission through mechanisms of oxytosis and ferroptosis, respectively. The fragmented mitochondria accumulate around the nucleus and release apoptosis inducing factor (AIF) which executes cell death in a caspase-independent manner. The mechanisms upstream of detrimental mitochondrial fission involve concomitant mitochondrial translocation of Bid and dynamin related protein 1 (Drp-1) which mediate mitochondrial membrane permeabilization. Inhibition of Bid or Drp-1, as well as inhibition of ferroptosis by ferrostatin or inhibition of necroptosis by necrostatin-1 preserve mitochondrial morphology and provide neuroprotective effects, also after oxygen glucose deprivation in primary cultured neurons and in models of cerebral ischemia in vivo. In conclusion, our data suggest that regulators of pathological mitochondrial fragmentation such as Bid and Drp-1 as well as pathways of ferroptosis and necroptosis are promising therapeutic targets for neuroprotection.

|A7|

Cyclin-dependent kinase 5 signaling in neuroinflammation and neurodegeneration

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The common feature of age-related neurodegenerative disorders is accumulation, improper folding and aggregation of specific proteins, with amyloid beta (Aβ) and alpha synuclein (ASN) being the most prominent representatives. The growing body of evidence suggests that significant component of molecular mechanisms of misfolded proteins' toxicity may be dysfunction of cyclin-dependent kinase 5 (Cdk5). Cdk5 is fundamental for central nervous system's development, but also actively contributes in regulating neuronal function in adult brain. Cdk5 controls neurite elongation, synaptogenesis, synaptic plasticity, oxidative stress and neuronal survival, though its deregulation has detrimental effects for neuronal function. In our studies we analyzed the role of Cdk5 in toxicity of two major misfolded proteins, Aβ and ASN. We used PC12 cells stably transfected with human APP gene or incubated with exogenous ASN. For in vivo analysis we used mouse model of systemic inflammatory response and model of acute toxicity of Aβ after intracerebroventricular administration.

The in vitro studies discovered two pathways responsible for Cdk5 overactivation in cytotoxicity of ASN: i) calcium-induced, calpain-dependent proteolysis of Cdk5-activating protein p35 leading to formation of excessive activator of Cdk5-p25, ii) phosphorylation of Cdk5 at

Tyr15. Enhanced activity of Cdk5 was responsible for mitochondrial dysfunction, oxidative stress and cell death evoked by ASN. However, the studies carried out in cells overexpressing Aβ revealed that decrease in Cdk5 activity evokes a decline in the level of phosphorylation of glycogen synthase kinase-3β at Ser9, leading to its activation and to hyperphosphorylation of Tau protein. These results indicate that not only an increase but also a decline of Cdk5 activity may lead to detrimental effects.

The recent data suggest that Cdk5 may actively assist inflammatory signaling. Our in vivo studies showed overactivation of Cdk5 and its involvement in controlling the expression of inflammation-related genes in the brain during systemic inflammatory response. Moreover, we demonstrated the significant role of Cdk5 in regulation of gene transcription in mouse model of Aβ toxicity.

In summary, our results demonstrated that Cdk5 actively contributes to pathomechanism of protein misfolding-linked disorders, being involved in both neurodegeneration and neuroinflammation.

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|A8|

Neurosteroids and Alzheimer' disease: mitochondria at the interface

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Alzheimer's disease (AD) is an age-related neurodegenerative disorder that currently accounts for more than 60% of all dementia cases and is characterized by a progressive cognitive and physical deterioration. This neuropathology will become increasingly burdensome and costly in the coming years as AD prevalence is expected to double within the next two decades.

Mitochondrial dysfunction is a prominent and early event of the disease, since energy deficiency is a fundamental characteristic of AD that was observed in the brain of AD patients as well as in transgenic AD mouse models. Indeed, an impaired mitochondrial function can already be detected before the onset of cognitive impairments and the appearance of the two histopathological hallmarks of the disease – the presence of extracellular amyloid-β (Aβ) deposits and intracellular neurofibrillary tangles (NFT). Of note, Aβ and abnormally hyperphosphorylated tau protein, which composes the NFT, may act synergistically to trigger mitochondrial dysfunction in AD.

Neurosteroids are steroid molecules that are synthetized within the nervous system independently of peripheral endocrine glands and are involved in brain-specific functions. Neurosteroids have recently shown promise in alleviating cognitive and neuronal sequelae of AD. In particular, extensive studies only focused on estradiol as a promising neurosteroid compound that is able to ameliorate cellular bioenergetics, while the effects of other steroids on brain mitochondria are still poorly understood.

To gain insights into the underlying mechanism of neuroprotection by neurosteroids, in particular those belonging to the sex hormone family, we focused on their effects on AD-related mitochondrial dysfunction. First, we showed that the binding of Aβ to the mitochondrial enzyme ABAD (Aβ-binding alcohol dehydrogenase) disturbed estradiol homeostasis and that a treatment with AG18051, a novel small ABAD-specific compound inhibitor, prevented cell death induced by the presence of Aβ thereby normalizing estradiol levels in vitro. Second, we showed that the bioenergetic deficits induced by either Aβ or abnormally hyperphosphorylated tau protein can be alleviated by a treatment with sex hormones-related neurosteroids, with testosterone representing the lead steroid acting against mitochondrial deficits induced by Aβ, while progesterone and estrogens were more efficient against AD-related tauopathies.

Together, our findings lend further evidences to the neuroprotective effects of neuroactive steroids in AD pathology and indicate that these molecules represent promising tools able to increase mitochondrial bioenergetics in pathological conditions. Our results may open new avenues for the development of gender-based therapeutic approaches in AD.

|A9|

M1 muscarinic agonists and a novel and highly potent activator of the M1R/sigma-1R complex: future therapeutics of Alzheimer's (AD) and Parkinson's disease (PD)

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The M1 muscarinic receptor (M1R) has a major therapeutic role in AD and PD. The sigma-1 receptor (S1R), a molecular chaperone, is another drug target as it plays a fundamental role in cognitive function, mitochondrial functioning and in protein conformation diseases. We previously developed orthosteric M1R agonists (*e.g.*, AF102B, AF267B, and AF292), which act as cognitive enhancers and potential disease modifiers in AD and PD *(review: Fisher, J. Neurochem, 2012)*. Notably, i) AF102B and AF267B decreased CSFAbeta levels in vivo and reduced brain alpha-synuclein in transgenic mice over-expressing human alpha-synuclein; ii) AF267B was effective against cognitive deficits, Abeta42 and tau pathologies in 3xTg-AD mice; and iii) AF102B decreased CSF Abeta in AD patients. We now report on AF710B, a highly potent and selective allosteric M1R and S1R receptor agonist. AF710B exhibits an allosteric agonistic profile on M1R; AF710B (0.1nM, *in vitro*) significantly potentiated the binding and efficacy of carbachol on M1R and their downstream effects (phopho-ERK1/2, phospho-CREB). AF710B (nM range) decreased Tau-hyperphosphorylation, GSK3beta activation, and reduced apoptosis and mitochondrial dysfunction *via* increased Bcl2/Bax. AF710B (1-30 microg/kg, po) was a potent and safe cognitive enhancer in rats treated with the M1R antagonist trihexyphenidyl (passive avoidance impairment). These effects of AF710B involve S1R activation. In agreement with its anti-amnesic properties, AF710B (at 30 nM), via activation of M1R and a possible involvement of S1R, rescued mushroom synapse loss in PS1-KI and APP-KI neuronal cultures, while AF267B (1 microM) was less potent in PS1-KI and ineffective in APP-KI models, respectively. In female 3xTg-AD mice AF710B (10 μg/kg, ip/daily/2 months) – i) mitigated cognitive impairments in Morris water maze; ii) decreased BACE1, GSK3beta activity, p25/CDK5, neuroinflammation, soluble and insoluble Abeta40, Abeta42, plaques and tau pathologies. The effects of AF710B can be attributed to concomitant activation of S1R and a super-sensitized M1R, via a hypothetical heteromerization of these receptors. AF710B represents a comprehensive therapy on cognitive deficits, synaptic loss, beta-amyloid and tau pathologies, neuroinflammation, ER-stress and mitochondrial dysfunctions. AF710B may also be effective in several other protein conformation diseases (e.g. PD, LBD, ALS and more).

|A10|

Tissue Stress Phenomena in the Brain in the Context of Mechanisms of Neurological Diseases and Therapeutic Neuroprotection

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Although its basic meaning was different, the word "stress" is frequently used to describe either exposure to, or response of organs (e.g. brain), tissues, cells or subcellular organelles to adverse influences. The overarching definition of this understanding of "stress" may be thus be given as "a strain of circumstances". Many neurologic diseases, both chronic and acute, are believed to involve certain types of stress, e.g. hypoxic/ischemic, oxiadative/peroxidative, mitochondrial and endoplasmatic reticulum stress, etc. It is less emphasized that brief exposure to stressful stimuli precondition cells and organs to variety of different stressors and produce tolerace to otherwise lethal stress conditions, providing robust protection of cells. Acute responses to stress, taking place within second-to-minute time intervals, involve changes in blood flow and (in)activation (through (de)phosphorylation) of enzymes or receptors already present in cells ("preformed"). Chronic responses involve modifications of gene expression, synthesis and/or degradation of proteins, and take distincly more time. Responses to hypoxia or ischemia are of particular importance for the brain, which

critically depends upon continuous delivery of oxygen to maintain normal function and survival. Apparently they occur against a background of global depression of cellular protein synthesis, executed in order to save energy. It had been assumed that hypoxia-respnsive genes are controlled by hypoxia-inducible factor-1alpha (HIF-1a), which is quickly activated by hypoxia. More recently it has been found that in the brain up-regulation of transcriptional signaling by HIF-1a is supplemented by activation of patways involving insulin growth factor, vitamin D3 receptor/retinoid X nuclear receptor and glucocorticoid signaling. Hypoxia-responsive genes are predominantly up-regulated in hindbrain and down-regulated in forebrain – possibly to support hindbrain survival functions at the expense of forebrain cognitive functions. Of possible practical importance is response to chemical stressors, which involves induction of heat-shock proteins that counteract proteotoxic stress. Considering that tolerance evoked by a given stressor is effective also to other stress types, means of inducing tolerance in order to provide neuroprotection are being sought. For example, inducers of Hsp70 proterin expression may be effective in chronic neurodegenerative diseases which involve proteotoxic stress, such as ALS.

|A11|

Effects and mechanisms of prenatal stress on neuro-development: applications to autism

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Prenatal stress has been shown to have a profound and lasting impact on neurodevelopment and leads to an increased risk for several neuropsychiatric conditions, including autism spectrum disorder (ASD). It is crucial to understand how genetics may interact with this environmental trigger to identify those most susceptible to the increased risk and to create a better understanding of the underlying mechanisms and potential treatments of such disorders. A 44 base-pair deletion polymorphism located in the promoter region (5-HTTLPR) of the serotonin transporter gene (SLC6A4) results in decreased gene expression and has been associated with several anxiety related disorders. In an animal model, pregnant dams heterozygous for the serotonin transporter gene (Slc6a4 +/–) exposed to stress produced offspring that exhibited several autistic-like behaviors. It has been suggested that the GABAergic system plays a key role in the underlying pathophysiological process of ASD. Previous research has shown that prenatal stress and manipulation of the serotonergic system affects the proper development of the GABAergic system individually. However, the combined effects are unknown. In the present study, wild-type female mice and females heterozygous for the serotonin transporter gene (Slc6a4 +/–) were bred with wild-type males. Upon detection of a mating plug, mice were either placed in a prenatal stress or control condition. In the stress condition, animals were exposed to restraint stress beginning on embryonic day 12 and continued every day until embryo tissue collection or parturition. GABAergic interneuron development was then analyzed using immunofluorescence. Results suggest that manipulations of the maternal serotoninergic system further exacerbate the deleterious effects of stress on the developing brain. Embryonic tissue in this group exhibited delayed interneuron migration and abnormal cortical layering into the cortex. These findings begin to reveal how stress exposure and genetics may interact to impact the development of neural circuits believed to be critical for social interaction.

|A12|

Differential susceptibility of microglial cells and cholinergic neurons to neurodegenerative signals

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Neurodegenerative lesions in cholinergic encephalopathies include an excessive activation of microglia, which may aggravate this process. Microglial inflamma-

tory response to neurotoxic signals may contribute to neuronal degeneration through excessive production of nitric oxide (NO) and a vast range of pro-inflammatory cytokines. Little is known about the sensitivity of microglia to neurotoxins in combination with changes in their energy metabolism. The aim of this work was to investigate wheather and how lipopolysaccharide (LPS), and its key mediator NO, may differentially affect energy and acetyl-CoA metabolism of microglial and cholinergic neuronal cells. We demonstrated the differences between enzymes of acetyl-CoA metabolism, acetyl-CoA and ATP levels and toll-like receptor 4 contents in non-activated N9 microglial and cholinergic SN56 neuroblastoma cells. Exposition of microglial N9 cells to LPS caused concentration-dependent several-fold increases of nitrogen oxide synthesis, accompanied by inhibition of pyruvate dehydrogenase complex (PDHC), aconitase and α-ketoglutarate dehydrogenase complex (KDHC) activities, and by depletion of acetyl-CoA, but by small losses in ATP content and cell viability. On the other hands, SN56 cells were insensitivity to LPS, which was probably caused by lower than in N9, expression of TLR4. However, exogenous NO caused inhibition of PDHC and aconitase activities, depletion of acetyl-CoA and loss of SN56 cells viability. Microglial cells appeared to be more resistant than neuronal cells to acetyl-CoA and ATP depletion evoked by these neurodegenerative condition. This date indicate the existence of substantial differences in TLR4 levels and activities of key enzymes of acetyl-CoA synthesis and utilization, as well as in energy metabolism in cholinergic neuronal and microglial cells. This factors contribute to differential distribution of acetyl-CoA/ATP in microglial and cholinergic neuronal compartments of the brain, resulting in their smaller and greater susceptibility to neurodegenerative conditions, respectively. One of the reasons for greater resistance of microglial cells to cytotoxic inputs could be their lower energy demand. The preferential susceptibility of cholinergic neurons to neurodegenerative insults may results from competition for acetyl-CoA between mitochondrial energy-producing and cytoplasmic acetylocholine synthesizing pathways.

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|A13|

Noradrenergic signaling in Parkinson's disease

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Parkinson's disease (PD), the second most common neurodegenerative disorder, is characterized by subsequent loss of the dopaminergic neurons of substantia nigra (SN) and ventral tegmental area (VTA, directly responsible for the observed symptomatology (bradykinesia, rigidity, and tremor). Most of the PD cases (90%) have a sporadic occurrence, and even for those in which the genetic factors have been determined, the distinct molecular pathways leading to the final, inevitable cell death remain unclear. As a consequence, currently available pharmacotherapies are based on disease symptomatology, and, although they do alleviate the typical symptoms, they do not restore neuronal function nor prevent neuronal loss.

However, PD is well known to be associated with factors beyond dopaminergic transmission. The involvement of extranigral structures in PD also includes the noradrenergic system as well. Noradrenaline is one of the most important neurotransmitters in the brain, and the projections of noradrenergic neurons originating in the locus ceruleus (LC) penetrate nearly all brain structures. It was shown, that degeneration of noradrenergic neurons in the LC is observed in PD patients to an even greater extent and exacerbates the loss of dopaminergic neurons of SN/VTA. Experimental data from toxin-treated PD animal models seems to confirm the important involvement of the noradrenergic system in PD brain damage. These data prompt the hypothesis that noradrenergic neurodegeneration may be regarded as an early pre-symptomatic phase of PD that progresses in neuropathological stages and finally reaches the threshold responsible for symptomatology directly associated with a profound loss of SN/VTA dopaminergic cells Noradrenaline was also proposed to serve as a compensatory mechanism in PD dopaminergic neurodegeneration.

