
Posters

Peripheral inflammation regulates BDNF expression in rat trigeminal ganglion neurons

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The trigeminal system, with first-order neurons in the trigeminal ganglion (TG), provides sensory innervation to the majority of craniofacial tissues. Many peripheral targets of TG neurons constitute the source of common chronic pain conditions, such as trigeminal neuralgias, migraine headaches, and temporomandibular disorders. Although it is well established that chronic trigeminal pain is frequently associated with inflammation of peripheral endings of TG neurons, the exact molecular mechanisms of trigeminal nociceptive transmission are poorly understood. The goal of the present study was to examine the effects of peripheral inflammation in the trigeminal system on regulation of the neurotrophin brain-derived neurotrophic factor (BDNF) in TG neurons *in vivo*, and to begin investigating the underlying molecular mechanisms using an *in vitro* model of trigeminal inflammation.

In our studies *in vivo*, we used a rat model of tooth pulp inflammation. Cavities were prepared in right-side maxillary molars of 4-week-old animals. BDNF expression in right TG was compared with contralateral TG of the same animal and with right TG of sham-operated controls, 7 and 28 days after cavity preparation. Using ELISA, we determined that BDNF levels were

significantly upregulated in right, compared to left, TG. Double BDNF/TrpV1 and BDNF/CGRP immunohistochemistry revealed that inflammation recruits new TrpV1- but not CGRP-immunoreactive cells to express BDNF.

In the *in vitro* portion of the project, newborn rat TG neurons were grown under neuron-enriched conditions for 3–5 days, followed by treatment with various pro-inflammatory cytokines, including tumor necrosis factor α (TNF α), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6). The cytokine treatment (4 and 24 hours) was combined with patterned electrical field stimulation to mimic activity of trigeminal neurons *in vivo*. At the end of the treatment period, cultures were processed for either quantitative PCR or ELISA to evaluate changes in BDNF mRNA and protein, respectively. Our results indicate that TNF α and IL-1 β regulate BDNF expression in a dose-dependent manner, and the effects are dramatically enhanced in the presence of electrical stimulation. In view of the previous evidence that BDNF is expressed at trigeminal nociceptive synapses in the brainstem, our current data point to BDNF as a likely key player of trigeminal inflammatory pain.

Postischemic changes in colocalization of GABA_A receptor, gephyrin and dystrophin (Dp71) in gerbil hippocampus

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Till now the glutamate transmission is studied most often in relation to the development of brain ischemia-induced cell death. For a last few years interest to GABAergic system which functions in opposition to that of glutamate has also appeared. Many of the GABAergic drugs have shown neuroprotective efficacy in animal models of brain injury. Moreover it is proven that the metabolism of GABA have shown marked changes during first minutes of brain ischemia. In turn this can lead to decrease in expression of GABA_A receptor (GABA_AR) what can have influence on efficient inhibitory synaptic transmission. GABA_AR similarly to glutamatergic receptors is clustered to membrane by complex of proteins including gephyrin, dystrophin (Dp71) and β -dystroglycan. We have studied the postischemic changes in GABA_AR immunoreactivity and its colocalization with the above mentioned proteins after 5 minutes gerbil brain

ischemia in the time course of reperfusion in ischemia-vulnerable (CA1) and resistant (CA2-3, DG) part of hippocampus. We observed postischemic increase in the expression of mRNA and protein of dystrophin in CA1 and CA2-3, DG, while the expression of GABA_AR, gephyrin, and β -dystroglycan in hippocampus remained unchanged in the tested time points. Using confocal microscopy, we confirmed colocalization of GABA_AR with gephyrin and Dp71 and observed postischemic spatial changes in the relationships between GABA_AR, gephyrin and Dp71 in CA1 and CA2-3, DG. We suggest that postischemic rearrangements of proteins forming GABAergic synapses are probably involved in the modulation of inhibitory transmission and can influence neurons fate.