Elucidating these mechanisms may further our understanding of the preclinical deficits observed in neurodegenerative diseases and provide insight into the pathogenic mechanisms underlying the initial, symptomless phase of their onset. These could lead to opportunities for more successful, neuroprotective and neurorestorative anti-PD therapies, not only those restricted to the compounds that simply delay the onset of degeneration.

|A14|

Therapeutic relevance of local hypothermia after spinal cord compression: a characterization of neurofilaments in white matter pathology

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The resurgence of interest in the use of hypothermia in spinal cord injury (SCI) is due to evidence that moderate hypothermia may improve electrophysiologic, histologic, and neurological outcomes. We studied the effect of local hypothermia (initiated half hour after spinal cord compression) on changes of neurofilaments (NF) and neurological outcome. Adult Gottingen-Minnesota-Liběchov minipigs underwent SCI (18N force) by computer-controlled compression apparatus at L3 level causing paraplegia of lower extremities. Hypothermia was performed locally through perfusion chamber with 4°C saline solution perfusion, oxygenated culture medium (DMEM/F12) or DMEM/F12 in combination with growth factors, energy metabolism factors and compounds with antioxidant effects. The animals were behaviorally assessed during 9 weeks of survival (neurological scale for minipigs; points range 0-20). We show that local hypothermia with 4°C saline solution (perfusion lasting first 5 hours after SCI) significantly protected against locomotor deficits and reduced area of tissue damage in minipigs 9 weeks after injury. Staining with SMI-312 antibody have shown significant decrease in number of NF/mm2 in sensory and motor tracts after SCI. The results clearly correlate with the development of paraplegia. Systematic quantification of SMI-312 positive axons in L2-L4 spinal cord sections after local hypothermia have shown the increase in number of structural components of myelinated axons in motor areas 1 cm rostrally (23% in lateral and 13% in ventral white matter) and 3 cm caudally (17% in both white matter columns) from the epicenter of injury, when the results were compared to SCI group. The results show that the regeneration of NF in spinal cord after trauma and subsequent treatment clearly indicate an important role of local hypothermia in initiation of functional improvement. Hypothermia with 4°C saline, but not with DMEM/F12 or DMEM/F12 in combination with growth factors, energy metabolism factor and compounds with antioxidant effects, if combined with early physical therapy could be promising treatment for improvement of the neurological behavior of animals after SCI. Hypothermia initiated after traumatic insult in preclinical model of minipigs may improve both, behavioral and histological outcome.

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|A15|

Synaptic plasticity impairments in a BTBR $T^{+}Iptr3^{tf}/J$ mouse model of idiopathic autism

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Autism spectrum disorders (ASD) are the most commonly diagnosed neurodevelopmental disorder affecting more than 1 % of children. Despite many years of research it is still diagnosed entirely based on behavior as its molecular basis is not clear. Only 5-10% of cases seem to have genetic background with many of the associated genes involved in forming and maintenance of synapses. Mice lacking these genes often do not represent a full ASD phenotype, therefore in the current study we took an opposite approach. We studied the extracellular matrix components crucial for proper neuronal development and neuronal plasticity, in the BTBR T+ tf/J mouse, an idiopathic model of autism (BTBR). BTBR mice have been extensively studied and show all of the behavioral symptoms of ASD: social interaction and communication impairments as well as strong insistence on sameness combined with repetitive behaviors. We found that unlike the prosocial c57BL6/J (B6) mice, BTBR mice have very little heparan sulfate content in the neurogenic niches and that they display increased matrix metalloproteinase-9 activity in the central nucleus of amygdala, Both of these findings have implication for the function of the synapse and neuronal connections in the development of autism-like phenotype of these mice.

|A16|

Sphingolipid signalling in experimental model of Parkinson's disease

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The last study highlight the importance of sphingolipids in the pathomechanism of neurodegenerative disorders. The alterations of sphingolipids biostat between ceramide and sphingosine-1-phosphate (S1P) might play a crucial role in neuronal cell death in Alzheimer's disease (AD). The significant lowering of S1P level and down-regulation of sphingosine kinases (Sphk1/2) has been recently reported in AD brain. S1P is very potent messenger that regulate cell proliferation, differentiation, migration and apoptosis. This bioactive lipid mediator, which is mainly synthetized by Sphk1 is easily transported outside the cell and act in autocrine or paracrine manner through five specific G-protein-coupled receptors, termed S1P1-S1P5. Moreover, S1P is also important intracellular messenger, which regulate gene expression, mitochondria function and Ca2+ ions concentration. However, the significance of S1P/Sphk1 in Parkinson's diseases (PD) is not fully elucidated. Our in vitro study, using dopaminergic SH-SY5Y cell PD model, induced by 1-methyl-4-phenylpyridinium (MPP+), indicated for the first time the inhibition of Sphk1 expression and activity. Our data showed also higher expression of S1P lyase, which irreversibly leads to S1P degradation and significantly reduces this bioactive sphingolipid concentration. Additionally, we have found that alterations of S1P signalling in PD cell model leads to activation of caspase-dependent apoptotic neuronal death. In current study using mice in vivo PD model evoked by i.p. injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) we have confirmed the alterations of bioactive sphingolipids observed in in vitro PD model. The lower Sphk1 activity and its expression were observed in mice midbrain after MPTP administration. Concomitantly, the inhibition of pro-survival kinase Akt and up-regulation of pro-apoptotic Bax protein were indicated. Moreover, the significant changes in motor activity in rota-rod test were found. The modulator of S1P receptors Fingolimod (FTY720) which exerted neuroprotective effect in *in vitro* study, in this in vivo model has also ameliorating effect. The agonist of dopaminergic receptors D2/D3 pramipexole (PPX) protects midbrain against Sphk1 activity/expression disturbances, apoptosis and motor activity alterations. Our data indicated impaired sphingolipid rheostat in PD model and suggest sphingolipid signalling as a promising target in PD therapy.

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|A17|

Dangerous Liaisons: tau interaction with muscarinic receptors

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The microtubule-binding protein tau has numerous binding partners, including signaling molecules, cytoskeletal elements and lipids, suggesting that it is a multifunctional protein. Indeed, tau can bind to and affect cytoskeletal components and regulate signaling pathways by acting as a protein scaffold for signaling complexes; tau binding also activates or inhibits several enzymes. Tau may also exert toxic effect acting as a agonist of cholinergic muscarinic receptors. There are different alternatives to explain tau pathology spreading in tauopathies like AD, a disease that long time ago was associated with severe loss of cholinergic markers in the brain, and that such loss may be due to the toxic interaction of tau with muscarinic receptors. By using specific antagonists of muscarinic receptors it was found that extracellular tau binds to M1 and M3 receptors and that it may explain the increase of intracellular calcium found in neuronal cells upon tau-binding. It is suggested that increase of calcium mediated by the interaction of tau with muscarinic receptors could result in cell death. M1 and M3 receptors are coupled with Gq/G11 proteins leading to activation of phospholipase C and an increase in the level of intracellular calcium. This calcium increase could activate some protein kinases, and these kinases could modify tau protein rendering the protein toxic. M1 receptors are involved in all key pathological changes found in AD – parenchymal and cerebrovascular amyloid deposition, neurofibrillary tangles, neuroinflammation, and cognitive decline studying 3xTgAD mice with

the deletion of M1 receptor gene. Notably, tau overphosphorylation and potentiation of amyloidogenic processing in the mice with AD lacking M1 were attributed to changes in the GSK-3β and protein kinase C activities. Corroborating these findings, genetic deletion of M1 receptor has recently increased Aβ pathological features in APPSwe/Ind mice. Finally, deleting the M1 receptor increased the astrocytic and microglial response associated with Aβ plaques. These data highlight the significant effect of M1 receptors disruption in exacerbating AD-related cognitive decline and pathological features and provide critical preclinical evidence to justify further development and evaluation of selective M1 agonists for treating AD. However so far, significance of tau signaling through muscarinic receptor in solely in vivo tauopathic models remains uncertain.

|A18|

PKCβII in mitochondria as a potential neuroprotective factor. Proteomics strategy to elucidate protein-protein interactions involved in PKCβII signaling

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Mitochondria are principal mediators of cell death that occurs during cerebral ischemia. Recent reports indicate participation of the PKC in regulation of mitochondrial metabolism what can determine the fate of the cell following ischemic stress. We demonstrated that PKCβII, due to ischemia/reperfusion injury (I/R), translocates to mitochondrial fraction, mainly in ischemia-resistant part of hippocampus. We hypothesize that this translocation is responsible for endogenous neuroprotection. Therefore, we aim: to prove the possible relationship of PKCβII translocation with endogenous neuroprotection and to understand the mechanism of this process. To reveal the role of PKCβ we used widely acknowledged models of brain ischemia: *in vitro* – rat organotypic hippocampal slice culture exposed to excitotoxic injury and *in vivo* – 5' transient brain ischemia in gerbils. We observed ischemia-induced increase of PKCβII isozyme immunoreactivity in mitochondrial fraction in ischemia-resistant part of hippocampus. Moreover, we determined that PKCβII enzymatic activity is preserved after its translocation to mitochondria. The PKCβII-isozyme selective inhibitor administration showed: inhibition of postischemic translocation of PKCβII to particulate fraction and an increase of neuronal death after I/R in an in vitro and in vivo models. It may bespeak neuroprotective role for PKCβII. Pull-down chromatography and mass spectrometry (MS) revealed potential mitochondrial PKC βII-interactors which are involved in regulation of mitochondrial metabolism. We assume that a biological effects of the PKCβII translocation are associated with the phosphorylation of mitochondrial proteins by it. Therefore, mitochondrial phosphoproteomic analysis using titanium affinity chromatography and MS is under investigation. Until now, two proteins identified as potential PKCβII-partners, have been confirmed by co-immunoprecipitation method. Moreover, in silico analysis (http://networkin.info) confirmed that due to amino acid structure, this two proteins can be potentially phosphorylated by PKCβ. Taken together, we suggest that at least these two proteins might be regulated by PKCβII phosphorylation and might be involved in PKCβII-mediated neuroprotection. The exact mechanisms by which PKCβII phosphorylation leads to neuroprotection still needs to be elucidated.

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|A19|

Insights for botanical polyphenols to target signaling pathways and regulate microglial activation

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Many neurodegenerative diseases and brain injuries are marked by increased oxidative stress that results in neuron cell death and glial cell activation. In recent years, intense interest has been directed to unveiling the role of microglial cells, which are the major innate immune cells

in brain. Microglial cells not only are capable of performing host defense mechanisms, but also exhibit multiple functions for regulating cellular redox homeostasis. Microglial activation has been implicated in a number of neurodegenerative diseases, in particular, in Alzheimer's disease, Parkinson disease, stroke and traumatic brain injury. Our studies have demonstrated microglial activation in cerebral ischemia in gerbil and mouse brain. Many botanical polyphenols including grape polyphenols, curcumin as well as elderberry and Sutherlandia can suppress microglia activation and mitigate expression of oxidative and inflammatory marker proteins in these cells. Studies using the BV2 microglial cells also demonstrated a number of botanical polyphenols including EGCG from green tea, honokiol from Magnolia bark and quercetin from berries to inhibit lipopolysaccharides (LPS)-induced production of nitric oxide (NO) and reactive oxygen species (ROS), and alter signaling pathways involving NADPH oxidase, NF-κB and MAPKs. In addition, there is evidence that some polyphenols, such as quercetin, not only are capable of inhibiting inflammatory responses through the NF-κB pathway, but also can stimulate the antioxidant pathway involving Nrf2 and upregulation of antioxidant response element (ARE) and synthesis of heme oxygenase-1 (HO-1), a potent antioxidant enzyme. Therefore, understanding the mechanisms for these polyphenols to modulate the oxidative and antioxidative pathways in microglial cells may be important for providing therapeutic potential for prevention or treatment of neuroinflammatory disorders.

|A20|

Neuroprotective and immunomodulatory properties of oligodendrocyte progenitors as a tool for neuroregenerative strategies

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Oligodendrocyte progenitor cells (OPCs) are known to undergo a multi-stage maturation to gain a myelinating potential. Their role however is supposed to go beyond the process of myelinating nerve fibers in the central nervous system. Pre-clinical studies based on oligodendrocyte progenitor cell (OPCs) therapies revealed significant behavioral improvement in spite of failure in the remyelination process in animal models of demyelinating diseases. Basing on the reported observations, a trophic support provided by OPCs has been hypothesized as an explanation of the beneficial effects resulting from OPCs transplantation. To address the issue, series of co-culture experiments with neonatal rat OPCs and organotypic hippocampal slices were designed. For this purpose, rat organotypic hippocampal slices were exposed to a brief oxygen and glucose deprivation (OGD) which allowed mimicking an ischemic injury ex vivo. Soon after the OGD procedure, the hippocampal slices were co-cultured together with differentiating oligodendroglial progenitors. The molecular analysis revealed that the mRNA levels for several cytokines, transcription factors and neurotrophins is up-regulated in OPCs in the response to the vicinity of the injured nervous tissue. During the following biochemical analysis and functional assays, some of the factors were proved to be secreted extracellularly and to exert either neuroprotective or immunomodulatory effect. In response to the OPC-derived IL-10, the number of microglial cells increased within the OGD-subjected slices, while the BDNF and SCF were shown to up-regulate the proliferation of the neuroblasts and their subsequent differentiation into the cells with the neuronal phenotype. The results obtained in the study prove that OPCs indeed are potent to secrete soluble factors and exert the neuroprotective effect, additionally promoting cell survival and proliferation in the damaged nervous tissue. In conclusion, the OPCs might support the preservation/restoration of a tissue structure. The immunomodulation of locally ongoing inflammatory process might also contribute to the initiation of neuroregenerative mechanisms which could be the other beneficent effect of the therapies based on OPC transplantation.

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|A21|

Cholinergic hypothesis of dementia

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Cholinergic neurons require continuous provision of acetyl-CoA and choline to their synaptoplasmic com-

partment for synthesis of acetylcholine (ACh) by choline acetyltransferase (ChAT) in order to maintain their neurotransmitter functions. Glucose- derived pyruvate, through pyruvate dehydrogenase complex (PDHC) reaction in mitochondria, is almost exclusive source of brain acetyl-CoA. The latter is used mainly for energy production in mitochondria of neurons and other types of brain cells. However, only in cholinergic neurons certain fraction of acetyl-CoA is used for ACh synthesis. Therefore, they appear to be particularly vulnerable to several neurodegenerative conditions (e.g. Alzheimer's disease, thiamine deficits) presumably due to competition for acetyl-CoA between energy and ACh synthesizing pathways. On the other hand, second substrate - choline has to supplied to the site of ACh synthesis from extracellular space exclusively by specific high affinity choline transporter. During neurotoxin-evoked excessive depolarization, when transporter is inhibited, choline for ACh synthesis may be provided by hydrolysis of structural phospholipids in neuronal membranes by activated phospholipases, thereby affecting cholinergic neurons viability. Studies on Tg2576 mice, as well as cell lines revealed that, several neurodegenerative inhibited PDHC and other enzymes linked with energy production. It caused loss of viability and transmitter functions in cholinergic neurons, being less or non toxic to non-cholinergic neurons or glial cells. Viability of mature cholinergic neuronal cells displayed direct significant correlations with activities of PDHC and mitochondrial acetyl-CoA levels under various cytotoxic and cytoprotective conditions. No such dependencies were observed for non cholinergic cells. In the same conditions, choline acetyltransferase activity and ACh content/release displayed direct correlations with acetyl-CoA levels in cytoplasmic/synaptoplasmic compartment of cholinergic neurons. Also choline depletion or supplementation in whole animal or cellular models of dementia, decreased or increased neuronal viability, their ACh metabolism, respectively. Thus, combinations of these two autocanibalism-like mechanisms, involving acetyl-CoA and choline shortages present in degenerating brain, may contribute to preferential functional and structural impairments of cholinergic neurons in these conditions.

|A22|

α-Synuclein and its role in synaptic function

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The cytosolic protein α-synuclein (ASN) is enriched at the pre-synaptic terminals of almost all types of neurons in the central nervous system. In physiological conditions this protein is involved in the synapses formation, neurotransmitter release and re-uptake, vesicle recycling and regulation of the synaptic cytoskeleton assembly. A growing body of evidence has underlined ASN missfolding and oligomerisation as important events that contribute to synaptic abnormalities (synaptopathies) occurring in various neurodegenerative diseases. Our previous studies demonstrated that this protein in monomeric/oligomeric form induces Ca^{2+} influx and NMDA receptor-dependent activation of neuronal nitric oxide (NO) synthase. Subsequently, it was shown that ASN enhanced Aβ toxicity leading to acceleration of oxidative stress, mitochondria failure and caspase-dependent apoptotic cell death. Our recent findings showed, that ASN-evoked oxidative/nitrosative stress leads to S-nitrosylation of multifunctional E3 ubiquitin ligase-parkin, resulting in the inhibition of this enzyme's function. Moreover, using ATRA-differentiated SH-SY5Y cells, we observed that ASN alters ATP-dependent signalling. Exogenously added ASN induced release of ATP from nerve endings, leading to activation of synaptic purinergic P2X7 receptor, intracellular Ca^{2+} influx and cell death. Interestingly, in SH-SY5Y cells stably transfected with ASN we observed that agonist of purinergic receptor, extracellular ATP significantly increased intracellular ASN level. Further investigation revealed that ASN accumulation is a result of alteration of its secretion and probably lysosomal as well as proteasomal dysfunction. The alteration of ASN level may subsequently affect the function of synaptic vesicles and mitochondria and may be implicated in synaptosis. Taken together, these findings provide new insight into our knowledge of the relationship between purinergic signalling and ASN in synaptic failure and may be helpful in identifying new therapeutic targets for neurodegenerative disorders.