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Enhanced apoptotic response to redox stress in lymphocytes from patients with Alzheimer's disease

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Multiple studies suggest that neuronal death in Alzheimer's disease (AD) is the result of an apoptotic mechanism, probably due to elevated redox stress. Some molecular changes can be detected not only in AD neurons but also in peripheral cells such as lymphocytes. The aim of this study was to assess lymphocytes as a study model in AD pathogenesis and as easily accessible diagnostic material. Therefore we ana-

lyzed apoptotic response to redox stress evoked by 2-deoxy-D-ribose (2dRib) in immortalized lymphocytes from 18 patients with sporadic AD, 2 patients with familial AD carrying mutations in presenilin1 (P117R and I213F), and 20 healthy individuals. The percentage of apoptotic cells was measured using two flow cytometry methods: AnnexinV/Propidium iodide (PI) labeling and analysis of fragmented DNA

content. We also determined the functional status of mitochondria by measuring mitochondrial membrane potential with the lipophilic dye JC-1 as well as estimated caspase9 and caspase3 levels by immunoblotting. Our findings demonstrate that all lymphocyte cell lines show significantly increased apoptotic response to the redox stressor 2dRib associated with decline in mitochondrial membrane potential. These changes in AD cells were correlated with increased

activities of caspase9 and caspase3. Our data indicate enhanced activation of mitochondrial apoptotic pathway in both sporadic and familial AD lymphocytes under redox stress. This study demonstrates that increased susceptibility to redox stress and mitochondrial dysfunctions are characteristic not only for AD neurons, but also for AD lymphocytes. Thus, human lymphocytes could be used in further studies on AD pathogenesis and diagnostics.

Impact of apolipoprotein E on the pathophysiology of multiple sclerosis

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Apolipoprotein E plays an important role in the metabolism of cholesterol and in modulation of inflammatory reactions, processes of significance in pathology of multiple sclerosis. Therefore the great interest on the input of APOE on the pathophysiology of multiple sclerosis.

The study group included 100 patients with multiple sclerosis diagnosed according to the criteria of McDonald et al.. The progression of multiple sclerosis was estimated by calculating the progression index (PI = EDSS score) duration of disease in years. APOE genotyping was determined by polymerase chain reaction and restriction enzyme digestion on DNA extracted from leucocytes of studied persons.

The genotype distribution, haplotype distribution and APOE $\epsilon 4$ allele frequencies in the group of MS victims and controls were similar. The overwhelming genotypes in MS were homozygotes $\epsilon 3/\epsilon 3$ (71% of patients) and heterozygotes $\epsilon 3/\epsilon 4$ (25%). In control material the homozygotes $\epsilon 3/\epsilon 3$ were found in 88% and heterozygotes $\epsilon 3/\epsilon 4$ in 10%. Almost identical was the distribution of genotypes between the group of patients with the most benign and most malignant course of disease estimated by PI index.

We conclude that in Polish population any particular genotype of APOE or a presence of particular alleles is not a predisposing factor to the development of multiple sclerosis or to the increased progression of the disease.

Testosterone level in idiopathic PD patients and idiopathic PD patients with cardiovascular risk factor – a pilot study

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Clinical studies demonstrated 1.5-3.7-fold greater prevalence of PD in men compared to women. It has been shown recently that testosterone (T) deficiency has a higher prevalence in male PD patients than in age-matched healthy males. This suggests that T deficiency may play a role in the etiology of PD. In line with such assumption are data indicating T-mediated neuroprotection in human primary neurons and an essential role of T for the development and maturation of the substantia nigra. Moreover, decreased T level is associated with some metabolic disorders and with increased risk of cardiovascular diseases which both are common in patients with PD. Our previous studies have demonstrated activation of nigrostriatal NO/GC/cGMP pathway in MPTP-induced PD and elevated serum cGMP in patients with idiopathic PD (iPD). To further explore the role of T in iPD, we investigated serum levels of testosterone and cGMP in male patients with iPD (n = 9), in iPD patients with cardiovascular risk factors (iPD-CVRF, n = 10), and in 20 age-matched healthy volunteers. There was no

difference in PD stage between the two groups of PD patients as measured by UPDRS. A non-parametric test U-Menna-Whitney revealed between-group differences in all measured variables. Decreased serum total T concentrations were found in both groups of iPD patients as compared to the control group (p < 0.05). However, the serum T level was lower in the iPD-CVRF group than in the iPD group (p < 0.05). Serum cGMP levels were significantly higher in both groups of PD patients than in the controls (p < 0.05). However, the serum cGMP level was higher in the iPD-CVRF group than in the iPD group. A significant negative correlation between T and cGMP levels was found (Spearman correlation, r = -0.678, p < 0.0005). Our findings suggest that the hypothesis postulating a causal relationship between T level and severity of PD should be taken with caution.

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PARP-1 and AIF in neuronal death signaling evoked by genotoxic stress

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Poly(ADP-ribose)polymerase (PARP-1) is a nuclear enzyme involved in DNA repair but its over activation leads to formation of poly(ADP-ribose) (PAR), a new signaling molecule, which is released from nucleus and may act at the mitochondria and induce apoptosis inducing factor (AIF) release.

The aim of this study was to investigate the role of AIF and PARP/PAR in death signaling pathway evoked by genotoxic stress in mouse hippocampal immortalized neurons (HT22), subjected to DNA alkylating agent, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

HT22 cells treated with MNNG at 50–500 μ M demonstrated concentration and time dependent enhancement of PAR level and alteration of mitochondrial function evaluated by MTT test. The level of AIF in mitochondria significantly decreased after 24h of MNNG treatment. PARP-1 inhibitors: 3-amino-benzamide (3AB) and PJ34 at 5mM and 20 μ M respectively, enhanced the level of AIF in mitochondria and protected most of the cells against MNNG – induced

death signaling. The inhibitors of caspase-3 activity (40 μ M) and p53 (20 μ M Pifitrin) had no protective effect. Summarizing, our data indicated that PARP/PAR/AIF signaling pathway is responsible for hippocampal HT22 cell death and that inhibitors of PARP-1 protect mitochondrial integrity and function.

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Cyclin-Dependent Kinase 5 in PC12 cells overexpressing of Amyloid Precursor Protein

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Deregulation of protein phosphorylation has been implicated in pathogenesis of Alzheimer's disease (AD). It was observed that expression and activity of many kinases and phosphatases are altered in the brain of AD patients. Among many kinases, Cyclin-Dependent Kinase 5 (CDK5) belongs to the most interesting candidates, as kinase responsible for aberrant phosphorylation of microtubule associated protein tau. Moreover, CDK5 may also phosphorylate other proteins potentially involved in AD pathogenesis.

The aim of the present study was to analyze involvement of CDK5 in cell death processes occurring in PC12 cells overexpressing Amyloid Precursor Protein (APP). We used cells transfected with human wild-type APP (APPwt) and human APP with Swedish mutation (APPsw). Real-time PCR and Western blotting were used for analysis of expression and phosphorylation of CDK5, CDK5R1, CDK5R2, GSK-3 β .

MTT and LDH tests were used for determination of cytotoxicity.

Our data demonstrated enhanced cell death in PC12 cells transfected with APP gene. Real-time PCR analysis indicated increased level of mRNA for CDK5 gene in APPsw cells, however, protein level was not altered. Significantly decreased phosphorylation of CDK5 on Tyr15 was observed in APPwt and APPsw cells, what suggests lowering of CDK5 activity. However, phosphorylation of CDK5 substrate-GSK-3 β was not changed. Our results indicate that CDK5 is not involved in degeneration processes evoked by APP overexpression in PC12 cells. However, the role of CDK5 in adaptation to high levels of APP needs further investigations.

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The levels of homocysteine and p53 protein in patients with epilepsy treatment antiepileptic drugs

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Antiepileptic drugs (AEDs) in patients with epilepsy may increase concentration of homocysteine (Hcy) and alter metabolism of Hcy to methionine.

Hcy may induce DNA damage, oxidative stress and apoptosis. P53 protein may induce apoptotic process in cells. Valproic acid induces apoptosis in human leukemia cells.

The aim of study was to determine of plasma levels of Hcy, and p53 protein from peripheral lymphocytes in patients with epilepsy treated with AEDs and in control.

The levels of Hcy estimated by HPLC and levels of p53 protein estimated by Western Blot, on 20 patients with epilepsy treatment AEDs in age 21–69 years,

and 3 patients with epilepsy untreated AEDs in age 18–65 years, and 22 controls in age 22–61 years.