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POSTERS

|B1|

Monitoring of human mesenchymal stem cells overexpressing vla-4 after their intracarotid administration in a rat model of deep brain structure damage

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Introduction: Mesenchymal stem cells (MSC)-based therapy is one of the most promising approaches in the treatment of wide range of diseases. However up to now clinical trials with MSC transplanted in neurodegenerative diseases provided inconclusive results. One of the reason may be insufficint migration of donor cells after systemic infusion and lack of their homing in the brain. Thus the modification of adhesive proteins on the surface of transplanted cells might be beneficial. VLA-4 is one of the most important proteins involved in leukocyte diapedesis and migration to inflamed area, therefore our research is focused on use it to enhance homing of MSCs.

Material and methods: In our study we used mRNA-ITGA4 transfected human bone marrow-derived MSC (hBM-MSC) (Lonza) overexpressing A4 subunit of VLA-4 intergin. The modified and naïve cells were labelled with SPIO (MIRB, BioPAL) and delivered into right internal carotid artery in rats withdeep brain structure damage. The infusion of hBM-MSC was monitored with 7T Biospec 70/30 MR scanner (Bruker) directly after transplantation and 24h later. The analysis of signal intensity was performed in ImageJ program then rat brains were collected and analysed by immunohistochemistry using antibodies against CD44 (specific for human MSC), von Willebrand factor and Claudin-5 antigens (recognized endothelial cells).

Results: Both naïve and A4 overexpressing MSC reached brain lesion area after intra-arterial delivery. Directly after transplantation and 24 hours later hBM-MSC were present in the right hemisphere of the recipients but significant decrease in signal level originating from transplanted cells between these two time points was noticed. However, the decline of the signal in rats transplanted with modified hBM-MSC was much smaller as compared to naïve cell recipients. The presence of transplanted hBM-MSC in the rat brain was confirmed by using of CD44 antibody. Staining with von Willebrand factor and Claudin-5 antibodies revealed that both types of cells (modified and naïve) remained inside brain blood vessels 24 hours after transplantation.

Conclusions: Our results showed that arterially transplanted hBM-MSC effectively reach the brain lesion area. The overexpression of VLA-4 increases homing of MSCs to the brain, however no diapedesis of transplanted cells was present. The role of peripheral sensory pathways and NKA1 and NKA2 receptors in cardiorespiratory effects induced by neurokinin A.

|B2|

Treatment of neural stem cells (HUCB-NSC) with methylmercure chloride results with cytotoxic and genotoxic effect

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Several studies have demonstrated that exposure to toxic levels of MeHgCl during pre- and post-natal life causes neurological abnormalities, cognitive impairment, and behavioral disturbance. In this study we present that the mechanism of cell damage induced by MeHgCl involves generation of free radicals, which trigger a double and a single DNA strand breakage and is related with cytotoxic and genotoxic effect. We have tested exposition of MeHgCl on HUCB-NSC in different culture conditions: monolayer 2D culture at various conditions resembling different developmental stages (serum free culture, low serum medium and low serum with dBcAMP) and 3D culture on collagen scaffolds, all in 21% or 5% of oxygen tension. Treatment of HUCB-NSC on MeHgCl showed a decrease in cell viability (Alamar blue test), in both oxygen conditions (21% of oxygen or 5% of oxygen) and both dimension of culture (monolayer vs culture on collagen scaffolds). The most sensitive to MeHgCl were cells cultured under 5% oxygen tension in medium without serum, where cells exhibit ear-

ly neural phenotype. Examination of HUCB-NSC exposition on MeHgCl showed numerous dose-dependent chromosomal abnormalities. Methylmercury chloride increases level of chromosome break or lost what produces micronuclei (MN) and chromosomal rearrangement – nucleoplasmic bridge (NPBs) and gen amplification – nuclear bud formation (Nbud). This effect suggest that methymercury chloride is mutagenic agent, causes the DNA damage and in consequence produces chromosome changes in Human Umbilical Cord Blood Neural Stem Cells.

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|B3|

The role of peripheral sensory pathways and NKA1 and NKA2 receptors in cardiorespiratory effects induced by neurokinin A

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Neurokinin A (NKA) is a peptide neurotransmitter that participates in the regulation of breathing and cardiovascular system. It is established that NKA released from the vagal excitatory NANC fibers innervating respiratory tract acts via NK2 receptors, causing contractions of the smooth muscle of the tracheobronchial tree. Neurokinin A is also present in the ganglion nodosum of the vagus nerve and the petrosal ganglion of the glossopharyngeal nerve which both project to the nucleus of the solitary tract in the medulla oblongata. However its impact on the cardiorespiratory pattern is not clear. The purpose of our study was to determine the cardiorespiratory pattern exerted by the systemic injection of neurokinin A, to look at contribution of neurokinin NK1 and NK2 receptors, and to show the engagement of the vagal pathway in mediation of these responses. The effects of intravenous injections of NKA (50 µg/kg) were studied in anaesthetized, spontaneously breathing rats in the following experimental schemes: in neurally intact rats, and vagotomized at either midcervical or supranodosal level. Intravenous injections of NKA in the intact rats evoked sudden and short-lived increase in the respiratory rate, followed by a prolonged depression coupled with slow continuous augmentation of the tidal volume. Respiratory alterations were accompanied by short-lived tachycardia and a prolonged hypotension. Midcervical vagotomy eliminated only respiratory rate response, while supranodosal vagi section abrogated all respiratory reactions. NK2 receptor antagonist eliminated the respiratory frequency and tidal volume changes not affecting the fall in arterial blood pressure. NKA1 receptor blockade revealed an avert effect significantly reducing hypotension with no influence on the respiratory system. These results indicate that NKA induced changes in the breathing result from an excitation of the NKA2 receptors of the vagal endings and also from modulation of NKA2 receptors within the nodose ganglia. Fall in blood pressure triggered by NKA occurs outside of the vagus nerve and is probably mediated via a direct action of the neurokinin A on vascular smooth muscles supplied with NK1 receptors.

|B4|

Differential effect of KU55933, an inhibitor of ATM kinase, against hydrogen peroxide-, doxorubicin- and staurosporine-induced cell damage in neuronal-like and astrocyte-like cell lines

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ATM (ataxia-telangiectasia mutated) kinase belongs to family of phosphatidylinositol 3-kinase-related kinases (PIKKs) and its dysfunction is a cause of rare neurodegenerative disorder- ataxia-telangiectasia. It is involved in processes like DNA repair system, cellular response to oxidative stress, insulin signaling and mitochondrial homeostasis. Taking these properties into account, ATM started to be the subject of research in diabetes, cancer and neurodegenerative diseases. Our previous data demonstrated that pharmacological inhibition of ATM kinase by specific inhibitor – KU55933, protects the retinoic acid differentiated human neuroblastoma SH-SY5Y cells (RA-SH-SY5Y) against the cell damage induced by hydrogen peroxide (H_2O_2) and doxorubicin (Dox), but not by staurosporine (St). In order to study the cell specificity of the above results in the present study we tested the effect of ATM kinase inhibitor in mouse hippocampal (HT-22) and rat glioma C6 cell lines. The data showed that KU55933

(1-10 μM) was not toxic to HT-22 cells when given alone, but induced cell damage in C6 cells at concentration of 10 μM. Moreover, it partially attenuated the cell death induced by (H_2O_2) (by 11-15%) and doxorubicin (by 20-26%) in HT-22 cells, whereas in glioma cells it mediated only a slight protection in Dox-model (1 μM with 8% protection). However, in the model of oxidative stress-evoked cell damage in C6 cells it was not only without protective effects but at concentration of 10 μM it enhanced the H_2O_2 toxicity (by 24%). In contrast to results obtained from RA-SH-SY5Y cells, in St-model of cell damage we found a slight protection mediated by KU55933 in both, HT-22 (10 μM with 10% protection) and glioma (0.1-1 μM with 7-10% protection) cells. Taking together results from neuronal-like (RA-SH-SY5Y and HT-22 cells) and glioma cells, a differential protective effect of ATM kinase inhibitor in dependence on the used model of cell damage has been demonstrated. The most significant protective effects of KU55933 were observed in neuronal-like cells (RA-SH-SY5Y and HT-22) in the model of H_2O_2- and Dox-induced cell damage.

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|B5|

The interplay between amyloid β42 peptide, sphingosine kinase-1 and mitochondrial sirtuins in cell survival and death: implication in Alzheimer's disease

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Amyloid beta (Aβ) is a key player in pathomechanism of Alzheimer's disease (AD). The recent studies demonstrated that soluble oligomers are the most toxic form of Aβ. The oligomers are responsible for synaptic dysfunction, memory loss and neurodegeneration, but the mechanisms of their toxicity largely remain unsolved. The Aβ precursor protein and the enzymes involved in Aβ release are situated in lipid rafts rich in sphingolipids. Recent data underline the role of sphingolipid rheostat alterations in AD pathology. In this study we analyse the interaction between $A\beta_{1-42}$ and sphingosine kinases (SphKs) – the key enzymes in sphingolipid biostat. Moreover, we investigated the role of mitochondrial sirtuins and other antioxidative enzymes in cell survival under Aβ toxicity.

PC12 cells were incubated with $A\beta_{1-42}$ oligomers (AβO) and SphK inhibitor SKI II for 24-96 h. AβO increased SphK1 expression and activity after 24 h, but down-regulated them after 96 h and had no effect on Sphk2. AβO and SKI II accelerated oxidative stress and affected the pro- and anti-apoptotic signalling leading to cell death. At the same time, up-regulation of anti-oxidative enzymes catalase and superoxide dismutase 2 was observed. AβO significantly increased the level of mitochondrial proteins: apoptosis-inducing factor (AIF) and Sirt3, -4, -5. To decipher the molecular pathways involved in AβO toxicity, several pharmacologically active compounds were tested. It was shown that at very early stages of AβO toxicity, p53 protein plays a major role. However, during prolonged exposure, alterations of caspases, MEK/ERK, mitochondrial permeability transition pores, oxidative stress, S1P signalling pathway and down-regulation of Sirts were responsible for cell death.

Our data demonstrated the molecular relationship between Aβ peptide, Sphk in cell survival and death, and indicated that inhibitor of p53 and activators of S1P receptors and Sirts may be useful in cell protection against Aβ toxicity.

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|B6|

The oxidative stress in myelin of the rat brain exposed to silver nanoparticles

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Nanotechnology has become a major research project in the last decade and scientists world-wide are continuing to discover unique properties and applications of nanomaterials. Among the metal nanoparticles, nanosilver (AgNPs) has the highest degree of commercialization because of

its antimicrobial properties. However, the unique properties of AgNPs could potentially lead to unexpected hazards to both the environment and human health. In the present study we investigate the pro-oxidative potential of AgNPs in chronically exposed rats. Markers of oxidative stress such as lipid peroxidation, the level of sulfhydryl groups (-SH), and the mRNA expression of superoxide dismutase (SOD) were examined in myelin of the CNS. Small (10 nm) citrate-stabilized silver nanoparticles were administered once daily via the gastric tube at a dose of 0.2 mg/kg b.w. per day for 14 days. Control groups received silver citrate or saline in the same dose. Our results indicated that exposure to AgNPs decreased level of sulfhydryl groups. We noticed statistically significant reduction of both protein- and non-protein –SH groups level by about 35% and 20%, respectively. Similar effect was also observed after administration of ionic form of silver. Exposure to AgNPs increased the rate of lipid peroxidation expressed by the level of malondialdehyde (MDA) – final products of polyunsaturated fatty acids peroxidation in the cell memranes. The level of MDA was significantly elevated (by about 40%) in myelin of AgNPs-treated rats. Exposure to AgNPs also changed mRNA expression of SOD – an antioxidative enzyme. Obtained results indicate that in AgNPs-exposed rats antioxidative mechanisms are inefficient to counter reactive oxygen species (ROS)-mediated cellular stress.

|B7|

8-Oxoguanine DNA glycosylase 1 and TP53 genetic variants and apolipoprotein E (APOE) genotype and oxidative stress in the patients with Alzheimer's disease

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Alzheimer's disease (AD) leads to generation of β-amyloid (Aβ) and oxidative stress. Oxidative stress and apolipoprotein E (ApoE) are associated with DNA damage which leads to apoptosis induction e.g. in cells expressing wild-type p53 protein encoded by TP53 gene. 8-Oxoguanine DNA glycosylase 1 (OGG1) is a main DNA repair enzyme that removes of 8-oxo-2'-deoxyguanosine (8-oxo2dG) from DNA. It is known that OGG1 and TP53 genetic variants, and APOE genotypes are involved in dementia diseases pathogenesis.

The purpose of this study was to analyze OGG1 and TP53 genetic variants, and APOE genotypes in peripheral lymphocytes and the extent of oxidative DNA damage (8-oxo2dG) in AD patients and controls.

The studies were conducted on 60 patients with AD, aging 47-90 years. The control group consisted of 200 individuals, aging 25-86 years, including 80 related (RC) and 120 unrelated (UC) persons with AD patients. The OGG1 and TP53 genetic variants and APOE genotyping analysis was performed using PCR and DNA sequencing, and Real-Time PCR method. The plasma levels of 8-oxo2dG were determined using ELISA technique.

Our studies revealed the presence only in AD patients of two different mutations in TP53 gene: a silence C708T mutation (21%) [$p < 0.05$] and a missense C748A mutation (4%) as well as first described in OGG1 gene a silence C798T mutation (6%). Additionally, significant differences in the frequency of the APOE ε4 in patients with AD and UC ($p < 0.001$), and RC ($p < 0.01$), respectively, as well as RC and UC ($p < 0.05$) were observed. AD patients with silent or missense mutation in the TP53 and OGG1 gene have protective (ε2) and/or neutral (ε3) and/or pathogenic (ε4) allel of APOE gene. The plasma level of 8-oxo2dG was almost 2 times lower in AD patients with APOE ε4/ε4 as compared to AD subjects with APOE ε3/ε3 genotype.

It seems that APOE ε4 as well as APOE ε2, ε3 associated with OGG1 and TP53 genetic variants may be involved in the AD pathology.

|B8|

New bifunctional peptides with both, opioid agonist and tachykinin antagonist components as new analgesics

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Morphine and its derivatives are the most widely used narcotic analgesics for relieving severe acute pain. Unfortunately, the use of opioids in treating is limited due to significant side effects e.g. analgesic tolerance, addiction, respiratory depression. Since many years Neuropeptide Department search for new bifunctional compounds, acting on two (or more) different receptors, which will have fewer side effects than compounds acting at a single target. Substance P plays an important role in pain signals generation and transmission from periphery to CNS. In contrast, opioids suppress pain signals mainly through suppression of substance P release. These mechanisms of neurophysiological actions were fundamentals for invention of hybrid compounds that act as both antagonists of substance P to reduce postsynaptic activation of NK1 receptors as well as opioid agonists to activate presynaptic opioid receptors that result in decrease substance P release. We will present bifunctional compounds acting on opioid and tachykinin system. In all presented compounds opioid pharmacophore is covalently hybridized with tachykinin pharmacophore that positively modulate effects of the opioid part. Synergistic enhancement of opioid analgesia and/or decrease of unwanted side-effects should result from such hybridization. Therefore, to the list of already synthesized and characterized compounds presented in the literature we elaborated new series of compounds that combine peptide opioid pharmacophore (biphalin) with 3,5 bis-trifluoromethyl-benzyl derivative, responsible for antagonism at NK1 receptor. Opioid and tachykinin pharmacophores are connected by few kinds of linkers which are derivative of hydrazine. We will present pharmacological and analgesic properties of new bifunctional peptides, which exhibit affinity to opioid (mu and delta) and NK1 receptors. For the most active compound the hypothetical model of interaction of bifunctional compound and the NK receptor will be shown.

|B9|

Nanofiber mat dressing-mediated glutamate delivery to the spinal cord damages blood-brain barrier

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Excessive interstitial glutamate levels cause injury and death of CNS cells, and are involved in many CNS disorders. Excitotoxic neuronal injury may correlate with blood-brain barrier (BBB) damage. The aim of this study was to investigate the effects of glutamate released into the cerebrospinal fluid from electrospun nanofiber mat dressing of the spinal cord. Adult male Wistar rat were subjected to subarachnoid application of an 'empty' (carrying no active principle) nanofiber mat or a glutamate-loaded nanofiber mat at the lumbar enlargement level. Half of the latter group was additionally given a systemic treatment with the histone deacetylase inhibitor valproic acid. A group of age-matched intact rats served as an additional control. All rats were killed 21 days after dressing application, and the L1-L6 parts of their spinal cords were collected and processed for electron microscopy, histological and immunohistochemical (IHC) studies by routine procedures. Spinal cords from the intact rats and rats carrying 'empty' nanofiber mat dressing showed proper parenchyma and BBB morphology. Spinal cords from the rats carrying glutamate-loaded spinal cord dressing revealed the presence of multiple intraparenchymal hemorrhages of varying sizes. The capillaries in the vicinity of the dressing (both in the

meninges and the white matter) were leaky and showed considerable swelling; there was no difference in these characteristics between rats with and without systemic valproic acid treatment. IHC studies revealed no difference in the staining intensity or the labeling pattern for basal lamina markers (laminin, collagen IV and fibronectin) between the study groups. Endothelial nitric oxide synthase immunolabeling showed an increased staining intensity in rats carrying glutamate-loaded dressing, irrespective of the valproic acid treatment. Electron microscopy revealed signs of excitotoxic neuronal injury and perivascular edema in both rat subgroups with glutamate-loaded mat dressing. Endothelial cells in these animals showed considerable mitochondrial swelling, increased numbers of plasmalemmal pinocytic vesicles and cytoplasmic vacuoles, and the formation of membrane-bound intraluminal protrusions and blebs; the integrity of tight junctions was generally preserved. No similar changes were seen in controls. These results indicate that glutamate-loaded nanofiber mat dressing can create glutamate concentrations capable of damaging spinal cord BBB.

|B10|

The phenotypic characterization and therapeutic application of human mesenchymal stem cells derived from adipose tissue

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The application of mesenchymal stem cells (MSCs) in regenerative medicine offers hope for effective treatment of yet incurable diseases. The therapeutic effect of stem cells has been confirmed in numerous independent studies, but their widespread clinical use requires the development of unified protocols of safe and efficient cells acquisition. The adipose tissue is one of the most promising sources of MSC for regenerative medicine.

The aim of this study was to isolate MSC from human subcutaneous adipose tissue (ASC) in real clinical surrounding, and setting the optimal conditions for their further propagation or direct transplantation into the wound.

The ASC isolated by the enzymatic method established previously in our laboratory were characterized by flow cytometry and immunocytochemistry. Long term cell culture was carried out in two variants: 5% or 21% of oxygen concentration, for which population doubling time (PDT), the cumulative time of population doublings (CPDs) and the rates of cell senescent were estimated. The cells ability to differentiate into the adipocytes, chondrocytes and osteocytes were evaluated together with their potential to vasculogenesis. In order to develop methods for topical transplantation of ASC, the cells were co-cultured with human fibroblasts or plated directly on the top of skin patchs.

The cell survival, integration and differentiation were evaluated in vitro after direct application of ASC on the top of co-cultured fibroblast surfaces by the use of irrigator.

The results demonstrated that modified enzymatic method of ASC isolation is reproducible and efficient. The cells possess all features typical for MSCs and have capacity to vasculogenesis in vitro. The low oxygen condition determines faster proliferation and slower cells senescence. The ASC delivered by use of irrigator survived well and were able to stimulate the growth of fibroblasts. Moreover, ASC can be administered on the wound surfaces directly by use of irrigator or special skin patches.

We can conclude that ASC obtained and applied according to the described protocol can be easily used for cell therapy of non-healing skin wounds in clinic.

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|B11|

Energy metabolism in neurons exposed to hypoxia-inducing chemicals

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Excess of Zn ions under hypoxic episodes may lead to neurodegeneration by disruption the neuronal cells energy metabolism.

Aim of the project was to evaluate the effect of Zn excess on energy metabolism in neuronal cells during hypoxia.

Experimental model was human neuroblastoma SH-SY5Y cell line, modified to cholinergic phenotype by using RA and cAMP (DC) or unmodified (NC). To induce hypoxia Co was used, added 3 h prior to Zn. Cells viability was measured by lactic dehydrogenase assay. Also activities of enzymes: pyruvate dehydrogenase complex (PDHC), aconitase, NADP-isocitrate dehydrogenase (IDH) were measured. Following the total level of acetyl-CoA was determined.

Exposition of NC to 0.125 mM Zn or 0.20 mM Co ions used apart didn't increase cells mortality, however when cells were 3 h preincubated with 0.20 mM Co mortality increased to 17.5% in response to 0.125 mM Zn. After exposition to 0.125 mM Zn activities of selected enzymes IDH and PDHC were decreased: by 18% and by 30% respectively while activity of aconitase remained unchanged. Exposition to 0.20 mM Co caused decrease of enzymes activities: – aconitase by 17%, PDHC by 38%. There was no inhibition of IDH. However when cells were preincubated with 0.20 mM Co prior to 0.125 mM Zn, the inhibition of the enzyme's activity was aggravated to 69% for aconitase and 30% for IDH. Activity of PDHC in these conditions was inhibited by 22%. The level of acetyl-CoA in the cells was changed neither after the Zn or Co alone nor after the preincubative conditions.

DC exposed to 0.125 mM Zn or 0.20 mM Co didn't exert changes in their mortality. After preincubation of the cells with Co mortality increased to 30% under 0.125 mM Zn. Activities of IDH, PDH were inhibited by 40%, 17% under 0.125 mM Zn and 34%, 44% after 0.20 mM Co. Activity of aconitase was not inhibited by 0.125 mM Zn while 0.20 mM Co inhibited its activity by 23%. Zn added 3 h after Co significantly increased the inhibitory effect on selected enzymes. The acetyl-CoA level was only diminished after 0.125 mM Zn under hypoxic conditions.

These results indicate that highly differentiated cholinergic cells are more prone to Zn-overload in hypoxic conditions, expressed by aggravation of inhibition of their energy metabolism.

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|B12|

The effect of Perinatal Exposure to lead (Pb) on trafficking and scaffolding proteins dysfunction in rat brain

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Lead (Pb) is a highly reactive heavy metal found in air, soil, drinking water and food widely recognized as a potent toxin of central nervous system. The most critical effects of Pb toxicity occur among children exposed during fetal and/or postnatal development leading to impairment learning and memory, cognition and behavioural control. However, the precise mechanism by which Pb exerts its neurotoxic effect and induces deficit in learning and memory processes are not fully elucidated. The objective of the present study was to evaluate the effect of perinatal low level Pb exposure on the expression of pre- and postsynaptic proteins as well as Tau protein changes in the young brain. Experiments were carried out on Wistar rats. Pregnant females were divided into two groups: control and Pb-treated animals. The control group was maintained on distilled water until weaning of the offspring while females from the experimental group received 0.1% lead acetate (PbAc) in drinking water *ad libitum*, starting from the first day of gestation. Offspring stayed with their mothers and were fed by them. During the feeding of pups, mothers from the experimental group were still receiving PbAc in drinking water *ad libitum*. Pups were weaned at postnatal day 21. 28-day old pups were sacrificed and expression of presynaptic syntaxin-1 and VAMP1/2 and postsynaptic PSD95, as well as the BDNF concentration and ultrastructural alterations were analysed in three brain areas: forebrain cortex (FC), cerebellum (C) and hippocampus (H). Concomitantly, we examined the effect of perinatal exposure to Pb on Tau pathology in these regions. Our data revealed that pre- and neonatal exposure of rats to Pb (concentration in rat offspring's blood below a 'safe level') evoked significant decrease in the expression of key synaptic proteins: syntaxin-1 in (H) and (C), VAMP1/2 in (C) and PSD95 in (C) and (FC) without changes in other brain

parts. Moreover, we observed the lower concentration of BDNF in all analysed structures in comparison to control. Molecular alterations were accompanied by pathological changes in ultrastructure of all examined brain structures from rats subjected to Pb. Parallel with synapses dysfunction, our results showed that Pb treatment caused significant increase of Tau expression and its phosphorylation at Ser396 in (FC) and (C). In these structures, we observed activation of two major Tau kinases: GSK-3b and CDK5, through increase in phosphorylation of Tyr-216 and formation of p25 protein, respectively. Concluding, perinatal exposure to lead induces disturbances of synaptic endings structure and functions that is implicated in neurodegenerative disorders including Alzheimer's and Parkinson's disease as well as in autism. We suggest that neurotoxic effect of Pb might be mediated, at least in part, by GSK-3β and CDK5-dependent Tau hyperphosphorylation, cytoskeleton disruption and synapses dysfunction.

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|B13|

Changes of sFas, its ligand – sFasL and Bcl-2 in cerebrospinal fluid and serum of multiple sclerosis patients, during relapse and after glucocorticoid therapy

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The mechanisms underlying cell death in MS (multiple sclerosis) are not fully understood. Apoptosis is believed to be one mechanism contributing to a marked and prolonged neuronal cell loss following MS. Recent data suggest a role for soluble Fas (sFas) (sAPO-1, sCD95) and soluble FasL (sFasL), a type I transmembrane receptor glycoprotein of nerve growth factor/tumor necrosis factor (TNF/NGF) superfamily, and its ligand sFasL in the central nervous system in MS before and after glucocorticoid therapy. In addition, we evaluate the potential therapeutic effects of the glucocorticoids. A truncated form of the Fas receptor, soluble Fas (sFas) may indicate activation of the Fas/FasL system and act as a negative feedback mechanism, thereby inhibiting Fas mediated apoptosis. We measured the expression rate of Fas in cerebrospinal fluid (CSF) and serum collected from 37 patients. The patients were evaluated at three different timepoints: at relapse (active stage), after 5 days of glucocorticoid therapy and in the remitting period – not shorter than 30 days of neurological status stability (inactive stage). No sFas was detected in CSF samples from persons without neurological pathologies – control group, consisted of 10 volunteers. CSF samples of MS patients at relapse showed elevated Fas concentrations (mean 458.26 ± SD 157.42 pg/ml). Serum samples showed slightly elevated sFas at relapse, five days after glucocorticoid therapy and in the remitting period. Serum levels of sFas were always much higher than CSF levels. However, there was no correlation between concentrations measured in CSF and in serum of appropriate groups of patient at relapse, suggesting that the concentrations in the two compartments are independently regulated. Concomitantly we estimated sFasL in the serum and CSF. We noted increase of sFasL levels in relapse and in the remitting period compared to controls. We observed significantly higher immunoreaction of Bcl-2 in the active stage of MS than in the inactive stage after glucocorticoid therapy or in controls.

|B14|

Adrenergic control of membrane potential in medial prefrontal cortex (mPFC) pyramidal neurons

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Aims: Impairment of the signal transduction from adrenergic receptors to cellular effectors in prefrontal cortex (PFC) neurons occurs in many neuropsychiatric disorders (acute stress disorder, ADHD). Application of clonidine (alpha2-adrenergic receptor agonist) evokes hyperpolarisation of the resting membrane potential in mPFC pyramidal neurons. The aim of the study was to define the cellular effectors and the exact signal transduction pathway from the receptor to the effector, which still remains unclear.

Methods: The membrane potential was recorded in layer V mPFC pyramidal neurons in slices isolated from 3-week-old rats. Recordings were performed in perforated-patch configuration at a temperature of 34°C. Adre-

nergic antagonists, inhibitors of cellular effectors and intracellular signalling were applied to the bath medium before, during and after clonidine application (100 μM). Their effects on clonidine-dependent membrane potential changes were analysed.

Results: The alpha2-receptor antagonists yohimbine (60 μM, *n* = 15) and atipamezole (20 μM, *n* = 6) did not completely block clonidine-dependent hyperpolarisation. The effect of clonidine was attenuated by the blocker of hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels (ZD7288, 50 μM, *n* = 10) and by the selective Na^+/K^+-ATPase inhibitor (ouabain, 100 μM, *n* = 7). The hyperpolarisation was affected neither by the adenylyl cyclase inhibitor (SQ22536, 100 μM, *n* = 6), protein kinase A inhibitor (H-89, 40 μM, *n* = 6), phospholipase C inhibitor (U73122, 10 μM, *n* = 7) nor the protein kinase C inhibitor (chelerythrine, 5 μM, *n* = 5), but it was attenuated by the G-protein beta-gamma-subunit inhibitor (gallein, 20 μM, *n* = 12).

Conclusions: alpha2-adrenergic receptor activation evokes hyperpolarisation due to HCN channel inhibition and modification of the Na+/K+-ATPase function. The transduction pathway occurs in a membrane-delimited fashion and involves the G beta-gamma subunit released from the G-protein.

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|B15|

Human and bovine nuclear matrix protein complexes bind to the first intron of the tyrosine hydroxylase gene

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Tyrosine hydroxylase (TH) [EC 1.4.16.2] is a rate-limiting enzyme required for catecholamine synthesis. The TH gene and its product are under strict, multilevel regulation to control tissue-specific expression and enzymatic activity in neurons. Our previous work showed that the TH gene interacts with protein components of the nuclear structure called the nuclear matrix (NM). We showed that the proximal region (+725 to +1678) of the bovine TH gene bound to bovine NM proteins (NMPs) isolated from both TH-expressing (TH+) and non-expressing (TH–) tissues. In contrast, the -2300 to +2300 region of the human TH gene bound to bovine NMPs isolated from TH+ tissue but not from TH- tissue. We hypothesize that these interactions affect the spatial organization of the TH gene chromatin and thereby control its transcription. To determine whether NMP-mediated epigenetic regulation of the TH gene is species-specific, here we aimed to: (i) map the minimal sequence of the bovine TH gene responsible for interactions with bovine NMPs and (ii) identify regions of the human TH first intron that interact with human NMPs.

To accomplish our objectives, we isolated NMPs from the bovine adrenal medulla and the human SH-Sy5y cell line, which are both TH+, and the bovine liver and human HepG2 cell line, which are both TH-. Radiolabeled probes encompassing parts of the TH first intron (+113/+613, +589/+1085, +807/+1016, +1122/+1602, and +1449/+1973) were incubated with NMPs isolated from TH+ or TH- cells or tissues in the presence of non-specific competitor and increasing excess of unlabeled probe. EMSA, in which complexes were separated on 3 or 5% non-denaturing polyacrylamide gels, was used to assess binding.