The studies disclosed that level of Hcy increased higher than 16 μM in 30% patients with epilepsy taking AEDs. In patients with epilepsy treated with AEDs, level of Hcy was higher ($p < 0.01$) than in controls. In patient with epilepsy untreated AEDs level of p53 protein was no significantly statistically lower than in controls and increased after treated AEDs. In patients treated with AEDs with level of Hcy higher than 16 μM , the level of p53 protein increased.

It appears that, high level of Hcy may increase the level of p53 protein in patients with epilepsy taking AEDs.

Cytosolic phospholipase A₂ inhibition is involved in the protective effect of nortriptyline in primary astrocyte cultures exposed to combined oxygen-glucose deprivation

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Protective potential of nortriptyline has been reported in a few experimental models of brain ischemia both *in vivo* and *in vitro*. However, the detailed molecular mechanisms of protective action of the drug are still unresolved. The aim of the study was to determine whether treatment with the low or medium concentrations of nortriptyline (0.1–10 μM) with proved neuroprotective potential might have an effect on cPLA₂ protein and/or mRNA expression in ischemic astrocytes and that this influence might be related to its potential positive influence on cell viability. On the 21st day *in vitro*, primary cultures of rat cortical astrocytes

were subjected to ischemia-simulating conditions (combined oxygen glucose deprivation, OGD) for 24 h and exposed to nortriptyline. The drug at concentrations of 0.1 and 1 μM attenuated the expression of cPLA₂ (both phosphorylated and unphosphorylated form) together with a significant decrease in cPLA₂ mRNA level in ischemic astrocytes. We have demonstrated that nortriptyline influences on decrease in cPLA₂-mediated arachidonic acid (AA) release through a mechanism which appears to involve attenuation of both PKC and Erk1/2 kinases expression. Nortriptyline also significantly prevented mitochondrial de-

polarization in ischemic astrocytes. Moreover, the antidepressant protected glial cells against OGD-induced apoptosis and necrosis. Our findings document a role for cPLA₂ expression attenuation and AA

release inhibition in the protective effect of nortriptyline in ischemic astrocytes.

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Methyltetrahydrofolate reductase gene polymorphism – a novel genetic factor modifying phenotypic effects of copper toxicity in Wilson’s disease

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Wilson disease (WD) is an autosomal recessive disorder of copper (Cu) metabolism. The WD gene ATP7B encodes a copper-transporting P-type ATPase ATP7B. WD characterizes with broad spectrum of phenotypic expressions including early or late, hepatic or neuropsychiatric symptoms manifestation. It has been established that increased tissue concentrations of homocysteine (Hcys) exert hepatotoxic as well as neurotoxic effects, as hyperhomocysteinemia induces strong oxidative stress conditions. Hcys and Cu may exert synergistic cytotoxic effect. One of key folate/Hcys pathway enzyme is 5,10-methylenetetrahydrofolate reductase (MTHFR). Two common MTHFR polymorphisms, C677T in exon 4 and A1298C in exon 7, have been described. Both predispose their carriers to hyperhomocysteinemia. Results of our study, including 250 patients with WD, suggest that MTHFR C677T and A1298C genotypes determine phenotypic

expressions of WD. Patients possessing the 1298C allele present first disease symptoms on 4 years earlier than those not having this allele. Carriers of “high activity” MTHFR diplotype (677CC/1298AA) develop initial WD symptoms on 8 years later than non-carriers. As genotypes of MTHFR were not related to copper metabolism parameters, we may suppose that hyperhomocysteinemia does not affect Cu metabolism, but rather does modulate organ effects of increased cellular Cu concentrations leading to more extensive damage and earlier clinical disease manifestation. Our findings are novel and important, as there are currently only few known genetic modifiers of this highly variable disorder. Our observation is the more important, as Hcys metabolism may be improved with treatment combining methyltetrahydrofolic acid, hydroxycobalamin, pyridoxine, and betaine.

Overexpression of purinergic P2X₇ receptor in rat brain subjected to experimental autoimmune encephalomyelitis

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It is known that inflammation and oligodendroglial cell death are the main features of multiple sclerosis (MS), inflammatory demyelinating disease of the central nervous system, and its animal model, EAE. Recently, a great deal of attention is focusing on the relation between inflammatory/neurodegenerative changes observed in many pathological states, including MS/EAE and purinergic P2X₇ receptor. The reason is that activation of this receptor under pathological conditions (high ATP levels) leads to a release of inflammatory mediators (IL-1b) and to the cell death via necrotic or apoptotic pathway.