We found that a minimal 210 nt intronic fragment (+807/+1016) of the bovine TH gene interacted with bovine NMPs isolated from both the adrenal medulla and the liver. Interactions between regions of the human TH first intron and human NMPs were more complex. The proximal fragment (+113/+613) interacted with NMPs isolated from both SH-Sy5y and HepG2 cells, whereas the +1122/+1602 region did not interact with NMPs from either cell line. The +589/+1085 region only interacted with NMPs from SH-Sy5y cells, and the distal region (+1449/+1973) only interacted with NMPs from HepG2 cells. Together with our previous results, these data indicate that NMPs isolated from HepG2 cells contain species-specific proteins that are able to bind the human TH first intron.

|B16|

Neuroprotective effects of necrostatin-1 against oxidative stress- and pro-apoptotic factors-induced cell damage in human neuroblastoma SH-SY5Y cells

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Recent data suggest that necroptosis, a novel form of non-apoptotic programmed cell death, could be implicated in many pathological conditions including neuronal death in experimental models of stroke and brain trauma. Moreover, an inhibition of this process by necrostatin-1 (Nec-1) has been shown to be neuroprotective in in vitro and in vivo models of cerebral ischemia. However, the involvement of this type of cell death in other neurodegenerative conditions and its interplay with other cell death types (apoptosis, autophagy) is less recognized. Thus in the present study we tested the effect of necrostatin 1 (Nec-1), an inhibitor of necroptosis, in the models of oxidative stress (H_2O_2)- and pro-apoptotic factors-induced human neuroblastoma SH-SY5Y cell damage. The data showed that Nec-1 (0.1-20 μM) partially attenuated the cell death induced by H2O2, staurosporine and doxorubicin as confirmed by MTT reduction assay. The range of protection mediated by Nec-1 was similar to the effect of caspase-3 inhibitor, Ac-DEVD-CHO in all tested models of cell injury. Moreover, the neuroprotective effect of Nec-1 was not connected with reduction of the toxin-induced caspase-3 activity. The concomitant treatment of cells with necroptosis and apoptosis inhibitors evoked similar protection in all tested models as did each agent given alone, suggesting a shared common downstream intracellular cell death mechanisms for both processes. Our data showed an involvement of necroptosis in oxidative stress- and pro-apoptotic factors-induced cell damage of SH-SY5Y cells and that inhibition of this pathway could be neuroprotective.

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|B17|

The stimulation of hippocampal neurogenesis by a histone deacetylase inhibitor – Trichostatin A, after neonatal hypoxia-ischemia

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Neonatal encephalopathy is the most important cause of severe neurological disability in children. There are currently no effective therapies to reduce brain damage and its long-term sequel in infants. Mounting evidence indicate that treatment of adult animals with histone deacetylase inhibitors (HDACis) administered after stroke provides neuroprotection and also promotes neurogenesis. Based on the above data it can be hypothesised that HDACis provide a suitable option for the treatment of neonatal HI injury. This prompted us to check whether the administration of Trichostatin A, one of the HDACi, stimulates neurogenesis after neonatal hypoxic-ischemia.

We utilized a model of hypoxia-ischemia induced in rats of postnatal day 7. TSA (0.2 mg/kg) was injected subcutaneously in one single dose for 5 consecutive days starting immediately after the insult. The proliferation profile was estimated by BrdU injection at specific time points after HI. BrdU positive cells were determined by immunohistochemistry method.

At 3-14 days after hypoxia-ischemia BrdU-positive cells were seen in both hemispheres, with the greatest number of dividing cells in ischemic side. The strongest proliferation level occurs within 3 and 6 days after the injury. The labelling pattern revealed structure-dependent differences. Three days after HI the highest density of BrdU-positive cells was seen in the hilus, whereas at longer survival time (9-14 days) labelled cells changed their localization towards the subgranular zone (SGZ) of DG.

To determine the phenotype of newborn cells, BrdU was administered twice daily, at 5 to 7 days after HI and the animals were sacrificed 14 days after the HI insult. To analyse if BrdU-positive cells represent newly generated neuroblasts and oligodendrocyte progenitors, we used double immunohistochemical staining BrdU/ DCX and NG2/BrdU, respectively. We found that administration of TSA stimulates the generation of neuroblasts and oligodendrocyte progenitors, restoring their reduced number after HI to control level.

Conclusion: Our preliminary results show that Histone deacetylase inhibitor Trichostatine A exerts beneficial

effects after neonatal HI by stimulating the proliferation of neural progenitors.

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|B18|

Alteration of arginases and nitric oxide synthase in cells transfected with human wild-type beta amyloid precursor protein (βAPP)

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L-arginine is a semi-essential amino acid with a number of bioactive metabolites. Accumulating evidence suggests the implication of altered arginine metabolism in the pathogenesis of Alzheimer's disease (AD). However, the impact of amyloid β (Aβ) peptides on arginine degradation and re-synthesis is unknown.

In the present study we investigated the activity and expression level of arginase I and II as well as neuronal, endothelial and inducible NO synthase isoforms (*NNOS, ENOS, INOS*), enzymes that metabolize arginine or resynthesize it from citrulline and the levels of corresponding amino acids in rat pheochromocytoma (PC12) cells overexpressing human Aβ precursor protein (APPwt cells). Moreover, we investigated the changes in miRNAs responsible for modulation of arginine metabolism in AD brains. Arginase activity was assessed spectrophotometrically, and mRNA levels of arginase I and II and other urea cycle enzymes were measured using real-time PCR. Our results showed that the expression of *ARG1* and *ARG2* genes was significantly lower in APPwt than in control PC12 cells: *ARG1* mRNA was reduced 3-fold whereas *ARG2* was down-regulated by 50%. Moreover, total activity of arginases was significantly reduced by 52% in APPwt cells. Concomitantly, *NNOS* and *ENOS* mRNAs were significantly elevated in APPwt cells while *iNOS* was undetectable in both cell lines. Other urea cycle enzymes were also altered in APPwt cells. The expression of argininosuccinate synthase (*ASS*) that metabolizes citrulline was down-regulated while argininosuccinate lyase (*ASL*) remained unchanged. Ornithine decarboxylase (*ODC*), which decarboxylates ornithine to form putrescine, a precursor of other polyamines was also reduced. Arginine, the substrate for both arginases and NOS, was unchanged in APPwt cells. However, NOS product citrulline was significantly elevated. Using miRNA arrays and cluster analysis we have investigated microRNAs (miRNAs) known to interact with 3'-UTRs of mRNAs linked to arginine metabolism and *NOS* mRNAs. We have found elevated hsa-miRNA-9 and hsa-miRNA-128a in AD patients' brains as compared to the same brain areas of age-matched controls. Their changes might modulate the expression of *ASS* and *NOS*, respectively. Our results suggest the significance of arginases for the promotion of NOS-dependent cellular stress by βAPP/Aβ in a system where up-regulated, potentially pathogenic miRNAs are involved.

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|B19|

Carbon monoxide-releasing molecule (CORM-2) deflates neuropathic pain symptoms and potentiates opioid effectiveness in rat CCI model

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Chronic pain is one of the most urgent clinical problems for of poor understanding of its pathogenesis. It may appear as a consequence of nerve mechanical damage or as a co-symptom of many diseases, and may be divided into inflammatory and neuropathic pain. Due to P2X purinergic receptors, with the special focus on subtypes 4 and 7, are indicated to play a significant role in pain development, we decided to introduce a pharmacological antagonist of P2X4 receptor (P2X4R) and measure its analgesic potency - alone and together with opioid drugs. Experiments were performed to evaluate the contribution of P2X4R in modulation of neuropathic pain, and their ability to amplify the morphine and/or buprenorphine

effectiveness in Wistar rat chronic constriction injury (CCI) neuropathic pain model. Study consisted of behavioural tests (von Frey's and cold plate) and dorsal, lumbar (L4-L6) spinal cord biochemical analysis (Western blot) after CCI to the right sciatic nerve. We have demonstrated that single administration of CO donor: CORM-2, significantly reduced allodynia and hyperalgesia two hours after intraperitoneal injection as efficiently as morphine and buprenorphine. What is more, CORM-2 intensified the analgesic effect of both morphine and buprenorphine comparing to the effect of these drugs alone, completely revoking hypersensitivity. The analgesic potency of CORM-2 administered for 7 consecutive days was greater on day 7th than on day 2nd, suggests no tolerance development, accretion of anti-nociceptive effect over time. We are the first to demonstrate that even single intraperitoneal administration of CORM-2 potentiates the morphine and buprenorphine anti-hyperalgesic and anti-allodynic properties in rat CCI model. Our data complete those published so far in neuropathic pain field and are in agreement that P2X4R may indeed play significant role in neuropathic pain development.

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|B20|

The effect of designer drugs on neurotransmission in the rat brain

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Designer drugs, also known as "legal highs" or "legal drugs", are synthetic compounds developed to provide similar effects to illicit drugs of abuse, which are not subjected to legal control. Legal highs are widely available online and in clubs. These drugs come in various forms. Some of these are herbal mixtures, others come as a white powder or as tablets or capsules. They can be taken orally, smoked, inhaled, added to drinks or injected.

Synthetic drugs are classified roughly, based on their chemical formula, into phenethylamines, tryptamines, and piperazines. Although designer drugs still have the reputation of being safe, several experimental studies in rats but also in humans indicated risks including life threatening serotonin syndrome, hyperthermia, heart disease, neurotoxicity, and abuse potential.

The least investigated and most dangerous synthetic components found in the designer mixtures are phenylalkylamines: para methoxyamphetamine (PMA), para-methoxymethamphetamine (PMMA) and synthetic cathinone: mephedrone. Our study is aimed on examining the effects of the above-mentioned on extracellular levels of dopamine, serotonin and their metabolites using microdialysis in freely moving rats. Dialysates are assayed using HPLC witch coulochemical detection, following i.p. administration of each drug at doses of 5 and 10 mg/kg.

PMA, PMMA and mephedrone given intrastriatally, have strong inhibitory effects on re-uptake of serotonin and to lesser extent dopamine, causing their extracellular increase and decrease in their metabolites.

Our results suggest that designer drugs have strong impact on the central nervous system causing profound alterations in neurotransmission, but the exact mechanism by which they act still needs to be clarified.

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|B21|

Alternative methods for staining and characterization of extracellular vesicles derived from human bone marrow mesenchymal stromal cells

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Introduction: Mesenchymal stromal cells (MSCs) are of great interest in regenerative medicine and clinical strategy because of their ability to migrate to injured sites, function in tissue repair and to modulate immune

response. The predominant mechanisms by which MSCs participate in tissue repair seem to be related to their paracrine activity. Besides the long-time notion of growth factors and cytokines it now appears that MSCs secrete large amounts of extracellular vesicles (EVs). Several studies have suggested that MSC-derived EVs have functions similar to their cellular counterparts. Thus, MSC-EVs represent a promising opportunity to develop novel cell-free therapy approaches.

The aim of the study was to optimize the method of staining for EVs derived from mesenchymal stromal cells in term of their visualization after transplantation.

Material and methods: The experiments were performed on human bone marrow mesenchymal stem cells (hBM-MSCs) (Lonza). hBM-MSCs were labelled with three different dyes: lipophilic stain (PKH26), iron nanoparticles conjugated with rhodamine (SPIO) and quantum dots (Qdots). Then immunocytochemical staining for tetraspanins (characteristic markers for EVs): CD9, CD63, CD81 was performed. EVs were isolated from the culture media of previously labelled hBM-MSCs by centrifugation at 1800 × g for 30 min at 4°C to remove debris, then followed by two times ultracentrifugation at 100 000 × g for 60 min at 4°C in order to isolate EVs. The results were analyzed in confocal microscopy and transmission electron microscopy.

Results: Our results depicted the presence of intracellular structures positively stained with PKH26, SPIO or Qdots visible inside hBM-MSCs. These structures co-express CD9, CD63 and CD81 markers specific for EVs. The isolated EVs represent heterogeneous population differed with size and content as confirmed by electron microscopy together with their positive staining with SPIO and Qdots. Conclusions. All three different dyes proved to be efficient stains for EVs although iron nanoparticles conjugated with rhodamine and quantum dots have wider application for EM and MRI and can be useful to monitor EVs in vivo after their transplantation.

|B22|

Genetic variants of SLC6A4, HCRTR1, KCNK18 genes in migraine patients

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Introduction: Migraine is one of the most common neurological disorder that affects 11% of adults. There are two main clinical subtypes of this disease: migraine with aura (MA) and without aura (MO). Migraine has a significant genetic basis. It is believed that polymorphisms in SLC6A4, HCRTR1, KCNK18 genes may be involved in its pathomechanism. No genetic studies of migraine have been performed on polish population so far.

Aims: The aim of the study was to analyze exon 7 of HCRTR1 gene, exons 1 and 2 of KCNK18 gene, 5-HTTLPR length polymorphism and the plasma level of serotonin.

Methods: The studies included 34 migraine patients (MA: 17, MO: 17) and 34 healthy controls. The mean age of participants was 40 years. Genotyping was performed using PCR, HRM, RFLP and sequencing while 5-HT levels were determined using HPLC/EC technique.

Results: The studies confirmed presence of two polymorphisms in HCRTR1 gene: G29A and G1222A. Genotype AA of HCRTR1 G1222A was observed only in migraine patients ($p<0.01$), both MA and MO. HCRTR1 G29A GA may be involved in MA or MO. Presence of another two variants: A28G and T328C was confirmed in KCNK18 gene. Long allele of 5-HTTLPR polymorphism occurred more frequently in migraine ($p < 0.05$) as compared to the controls. There have been no statistical evidence for association of analyzed polymorphisms with the plasma concentration of serotonin.

Conclusions: The AA genotype of G1222A HCRTR1 seems to be a risk factor for migraine in Polish population. The presence of long allele of 5-HTTLPR polymorphism is associated with a tendency for increased concentrations of plasma serotonin.

|B23|

Regulation of the membrane potential in the medial prefrontal cortex (mPFC) pyramidal neurons by muscarinic receptors

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Damage to the cholinergic input to the prefrontal cortex has been implicated in neuropsychiatric disorders. Cholinergic endings release acetylcholine, which activates nicotinic and/or G-protein-coupled muscarinic receptors. Muscarinic receptors activate transduction system(s), which control cellular effectors which regulate the membrane potential in medial prefrontal cortex (mPFC) neurons. The mechanisms responsible for the cholinergic-dependent depolarization of mPFC layer V pyramidal neurons in slices obtained from young rats were elucidated in this study. Glutaminergic and GABAergic transmissions, tetrodotoxin (TTX)-sensitive Na^+ and voltage-dependent Ca^{2+} currents were eliminated. Cholinergic receptor stimulation by carbamoylcholine chloride (CCh; 100 μM) evoked depolarization (10.0 ± 1.3 mV), which was blocked by the M1/M4 (pirenzepine dihydrochloride, 2 μM) and M1 (VU 0255035, 5 μM) muscarinic receptor antagonists and was not affected by a nicotinic receptor antagonist (mecamylamine hydrochloride, 10 μM). CCh-dependent depolarization was temporarily diminished by current step (100 msec, 500 pA) and was attenuated by an inhibitor of the βγ-subunit-dependent transduction system (gallein) applied extra- (20 μM) or intracellularly (50 μM). It was also inhibited by intracellular application of the βγ-subunit binding peptide (GRK2i, 10 μM). mPFC pyramidal neurons express Nav1.9 channels. CCh-dependent depolarization was abolished in the presence of antibodies against Nav1.9 channels in the intracellular solution and augmented by ProTx-I toxin (100 nM) in the extracellular solution. CCh-induced depolarization was not affected by the following reagents: intracellular transduction system blockers, including U 73122 (10 μM), chelerythrine chloride (5 μM), SQ 22536 (100 μM) or H-89 (2 μM); channel blockers, including Ba^{2+} ions (200 μM), apamin (100 nM), flufenamic acid (200 μM), 2-APB (200 μM), SKF 96365 (50 μM), and ZD 7288 (50 μM); and a Na^+/Ca^{2+} exchanger blocker, benzamil (20 μM). We conclude that muscarinic M1 receptor-dependent depolarization in mPFC pyramidal neurons is evoked by activating Nav1.9 channels and that the signal transduction involves G protein βγ subunits.

|B24|

Influence of isolation and cultivation methods on stemness properties of human Wharton's Jelly-derived MSC population

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Human umbilical cord Wharton's Jelly Mesenchymal Stem Cells (WJ-MSC) are of a great research and clinical interest since their first isolation in 1991.

The main purpose of this paper was to elaborate and optimize enzymatic method of cell isolation in comparison to the classical method of mechanical isolation used routinely in our laboratory. Enzymatic method of MSC isolation relays here on the limited proteolysis of the tissue by collagenase I using different durations of the digestion.

Mesenchymal character of the cells isolated by both methods was verified by mesodermal lineage differentiation ability into fat, bone and cartilage. We tested also specific markers expression with flow cytometry, immunocytochemistry and quantitative RT-PCR analysis. For comparison the proliferation rate, long-term culture (population doubling time), one-week curve and expression of proliferating cells marker Ki67 analysis was performed. In addition we analyzed ability to create CFU-F by both types WJ-MSC and calculated percentage of senescence cells at late passages.

The results allow to conclude that, though comparable expression of the classical mesenchymal markers, the cells isolated by quantitatively more efficient enzymatic method proliferate much slower in culture and differentiate scarcely into typical for MSC mesodermal lineages in comparison with mechanically isolated MSC.

Moreover, in WJ-MSC(M) a significantly higher expression of early neuroectodermal marker nestin and primitive cell marker α-SMA has been observed, suggesting their pluripotent differentiation ability allowing the cells to cross mesodermal lineage barriers.

In contrast, absence of the above properties in WJ-MSC(E), together with the lack of their ability to CFU-F

formation (a golden mark of the stem cells) and faster cell senescence with limitation of typical mesodermal differentiation lineages may question the stemness characteristic of the cells isolated by the described enzymatic method.

Key words: stem cells, Wharton's jelly, WJ-MSC, mechanical method, enzymatic method, differentiation, proliferation, CFU-F

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|B25|

Alkaloids from *Huperzia selago* protect dopaminergic PC12 cells against sodium nitroprusside-evoked oxidative stress, mitochondrial dysfunction and apoptosis

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Mitochondrial dysfunction and enhanced cellular oxidative stress have long been recognized to play a major role in the pathogenesis of several neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Energy depletion, reactive oxygen species (ROS) generation and increased oxidative damage to several macromolecules may result in loss of calcium homeostasis and in consequence neurodegeneration occurs. Under this perspective, compounds which acting as radical scavengers and protected against oxidative stress and mitochondrial damage might be considered as a potential cytoprotective agents. Our previous data indicated that alkaloids isolated from *Huperzia selago* exhibited antioxidant activity and effectively protected against lipid and protein oxidation in rat brain tissue homogenate. Therefore, the aim of the present study was to investigate the antioxidative and cytoprotective capabilities of alkaloid fractions from *Huperzia selago* to protect dopaminergic PC12 cell against oxidative stress evoked by sodium nitroprusside (SNP). These cells were exposed to 0.5 mM SNP and the effect of alkaloid-rich extract (ARE) was evaluated. The experiments were carried out using spectrophoto- and spectrofluorometrical analysis, fluorescent microscopy and real-time PCR. Our data indicated that 24 h exposure of PC12 cells to SNP leads to significant, about 4-fold increase in free radicals and nitric oxide (NO) generation. Treatment with ARE (2.5 µg/ml) prevented SNP-induced NO liberation. Moreover, alkaloid fractions significantly protected against DNA fragmentation and mitochondrial damage evoked by SNP treatment. We observed that ARE notably attenuated SNP-induced changes in mitochondrial membrane potential and decreased the elevated mitochondrial calcium levels. Furthermore, we have analysed the effect of ARE on SNP-induced modification of Parkin, which is responsible for removal of damaged mitochondria via its poly-ubiquitylation and promotion of mitophagy. SNP-evoked apoptotic processes lead to decrease in PC12 cells viability and ARE protected cells against SNP-induced cytotoxicity. In conclusion, these results suggest that the alkaloid extract from *Huperzia selago* show good effects against SNP-induced oxidative injury in PC12 cells by adjusting oxidative stress, and suppression of mitochondrial dysfunction, and could be developed as a potential candidate as a neuroprotective compound.

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|B26|

Minocycline enhance botulinum neurotoxin a analgesia under neuropathic pain via glia modulation

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Recent studies show a promising analgesic effect using botulinum neurotoxins (BoNTs) for chronic pain state. The present study was undertaken to determine the influence of minocycline on botulinum neurotoxin serotype A (BONT/A) analgesia under neuropathic pain. The objec-

tive of this studies was also to assess whether botulinum neurotoxin serotype A (BONT/A) affects the markers of microglial and astroglial activation in CCI-exposed rats.

The experiments were carried out according to IASP recommendations and local Bioethics Committee The chronic constriction injury (CCI) to the sciatic nerve was performed on male Wistar rats (Bennett and Xie, 1988) and all experiments were conducted 7 days after CCI. The effects of BoNT/A administered intraplantarly into the injured hindpaw were assessed by von Frey test. Biochemical studies comprised the RT-PCR and Western blot analysis in the spinal cords and DRGs.

A single intraplantar (i.pl.) injection of BONT/A (75pg) caused strong antiallodynic effect in CCI-exposed rats which was enhanced after chronic intraperitoneal minocycline (30 mg/kg) administration. BONT/A decreased the injury-induced ipsilateral upregulation of C1q and GFAP mRNA expression in the spinal cord and dorsal root ganglia (DRG) and diminished elevated IBA-1 protein level in the spinal cord in CCI-exposed rats. The ipsilateral DRG and dorsal spinal cord of CCI-exposed rats showed no changes in GFAP protein levels. Minocycline inhibited C1q mRNA expression at the level of the spinal cord and DRG in CCI-exposed rats. Moreover, minocycline decreased GFAP mRNA expression in the DRG in a neuropathic pain model in rats. Decreased protein level of C1q and IBA-1 after minocycline injection in the spinal cord was significantly upregulated after BONT/A. The changing the activity of glial processes may affect BoNT/A-induced analgesia and biochemical markers. The results of this research indicate the ability to the development of new pharmacotherapeutic approaches of neuropathic pain. Acknowledgments: This research was supported by statutory funds from the Department of Pain Pharmacology and by a grant Harmonia 5 2013/10/M/NZ4/00261, both from the Ministry of Science and Higher Education. The scientific collaboration between PAN and CNR (Italy) is greatly acknowledged. Rojewska have a scholarship from the START sponsored by the Foundation of Polish Science and Zychowska from the KNOW sponsored by Ministry of Science and Higher Education, Poland.

|B27|

Ammonia reduces intracellular ADMA concentration in cultured astrocytes and brain endothelial cells (RBE-4) via y+LAT2-mediated efflux

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Toxic effects of ammonia in the brain are partly related to impaired NO production. Asymmetric dimethylarginine (ADMA), is an endogenous NOSs inhibitor and symmetric dimethylarginine (SDMA) is arginine (Arg) transport inhibitor. ADMA is synthesized by protein arginine methyl transferases (PRMTs), released during proteolysis and then degradated mainly by dimethylarginine-dimethylaminohydrolase (DDAH-1) (Teerlink *et al*., 2009). Previously we reported an increase of ADMA and SDMA concentration in brain of rats with acute liver failure (Milewski *et al*., 2014), but distribution of the ADMA/SDMA surplus between the particular intra and extracellular compartments has not been studied.

We hypothesize that ADMA/SDMA transport from astrocytes and brain endothelial cells in vitro, could be mediated by y+LAT2 carrier, belonging to y+L family of cationic amino acids transporters. Of note, y+LAT2 under hyperammonemic conditions is up-regulated both in astrocytes and RBE-4 cells (Skowrońska *et al*., 2012; Zielińska *et al*., 2012) and we speculated that this transporter may influence the concentration of both Arg derivatives.

The aim of this study was to evaluate the intracellular concentration of ADMA/SDMA/Arg in astrocytes and RBE-4 cells and the effect of ammonia treatment (48 h, 5 mM) on this issue.

In RBE-4 cells not treated with ammonia the ADMA concentration was twice higher and the Arg/ADMA ratio was much lower than in astrocytes, confirming well documented role of ADMA in endothelial NOS inhibition (Pope *et al*., 2009). Ammonia treatment led to an almost 50% reduction of ADMA and SDMA intracellular concentration in both cell type (measured by HPLC) with the accompanying ADMA/SDMA accumulation in the cultured medium (determined by mass spectroscopy). This phenomenon occurs without PRMT-1 and DDAH-1 gene expression changes or DDAH-1 protein level alteration. This observation suggests ammonia induced ADMA/SDMA transport changes. Indeed, silencing of the Slc7a6 (y+LAT2) gene

expression diminished the reduction of intracellular ADMA concentration caused by ammonia treatment in astrocytes and decrease NO production.

This results suggest that increased ADMA (and possibly SDMA) efflux mediated by upregulated y+LAT2 may be one of the ways in which ammonia interferes with intracellular ADMA content and, subsequently, NO synthesis.

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|B28|

The effect of preconditioning with hyperbaric oxygen on brain tissue expression of proteasomal and apoptotic proteins after experimental global brain ischemia

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Research hypothesis implies that hyperbaric oxygen preconditioning (HBO-PC) induces proteasome changes accompanied by a decrease in the level of apoptotic proteins, what may implicate participation of proteasome in the preconditioning mechanism.

The aim was to study whether HBO-PC has an impact on brain tissue expression of selected proteasome and apoptotic proteins after global brain ischemia.

The experiments were conducted under general anesthesia on male Wistar rats subjected to 5 min global brain ischemia obtained via bilateral common carotid artery ligation and a reduction of arterial blood pressure. Five sessions of HBO-PC, alone or combined with the inhibitor of proteasome MG132 were performed for 5 days preceding ischemia, 1 hr daily at 2.5 ATA. With immunohistochemistry techniques we investigated 20S proteasome β1 (catalytic) and proapoptotic proteins; p53 upregulated modulator of apoptosis (PUMA) and B cell lymphoma-2 interacting mediator of cell death (BIM) as well as neurofilaments (NF) and glial fibrillary acidic protein (GFAP). Klüver-Barrera stain was used to determine myelin and neuronal morphology.

At 7 days after ischemia partial sparing of the hippocampus CA1 zone neurons was noted with HBO-PC. As for the proteasomal 20S protein, perinuclear cytoplasmic stain was noted in neuronal cells of the hippocampus and cerebral cortex in the control. With HBO-PC, both cerebral cortex and the hippocampus exhibited the enhanced, predominately neuronal, 20S immunoreactivity.

PUMA showed a diffuse neuronal stain in the hippocampus and cerebral cortex in control. While after ischemia there was a strong PUMA reactivity in remaining pyramidal cells and dentate gyrus granule cells. Weak immunoexpression was found with the HBO-PC, especially in the CA1 zone, unless associated with focal cell loss. BIM exhibited cytoplasmic and perinuclear stain, enhanced after ischemia. A weak BIM expression was found after ischemia preceded with HBO-PC.

NF of the cytoskeleton showed postischemic depletion throughout the hippocampal pyramidal layers. Astroglial reaction to ischemia, as determined with GFAP stain, especially in the CA1 was reduced with HBO-PC. The HBO-PC combined with proteasome inhibitor exhibited changes of tissue expression within investigated characteristics similar to the ischemia alone.

These results seem to suggest the participation of proteasome and diverse alterations of apoptotic proteins within the postischemic brain after HBO-PC.

|B29|

Neuroprotective benefits of physical activity in a chronic mouse model of Parkinson's disease

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Parkinson's disease (PD) is neurodegenerative syndrome, where progressive degeneration of the nigro-striatal dopaminergic pathway leads to motor disturbances. The mechanisms underlying the pathological changes in PD are not clearly understood yet. The current pharmacological treatment is used mainly for symptomatic control and provide short-term benefits. Therefore, it is highly relevant to investigate the therapy, which prevents cell death. Accumulating clinical evidences suggest that physical activity and exercise can generally slow down aging, prevent chronic disease and promote health.

The aim of the study was to examine the protective effects of physical exercise and particularly the role of its

timing with respect to neurotoxin treatment, on motor, behavioral, and neurochemical characteristics in chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP)-induced mouse model of Parkinson's disease. Mice were subdivided into four groups: (i) control, (ii) sedentary (non-exercising, with induction of PD), (iii) early trained PD mice (exercising before during and after the induction of PD), and (iv) late trained PD mice (start of exercising after the induction of PD). Motor performance was tested before and after induction of PD on Rotarod. Brains were collected for immunohistochemistry of tyrosine hydroxylase (TH), vesicular monoamine transporter 2 (VMAT-2), brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) in substantia nigra pars compacta (SNpc) and for Western Blot analysis to determine dopamine transporter (DAT) level in the striatum and TH level in the midbrain.

Early training started before the induction of PD protects from massive degeneration of SNpc dopaminergic neurons and not only maintains but even improves motor performance. Late training started after the induction of PD has very limited protective effect. It is possible that the physical activity can prevent the degeneration of dopaminergic neurons in SNpc by the activation of signaling pathways of neurotrophic factors, however their effectiveness may be limited once the damage has been incurred.

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|B30|

Participation of pro- and antinociceptive interleukins in maraviroc analgesia in rat neuropathic pain model

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Neuropathic pain is caused by the nervous system damage. Treatment of neuropathic pain remains a challenge and requires combination therapy, because of not fully understood pathomechanism as well as many components involved in nociceptive transmission. Targeting chemokine signaling pathways is crucial in neuropathy development. The study examined the influence of CCR5 antagonist (maraviroc) on neuropathy development after chronic constriction injury of sciatic nerve in rats, and changes in pro- and antinociceptive interleukins. Research was carried out on Wistar rats implanted with intrathecal (i.t.) catheters. Neuropathic pain was developed using Bennett's model (CCI; chronic constriction injury to the sciatic nerve). Maraviroc (20 µg/5 µl) was dissolved in 12% DMSO and preemptively administered i.t. 16 h and 1 h before CCI and then once daily for 7 days. Two behavioral tests were conducted to measure allodynia (von Frey test) and hyperalgesia (cold plate test). Experiments were carried out according to IASP recommendations and were approved by the local Bioethics Committee. Behavioral studies have shown that chronic i.t. administration of maraviroc attenuated the allodynia and hyperalgesia on days 7 after CCI. Western blot results demonstrated down-regulation of pronociceptive interleukins: IL-1beta, IL-18, parallel to up-regulation of antinociceptive interleukins: IL-1RA, IL-18BP as measured on day 7 after chronic maraviroc administration. In summary, these results suggest that maraviroc reduces neuropathic pain by restoring the balance between pro- and antinociceptive interleukins. Our work provides the first evidence for its effect on interleukins. Maraviroc is especially promising because it is already used in human therapy; our results suggest that CCR5 is a potential novel target for neuropathic pain drug development. Acknowledgements: This study is supported by Institute of Pharmacology PAS statutory funds.

|B31|

The influence of 3D microenvironment and oxygen tension on WJ-MSC and HUCB-NSC fate decisions

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In this study the effect of 3D microenvironment and the oxygen tension on the growth, proliferation and differentiation of stem cells was investigated. The response to changing microenvironment of two different types of cells was compared: freshly isolated mesenchymal stem cells from Wharton jelly (WJ-MSC) and neurally-committed progenitor cells derived from umbilical cord blood (HUCB-NSC). Different biological three-dimensional (3D) scaffolds

were applied: (1) keratin based scaffolds; (2) a new family of scaffold consisting of two components: collagen (Col) and chondroitin sulphate (CS) and (3) electroconductive chitosan scaffold.

The analysis of settlement performed with scanning electron microscope revealed that both WJ-MSC and HUCB-NSC weakly adhere to keratin small rectangular fibers, but remained seated on the long hair-like fibers. The HUCB-NSC poorly adhere chitosan-based scaffolds, while WJ-MSC are present on the scaffold, but remain undifferentiated. We demonstrated that both cell types highly adhered and grow on Col-CS scaffolds. Moreover, Col-SC scaffolds did not affect the proliferation of the cells.

Immunohistochemical analysis revealed that HUCB-NSC growing on the surface of the Col-SC scaffolds had branched phenotype and expressed neuronal markers (β-Tubulin III, MAP2), while the cells that penetrated inside the scaffold remained rounded. Under the same conditions WJ -MSC penetrated inside the scaffold and proliferated (Ki67 positive cells), with no signs of differentiation.

The response to different oxygen concentration was cell type and developmental stage specific. The 5% O_2 level and Col-SC scaffolds prevent HUCB-NSC from further neuronal differentiation (lack of MAP2 expression). Moreover, only HUCB-NSC cultured on Col-SC scaffolds in 21% oxygen level expressed OLIG1 and OLIG2 genes (oligodendroglial differentiation).

On the other hand we have not observed the influence of oxygen level or 3D microenvironment on neural differentiation of WJ-MSC. However, 3D microenvironment promote CD 105 expression in WJ-MSC cultured in both tested oxygen concentrations.

We have demonstrated that geometry, structure and composition of the scaffold have an impact on cells adhesion, proliferation and differentiation, but the cellular response varies with the oxygen concentration and the type of investigated cells.

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|B32|

Possible involvement of kinin B1 receptor in the development of experimental autoimmune encephalomyelitis in rats

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Introduction: Experimental autoimmune encephalomyelitis (EAE) is the most commonly used animal model of multiple sclerosis (MS). According to the previous studies mammalian central nervous system presents all components of the kallikrein-kinin system. The biological activity of kinin is mediated by two types of G protein-bound receptors – B1 and B2. It is believed that activation of B1R leads to the induction of inflammation by the release of pro-inflammatory cytokines and increased vascular permeability.Therefore, there are reasons to investigate the role of B1 receptor in the enhancement of the BBB permeability during development of EAE.

Methods: One group of female Lewis rats was immunized by intradermal injection of 100 µl inoculum containing homogenate of guinea pig spinal cord. The second group was injected i.p. with DALBK (B1R antagonist) after immunization. Control group was not immunized. Rats were monitored daily for clinical signs and loss of weight. Animals were sacrificed in different stages of the disease. Parts of brains were used for Western blotting analysis and measurement of inflammatory cytokines using RayBio Rat Cytokine Antibody Array (RayBiotech, Inc.). Immunohistochemical study on isolated fraction of microvessels was also performed.

Results: We noticed the increased level of B1R protein in rat brain in the symptomatic phase of EAE. Animals treated with DALBK exhibited improvement of neurological symptoms and decreased level of B1R protein in most cases. Using a confocal microscope, we observed lowered immunoreactivity of thight jounctions proteins (ZO-1, Occludin, Claudin 5) in microvessels' fraction obtained from EAE rats which increased after DALBK treatment. We also noticed changes in the level of astroglial markers (GFAP and AQP4) in both experimental groups. Preliminary analysis showed increased protein level of cytokines: IFN-γ, IL-1β, IL-6, TNF-α, VEGF in EAE animals which tends to decrease in DALBK-treated EAE animals in syptomathic phase of the disease.

Conclusion: Administration of kinin B1 receptor antagonist (DALBK) significantly improved the condition of animals by reducing the intensity of neurological symptoms and delaying the onset 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 which lead to the changes in the blood-brain barrier.

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|B33|

Effects of microglial cell polarization on nociception and morphine-analgesia – *in vivo* and *in vitro* studies

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Neuropathic pain is clinically challenging because it is resistant to alleviation by morphine. Glial activation and increased spinal pronociceptive factors strongly influence on neuronal transmission, therefore they are crucial in the development and maintenance of neuropathic pain. Microglial activation is a polarized process divided into potentially neuroprotective phenotype M2 and neurotoxic phenotype M1. Recent studies suggest that the microenvironment of the spinal cord after injury favors M1 polarization with only a transient appearance of M2 microglia/macrophages. We investigated the effect of parthenolide (PTL), an inhibitor of NF-κB, on the chronic constriction injury to the sciatic nerve (CCI)-induced neuropathy in rat. PTL (5 µg; i.t.) was preemptively and then daily administered for 7 days after CCI. The administration of PTL decreased allodynia and hyperalgesia and significantly potentiated morphine effects. We analyzed spinal changes in glial markers and M1 and M2 polarization factors, as well as intracellular signaling pathways. PTL increased the protein level of IBA1 (a microglial/macrophage marker) but did not change GFAP (an astrocyte marker) on day 7 after CCI. PTL reduced the protein level of M1 (IL-1β, IL-18, and iNOS) and enhanced M2 (IL-10, TIMP1) factors. In addition, it downregulated the phosphorylated form of NF-κB, p38MAPK, and ERK1/2 protein level and upregulated STAT3. In primary microglial cell culture we have shown that IL-1β, IL-18, iNOS, IL-6, IL-10, and TIMP1 are of microglial origin. Summing up, PTL attenuates neuropathy symptoms and promotes M2 microglia/macrophages polarization. Our result suggest that microglia-derived pronociceptive factors may influence on nociceptive transmission and morphine analgesia, therefore neuropathic pain therapies should be shifted from neurotoxic phenotype M1 to neuroprotective phenotype M2.

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|B34|

The study of influence of minocycline on Botulinum toxin A on pro- and antinociceptive interleukins under neuropathic pain

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Neuropathic pain are some of the pathological states that have been recently treated with BoNTs with beneficial effects. The main target of BoNT/A action induce SNAP-25 cleavage and prevention of neurotransmitter release. The main target of minocycline is p38MAPK and MMP-9. Both of substance shows antinociceptive effect in neuropathic pain, however their influence on some pro- and antinociceptive cytokines remain to be clarified. The aim of the present study was to demonstrate the effects of intraperitoneal chronic minocycline administration and at day 5 intraplantar (i.pl.; 75 pg/paw) BoNT/A administration on pain behavior and CCI-induced changes on cytokines protein level in the DRG and spinal cord. Chronic constriction injury (CCI) of the sciatic nerve was performed according to Bennett and Xie (1988) were conducted to measure hyperalgesia (cold plate test). The experiments were carried out according to IASP rules. To study a potential role of neuroimmunological factors under neuropathy we studied in the lumbar spinal cords and DRG the IL-6, IL-10, IL-1beta and

IL-1RA protein levels using Western blot method. A single intraplantar BoNT/A injection similar to minocycline chronic administration (30 mg/kg) induced antinociception. The analgesic effect of BoNT/A was potentiated by minocycline. Our results suggest that CCI-induced upregulated the protein level IL-6 and IL-1beta in ipsilateral spinal cord. In DRG we observed increase the protein level of IL-6 and IL-1beta. After BoNT/A injection in the ipsilateral spinal cords of CCI-exposed rats, the protein level of IL-6, IL-10, IL-1beta and IL-1RA were not changes, but in the DRG BoNT/A enhance the protein level of antinociceptive factors (IL-10 and IL-1RA).Interestingly, minocycline diminished the protein levels of pro-nociceptive factor (IL-1beta) in the spinal cord and/or DRG. Our results show that BoNT/A significantly attenuates pain-related behaviors and enhance the level of some antinociceptive factors. Moreover, the minocycline enhance BoNT/A analgesic effects. The modulation of neuroimmune interactions by BoNT/A and minocycline can have some clinical implication in future. Acknowledgments: This work was supported by the National Science Centre, Poland, Harmonia 5 2013/10/M/NZ4/00261. Rojewska have a scholarship from the START sponsored by the Foundation of Polish Science, Poland. Zychowska is scholarship holder from the KNOW sponsored by Ministry of Science and Higher Education, Poland.

|B35|

Neuroprotective action of 3,3'-diindolylmethane on hippocampal cells exposed to ischemia involves inhibition of caspases and p38 stress-activated protein kinase

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Stroke is the fourth-leading cause of death and the primary cause of long-term disability worldwide. Ischemic insult triggers apoptosis regulated by activation of caspase cascade, which depends either upon the interaction of a death receptor with its ligand and subsequent activation of procaspase-8 or on the participation of mitochondria and the activation of procaspase-9. Our previous study demonstrated that stimulation of aryl hydrocarbon receptor (AhR) leads to caspase-3-dependent apoptosis of neuronal cells [1]. Apart from caspases, important regulators of neuronal apoptosis are also MAP kinases, p38/MAPK and JNK. Selective AhR modulators (SAhRMs) are class of compounds that act as AhR agonist or antagonist in a tissue-specific manner. Among the ligands that exhibit properties of SAhRMs is 3,3'-diindolylmethane (DIM). Recent data have shown neuroprotective potential of DIM against brain inflammation and Parkinson's disease [2,3]. However, there are no data concerning the protective capacity and mechanisms of DIM action in neuronal cells exposed to ischemia. The aim of the present study was to investigate the neuroprotective potential of DIM against the ischemia-induced damage with a special focus on caspases and p38 stress-activated protein kinase. In our study ischemic conditions were evoked by exposure of primary hippocampal cell cultures to oxygen and glucose deprivation. DIM and specific inhibitors of caspases-8,-9 and p38/MAPK kinase were added to the medium just prior to ischemia. We have shown that ischemia increased the lactate dehydrogenase release (LDH) and caspase-3 activity, whereas DIM (0.1-10 μM) inhibited these parameters in tissue-dependent manner. Inhibition of p38/MAPK kinase and caspase-8, but not caspase-9, intensified the neuroprotective effect of DIM. These preliminary data indicate strong neuroprotective potential of DIM against ischemia. They also suggest that mechanism of its anti-ischemic action could be mediated not only by targeting caspases but also by inhibition of stress-activated protein kinases.

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|B36|

Vasculoprotective effect of WJ-derived MSC and EPC-like culture on post-ischemic organotypic hippocampal slices

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The aim of study was to evaluate ability of mesenchymal stem cells derived from human Wharton jelly (WJ-MSC) to differentiate into endothelial progenitor cells (WJ-EPC) and then support vascular network and cell survival in rat hippocampal organotypic culture (OHC) model of ischemic injury.

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. The functional behavior of WJ-EPC was tested by DiI-Ac-LDL-uptake and Matrigel assay. Priming of WJ-MSC/EPC was performed by incubation with LPS and/or poly(I:C) to stimulate TL ¾ receptors. After 5 div the hippocampal slices undergo oxygen-glucose deprivation (OGD). Next, WJ-MSC or WJ-EPC were co-cultured with intact or post-ischemic OHC. Supporting effect of WJ-MSC and WJ-EPC supplementation on vascular network was evaluated with rat EC-specific antibody (RECA-1). The cell survival was measured by propidium iodide (IP) staining. Concentration of secreted human MSC-derived cytokines into culture medium was estimated by the BD CBA assays system.

Our results showed that WJ-MSC after 7 days of differentiation acquired typical for EPC cobblestone morphology, were able to form capillary-like structures and to took up DiI-Ac-LDL. Both cell types were positive for MSC and EC markers CD73, CD90, CD105, VEGFR2, VEGF, but only EPC-like cells expressed vWF and CD31 antigens. Moreover, indirect co-culture of WJ-derived MSC or EPC with OGD-OHC inhibited cell death and blood vessel atrophy in hypoxia-sensitive CA1 hippocampal region. These results suggest involvement of paracrine-related mechanism of MSC/EPC vasculoprotection in post-ischemic OHC injury.

Looking for putative mechanism of the MSC/EPC protection, the concentration of several human cytokines like TGF-β1, IL-6 and IL-1β was estimated in OHC co-culture media. Their secretion were found significantly higher under WJ-EPC co-culture except of VEGF growth factor released equally high in both, WJ-MSC/EPC cases. However, release of these cytokines remained stable independently from Toll-like receptor stimulation by LPS or Poli(I:C) in all investigated agonists concentration. Thus, in the next step we will estimate expression level of TLR ¾ genes in the WJ-derived MSC/EPC cultures.

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|B37|

The impact of di-(2-ethylhexyl) phthalate (DEHP) on PPAR-gamma, MMP-2 and MMP-9 expression in mouse astrocytes and cortical neurons *in vitro*

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Di-(2-ethylhexyl) phthalate (DEHP) is the most frequently used plasticizer of polyvinyl chloride (PVC) products such as food packaging, cosmetics, building materials, clothing, toys and medical devices. DEHP is not chemically bound to plastic polymer, therefore it is continuously released into environment through manufacturing, processing and disposal. Epidemiologic data showing the impact of DEHP on the development of psychiatric disorders in children such as Attention Deficit Hyperactivity Disorder (ADHD) and autism have been published. Several phthalates, have been reported to cause their toxicity via peroxisome proliferator-activated receptor gamma (PPAR gamma) in liver and testis. PPAR gamma is widely expressed in brain, where it has a crucial role in the regulation of nervous cell proliferation, differentiation and apoptosis. Therefore, the elucidation of the involvement of this receptor in phthalates actions in neuronal cells is necessary. Matrix metalloproteinases-2 and -9 (MMP-2 and MMP-9) actions are important in developing and adult nervous system and their potential to improve repair or regeneration after nervous system injury is well known. It is, however, unclear how MMPs are involved in DEHP

action and whether PPARγ regulates the function of these enzymes in the brain.

The aim of this research was to investigated the impact of DEHP on expression of PPAR-gamma and MMP-2 and MMP-9 in mouse neocortical astrocytes and neurons in vitro. The primary cultures were prepared from Swiss mouse embryos on 15/16 days of gestation. Phenol red-free DMEM/F12 medium supplemented with glutamine and 10% FBS was used for astrocytes and phenol red-free Neurobasal medium supplemented with glutamine and B27 was used for neurons. The cells were cultured in the presence of 10 μM concentration of DEHP for 1, 3, 6, 24 and 48 h. Afterwards, the cells have been lysed and the protein concentration has been determined. The expression of PPAR-gamma, MMP-2 and MMP-9 has been assessed by Western blot analysis.

The results showed that the effect of DEHP was depend on cell type. In astrocytes, PPAR-gamma and MMP-2 expression was increased while MMP-9 expression was decreased. In neurons, PPAR-gamma and MMP-2 expression decreased with a simultaneous increase in MMP-9 expression.

To sum up, DEHP mechanism of action might involve the PPAR-gamma receptors and therefore modulate the expression of matrix metalloproteinases.

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|B38|

Changes of glutathione homoeostasis in rat brain during subchronic oral exposure to silver nanoparticles or silver ions

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Despite the developmental potential of silver nanoparticles (AgNPs) in many fields of nanotechnology, there is a concern about possible negative influence of nanosilver on the environment, organisms and human health.

The selected studies on biodistribution show the accumulation of AgNPs in mammalian brain. Moreover, neurotoxic effects of silver nanoparticles has been confirmed in both in vitro and in vivo studies. There is also evidence that AgNPs can cause oxidative stress. However, the mechanisms of their toxicity have not been fully understood.

The aim of this study was to examine the effect of subchronic oral exposure of rats to citrate-stabilized AgNPs (10 ± 4 nm in diameter) or silver ions on glutathione homoeostasis.

Solutions of AgNPs or silver citrate were administered to male Wistar rats (180-210 g) via the gastric tube at a dose of 0.2 mg AgNPs or Ag(I)/kg b.w. per day for 14 days. The control group received the saline. Animals were sacrificed 24 h after last exposure and brain was collected for further studies. We confirmed the presence of silver nanoparticles in brains of exposed rats using TEM method.

The level of total glutathione did not change after exposure to both forms of silver, whereas the ratio of reduced to oxidized glutathione decreased in AgNPs- or silver ions-exposed rats in comparison to control group. Moreover, the statistically significant changes in glutathione peroxidase and glutathione reductase activities were observed.

The results of the present study showed that AgNPs and silver ions are able to induce a pro-oxidative environment inside the cells influencing the cellular glutathione system.

|B39|

The k-nn and roc estimation of significance of some matrix metalloproteinases as markers in als and edmd patients

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New statistical estimations of the significance of several matrix metalloproteinases (MMPs) in two neuromuscular diseases such as amyotrophic lateral sclerosis (ALS) and Emery-Dreifuss muscular dystrophy (EDMD) were used.

The ALS is a serious fatal progressive neurodegenerative disorder, the EDMD is a rare genetic disease manifested as skeletal muscle atrophy, dilated cardiomyopathy and often sudden death.

MMPs are recognized as taking part in many neurological diseases and may have diagnostic or prognostic significance. The altered levels of some MMPs are observed in ALS and EDMD patients [1,2]. Significance of serum MMPs such as membrane type matrix metalloproteinase-1 (MT1-MMP), gelatinases A (MMP-2) and B (MMP-9) are estimated as potential marker(s). The k-Nearest Neighbors (k-NN) classifier (the pattern recognition algorithm) with the ROC (Receiver-Operating Characteristic) curve analysis and the correlation matrixes was applied for differentiation between: (a) ALS patients with mild and severe symptoms and (b) AD- and X-EDMD patients based on set of the MMPs.

Our previous [3,4] and current results indicate that MMP-2 is effective as the biomarker in evaluation of ALS progression, MT1-MMP as the best of the MMPs as the biomarker in the recognition both AD- and X-EDMD forms.

In conclusion, a set of selected MMPs are helpful to recognize the ALS progression and the both EDMD forms in the proposed computer statistical-analytical approach.

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|B40|

Opioid agonist-neurokinin 1 antagonist designed multiple ligands alleviate both acute and neuropathic pain in rats

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Prolonged opioid administration (e.g. morphine) under neuropathic pain conditions (caused by nerve tissue damage) leads to changes in activity of endogenous nociceptive systems: increased release of pronociceptive substance P, together with up-regulation of its corresponding neurokinin 1 (NK-1) receptors, are associated with development of analgesic tolerance and opioid-induced hypersensitivity to painful stimuli. It is assumed that counteracting pronociceptive activity of this biochemical route with NK-1 receptor antagonists may improve opioids' effectiveness in neuropathic pain. In this study, the antinociceptive activity of novel hybrid opioid-NK1 peptidomimetics was evaluated in Wistar rats in acute pain (measured by tail-flick test) and neuropathic pain in chronic constriction injury (CCI) model on seventh day after injury. All results were compared to the analgesic potency of morphine and 'parent' compound containing only NK-1 pharmacophore. It was determined that hybrids provide dual alleviation of acute and neuropathic pain, providing the effect comparable to morphine, but in 3.5 to 4.5-fold lower doses, and are more efficient than morphine in attenuating hyperalgesia (measured by cold plate test) in CCI rats. NK-1 antagonist brought hardly any analgesic response in acute pain test, but proved to be very potent in diminishing allodynia and hyperalgesia in neuropathic rats. This may prove that NK-1 system is pivotal for pronociceptive signaling in neuropathy. Tolerance to analgesic effect of all compounds occurred at sixth day of daily intrathecal administration in CCI rats (the effect of hybrids was similar to the long-term profile of morphine administration), but there was no cross-tolerance between morphine and one of the hybrids. This proves that high antinociceptive effects of morphine and hybrid ligand may be reinstated during prolonged therapy. As a whole, these results give a promising outlook for using opioid-NK1 hybrids as a complementary treatment to pure opioid therapy in neuropathic pain states.

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|B41|

Impact of triclosan on cyp1a1 and cyp1b1 expression and activity 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 and urine. It is well known that TCS has a similar chemical structure to dioxin and in the presence of sunlight might be transformed to produce up to four dioxin compounds. Cytochromes CYP1A1 and CYP1B1 are involved in the metabolism of many endogenous compounds such as cholesterol, steroids hormones well as environmental xenobiotics especially dioxin. Interestingly, it has been well proven that some xenobiotics can inhibit activity of many metabolizing enzymes such as CYP1A1, CYP1B1 or UDP-glucuronosyltransferase.

The aim of the present study was to investigate the impact of TCS on the expression and activity of cytochrome CYP1A1 and CYP1B1 in 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, expression of mRNA CYP1A1 and CYP1B1 was measured. Additionally after 48 h of exposure protein expression of both enzymes and activity of CYP1A1 was measured.

Our preliminary data demonstrated that in the presence of 10 μM of TCS, mRNA expression of CYP1A1 and CYP1B1 was decreased. However, protein expression of CYP1A1 and CYP1B1 increased significantly. Moreover, TCS exhibited ability to inhibit activity of CYP1A1.

In summary, the presented study demonstrated that CYP1A1 and CYP1B1 are involved in TCS mechanism of action. Moreover, since the TCS inhibits activity of CYP1A1, it might also affect metabolism of other compounds.

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|B42|

Amyloid beta and its role in regulation of gene expression for APP cleaving enzymes. Implication in Alzheimer's pathology

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Amyloid precursor protein (APP) is a membrane protein that plays an essential role in regulation of synapse formation, neuronal growth and repair. APP is metabolized by secretase β and secretase γ which lead to amyloid-β peptides (Aβ) release. Excessive Aβ production and oligomerization play a key role in oxidative stress and cells death in Alzheimer's disease (AD). Moreover, alteration of presynaptic protein alpha synuclein (ASN) is involved in the pathology of AD. Oxidative stress influences two NAD dependent enzymes families: sirtuins (SIRTs) and poly(ADP-ribose) polymerases (PARPs) which are involved in the regulation of energy metabolism, transcription, and DNA repair. SIRTs (3,4,5) located in mitochondria regulate antioxidative enzymes and electron transport proteins. SIRTs and PARPs are responsible for DNA repair, however, under massive stress PARP1 can be over activated and may lead to NAD+ depletion and cells death.

The aim of our study was to determine changes in gene expression profile of enzymes involved in non amyloidogenic and amyloidogenic pathways of APP metabo-

lism in PC12 cells after Aβ42 treatment. Moreover, transcription of mitochondria SIRTSs and DNA bound PARPs was determined and their functional role in cell death/survival was evaluated.

Rat pheochromocytoma (PC12) cells were treated for 24h with Aβ 1-42 in oligomeric/monomeric form at 1μM. Moreover, the effect of endogenously liberated Aβ in PC12 cells transfected with human gene for APP wild type (APPwt) and bearing Swedish mutation (APPsw) was investigated. Our results show that Aβ42 reduces viability of PC12 cells by 60% and inhibits gene expression of α-secretase responsible for nonamyloidogenic APP metabolism. Concomitantly Aβ peptide enhances expression for β and γ secretases, the enzymes which lead to Aβ release. Increased expression for β- and γ-secretase was also observed in the APP wt cells. ASN (0.5 μM) induces also similar effect on transcription of APP cleaving enzymes. Moreover, Aβ peptides modulate gene expression for PARPs (1,2,3) and mitochondria SIRTs.

These results indicate the crucial role of Aβ peptides and ASN in regulation of APP metabolizing enzymes and suggest that ASN through alterations of APP processing may enhance Aβ toxicity. Moreover, interactions between NAD dependent enzymes may play important role in regulation of metabolism and cell fate under Aβ toxicity.

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|B43|

Pathomechanism of multiple sclerosis and polymorphism of osteopontin gene

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Osteopontin (OPN) is one of the key cytokines involved in T-cell activation in multiple sclerosis (MS). The OPN gene is therefore recognized as an early T-cell activation gene, which underlies immunological events involved in the aetiopathogenesis of MS. In an earlier study, we found OPN to be a useful marker to differentiate between malignant and benign ovarian tumours. In patients with optic neuritis, cerebrospinal fluid (CSF) OPN levels have been shown to be correlated with CSF chitinase-3-like protein 1, myelin basic protein, and neurofilament, light polypeptide. OPN also enhances the production of interleukin 12 and interferon gamma, and reduce interleukin 10. Moreover, OPN is expressed in MS plaques in the central nervous system.

Disability in 100 MS patients was evaluated using the expanded disability status scale (EDSS). Genotype and allele frequencies at exons 6 and 7 were examined by PCR. Using appropriate statistical tests, the distribution of variables was tested and means ± SD compared.

Genotype distribution and allele frequency differences between patients and control individuals were not statistically significant. No association of OPN with susceptibility to MS was found in the Polish population. The EDSS score was higher in 8090 T/T + 9250 C/C patients than in 8090 C/C + 9250 C/C MS patients (p = 0.0120), and the disability in 8090 C/C + 9250 C/T MS patients was higher than in 8090 C/C + 9250 C/C MS patients (p = 0.0137). Logistic regression analysis revealed age to be an independent factor influencing disability.

The polymorphism of OPN gene in positions 8090 T/T + 9250 C/C, 8090 C/C + 9250 C/T, and 8090 C/T + 9250 C/T were linked with relatively higher levels of disability in MS patients.

|B44|

Apoptotic and toxic effects of benzophenone-3 (BP-3) in mouse embryonic neuronal cells

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Benzophenone-3 (BP-3) is a commonly used chemical sunscreen agent, absorbing radiation in the range of UVB and UVA. The industrial use of BP-3 has been increasing over the past decade. Since UV filters are photostable and often lipophilic, they are persistent in the environment. Humans can be exposed to UV screens by dermal absorption or through the food chain. The most disturbing seems to be the fact that breastfed babies are exposed to BP-3.

Certain UV-filters (e.g. BP-3, BP-2, 4-MBC) were present in 85% of Swiss human milk samples (Schlumpf *et al*., 2010). There is no data that BP-3 crosses blood-placenta barrier, but bisphenol A which has similar structure to BP-3 has been found to pass this barrier. One may assume that chemicals like BP-3 also are able to enter the embryo/fetus and cause deleterious effects on prenatal development. Insufficient data (*in vitro* or *in vivo*) related to the possible impact of BP-3 on the nervous system both in terms of neurodevelopment and neurodegeneration processes confirm the relevance of presented report.

Our study demonstrated that BP-3 activated caspase-3 in the mouse neuronal cell cultures, which was found within 6 hours after the treatment and accompanied by significant increase in lactate dehydrogenase-release (LDH) at 6 and 24 h of experiment. These effects were observed in neocortical and hippocampal cells, however, hippocampal cells were less vulnerable to BP-3 than neocortical. Therefore, the use of neocortical and hippocampal embryonic cells allowed us to demonstrate neurodevelopmental and brain tissue-specific patterns of response to BP-3. These biochemical data were supported by increased apoptotic body formation and impaired cell survival, evidenced by Hoechst 33342 and calcein AM staining.

These data point to apoptotic and neurotoxic capacity of BP-3 that position this UV filter as a compound that may increase risk of developmental abnormalities.

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|B45|

Cardiorespiratory activity of PK20, a potent anti-inflammatory and antinociceptive hybrid peptide

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Objectives: PK20, chimeric peptide characterized by having in its structure an opioid and neurotensin pharmacophores has been demonstrated to induce anti-inflammatory activity in our in vivo studies and dose-dependent prolonged analgesic response in animal pain model (Kleczkowska *et al*., 2010). We have shown that local application of the PK20 in cream in a dose dependent fashion reduced oedema in mice ears with induced contact sensitivity response. In the present study we would like to investigate whether our new promising compound – PK20 exerts any unfavourable cardiovascular and/or respiratory action in experimental animals after intravenous injection.

Methods and results: Anaesthetized, spontaneously breathing rats were used. Tidal volume was measured at tracheostomy. The timing components of the breathing pattern, arterial blood pressure, and heart rate were recorded. Intravenous administration of PK20 in the neurally intact rats evoked a dose-dependent apnoea followed by short-lived insignificant increase in tidal volume and breathing rate, which resulted in ephemeral increase in minute ventilation. The blood pressure changes were biphasic: transient increase was replaced by prolonged hypotension. Midcervical vagotomy did not influence blood pressure response but abrogated post-PK20 respiratory effects including an arrest of breathing. Respiratory changes were substantially reduced by blockade of both: NTS1 and mu-opioid receptors, while hypotension was eliminated after neurotensin NTS1 receptors.

Conclusions: The respiratory changes triggered by chimeric PK20 seem to be resultant of simultaneous excitation of both opioid and neurotensin receptors of the vagus nerve. Hypotension is mediated beyond the vagi via activation central and peripheral neurotensin NTS1 receptors. This chimeric peptide should be used with care via intravenous administration in anaesthetized animals since in high doses PK20 may evoke respiratory apnoea and hypo-

tension. Nevertheless, applied locally or intraperitoneally in conscious mice induced no adverse effects.

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|B46|

Comparison between enzymatic and nephelometric serum ceruloplasmin assays in the diagnosis of Wilson's disease

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Wilson's disease (WD) is a rare autosomal recessive copper metabolism disorder due to inappropriate incorporation of copper into ceruloplasmin.

Ceruloplasmin binds about 90-95% of copper in the blood and protects cells against free radical injury. Many studies have shown that decreased levels (< 25 mg/dl) of ceruloplasmin besides WD occurs in Alzheimer's disease, Parkinson's disease and aceruloplasminemia. Assesement of activity of ceruloplasmin is a very important test in diagnosis of WD. The measurement of ceruloplasmin value is generally carried out by one of different analytical methods: manual - enzymatic and automated - nephelometric. The enzymatic assay measures active form of ceruloplasmin (Cu-containing). Nephelometric method measures non active ceruloplasmin and Cu-containing (active) form of ceruloplasmin. Nephelometric method is now widely used due to its simplicity.

In this study was tested discrepancy between the nephelometric and enzymatic measurements of ceruloplasmin concentration on four groups: non treatment WD (n = 38), treatment WD (n = 40), heterozygous carriers (n = 93) and healthy individuals (n = 183) (Wilcoxon's matched pairs test). Differences between groups on each methods was examined by Mann-Whitney U test. Based on the ROC curve (Receiver Operating Characteristics) was specified diagnostic accuracy (sensitivity, specificity, positive predictive value – PPV, negative predictive value – NPV) of the enzymatic and the nephelometric method.

Enzymatic measurements of ceruloplasmin was statistically significantly lower than nephelometric on three groups: non-treatment WD (14.9 mg/dl vs. 16.6 mg/dl), treatment WD (5.1 mg/dl vs. 7.1 mg/dl) and heterozygous carriers (30.6 mg/dl vs. 32.3 mg/dl). In healthy persons group enzymatic measurements was higher than nephelometric (33.4 mg/dl vs. 29.1 mg/dl). Statistically significant divergences was observed between groups by enzymatic and nephelometric assays. Interesting marked decrease of ceruloplasmin was observed in WD group treated by anti-copper drugs. Enzymatic method has better diagnostic accuracy in WD than nephelometric method – higher sensitivity (100% vs. 86.8%), PPV (1 vs. 0.86) and area under ROC curve (0.986 vs. 0.957) and should be recommended to diagnosis and monitoring WD.

|B47|

Effect of telmisartan on kynurenic acid production in rat brain cortex

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A renin-angiotensin-aldosterone system (RAAS) is one of the regulators of water – electrolyte metabolism. It is thought to control not only the thirst, blood vessels tone, vasopressin release, but it is responsible for thermoregulation, learning, memory, emotions, reproduction and sexual behaviors as well. Among angiotensin receptors, the most prominent role in pharmacotherapy possesses receptor type 1 (AT-1). It was reported that RAAS in the brain may have important role in animal seizure models or in the development of neurodegenerative disorders.

Kynurenic acid (KYNA) is an endogenous antagonist of glutamate receptors and of α7 nicotinic receptors.

The cerebral synthesis of KYNA from its precursor L-kynurenine (L-KYN) is catalyzed by kynurenine aminotransferases (KAT), from which the most important are KAT I and KAT II. The disturbances of KYNA production have been linked to the neurodegenerative diseases and epilepsy. Recent experimental data indicated that KYNA could play a role in the central blood pressure control.

In this study the influence of telmisartan, an AT1 – receptor antagonist, on brain KYNA production was investigated.

In cortical slices telmisartan at the concentration of 0.01; 0.1 and 0.5 mM decreased KYNA synthesis to 72% (p < 0.05); 65% (p < 0.05) and 54% (p < 0.05) of control,

respectively. The activity of KAT I was decreased by telmisartan at the concentration of 0.01; 0.05, 0.1 and 0.5 mM to 92%, 84%, 76% ($p < 0.05$) and 37% ($p < 0.001$) of control, respectively.

Similar inhibitory effect on KAT's II activity after telmisartan administration was observed.

Our data shows that telmisartan can modulate brain production of KYNA probably through the influence on KAT's activity.

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|B48|

Effect Of Zn On N-acetyl-L-aspartate Synthesis In SN56 Neuroblastoma Cells

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N-acetyl-L-aspartate (NAA) is one of most abundant amino acid derivative in the mammalian brain. NAA is synthesized exclusively in neurons from acetyl-CoA and L-aspartate in the presence of aspartate acetyltransferase (A-NAT). Acetyl-CoA production is catalyzed by pyruvate dehydrogenase complex (PDHC). In cholinergic neurons, acetyl-CoA is also used for energy production and acetylcholine synthesis. Considering the additional utilization of acetyl-CoA in cholinergic neurons, deficit of this metabolite can be the cause of particular cholinergic susceptibility to neurotoxic factors.

Therefore, the aim of our study was to investigated whether neurotoxic concentration of Zn might evoke NAA substrates shortages and changes of aspartate N-acetyltransferase activity.

SN56 neuroblastoma cells with high expression of cholinergic phenotype were used as experimental model of cholinergic neurons.

Chronic exposition of differentiated SN56 neuroblastoma cells to 0.15mM Zn increased the Zn level from 1.9 to 8.5 nmol/mg protein. In this experimental conditions the nonviable cells fraction increased for about 57%. The acetyl-CoA level in control condition was estimated for equal 24.0 pmol/mg protein. In 0.15 mM Zn-exposed SN56 assayed level of acetyl-CoA was decreased by over 50%. Furthermore, level of NAA was decreased by 74 %. Simultaneously, Zn caused 58% reduction of both PDHC and A-NAT activities.

Achieved results indicate that impairment of NAA level caused by cytotoxic agents in cholinergic neurons is due to both shortage of acetylo-CoA as well as inhibition of A-NAT activity.

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