The expression of P2X₇R was assessed on the level of protein and mRNA using Western blot and RT-PCR analysis, respectively. The profile of receptor's expression was examined in forebrain homogenates in different stages of EAE (4, 6, 8, 10, 20, 25 days post

immunization). We observed the early overexpression of P2X₇R protein in an asymptomatic phase which was followed by the subsequent increase of protein level in the peak of the disease and in the recovery phase. However, the significant increase of mRNA level was not observed before the symptomatic phase. Additionally performed immunohistochemical study revealed both enhanced intensity of reaction and changes in the pattern of P2X₇R expression during the investigated time points. Double immunofluorescent labeling shows the coexpression of P2X₇R and GFAP in some cortical cells. The results suggest that P2X₇R is overexpressed early during the course of EAE being almost partially connected with astrocytic pool of cells. The nature of these changes will be further elucidated.

DNA methylation changes in Oct3/4 gene promoter region of HUCB-NSC in response to neural differentiation

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Umbilical cord blood is considered as a promising source, not only for hematopoietic stem cells, but also for neural stem/progenitor cells. Latest groundbreaking news, focused scientists' attention on molecular mechanisms governing pluripotency and phenotype changes of stem cells. Emerging evidence suggests, that changes in expression of so called 'stemness' genes, like Oct3/4 or Nanog, are associated with unique epigenetic modifications. Independent, scientific groups demonstrated that the methylation status

of Oct3/4 and Nanog promoters correlates strongly with their ability to be expressed. The promoters are unmethylated in pluripotent stem cells, where those genes are expressed, and almost fully methylated in differentiated cells, where Oct3/4 and Nanog are silenced. The aim of the study was to analyze the promoter region's methylation pattern in Oct3/4 gene in HUCB-NSC line (Human Umbilical Cord Blood – Neural Stem Cells) before and after neural differentiation. Materials and Methods. HUCB-NSC were cul-

tured in ITS medium and in differentiating medium (DMEM/F12 + ITS (1:100) + FBS (2%) + AAS (1:100) + cAMP (300 μ M)) in the density of 5×10^4 cells per cm^2 in standard conditions. After 14 DIV cells were harvested and DNA was isolated. To analyze the methylation status of gene promoter region, sequencing of sodium bisulfite-treated DNA was performed. Obtained sequences were compared with sequences of non-treated DNA. Results. We observed changes in

methylation pattern of Oct3/4 promoter region due to differentiation process. Comparing to undifferentiated HUCB-NSC, cells after neural differentiation revealed higher methylation status in promoter region. Moreover those changes strongly correlate with the expression of Oct3/4 gene.

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Neurogenic reserve in adults rats potentially stimulated by transplantation of neural stem cells derived from human umbilical cord blood (HUCB-NSC)

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Neurogenic reserve represents the brain's compensatory potential in the face of cell lost or neurodegeneration. The lifelong generation of new neurons in the adult brain captures the scientific imagination with its seemingly obvious implications for regenerative medicine. This study was designated to examine whether HUCB-NSC transplantation modulates persistent neurogenesis in adult rat after focal brain ischemia. 2×10^4 neural stem cells derived from human cord blood (HUCB-NSC) were transplanted into corpus callosum 3 days after a focal brain injury induced by OUA injection (1 μ l/50nmol) into striatum of adult Wistar rats. At 1, 3, 7 and 14 days thereafter brains were removed and immunocytochemical analysis for the presence of doublecortin (DCX) and PSA-NCAM markers expressed by immature migratory neurons was performed. Results. Ouabain induced brain lesion resulted in increased number of DCX⁺ and PSA-

NCAM⁺ cells in SVZ and SGZ in comparison to intact rats. Three days following grafting of HUCB-NSC the number of DCX⁺ cells increased significantly in the ipsilateral SVZ and SGZ, whereas fewer neuroblasts were seen in contralateral hemisphere. At 7th day after HUCB-NSC transplantation intense migration of DCX⁺ cells from SVZ towards ischemic boundary regions of the striatum was observed. Conclusions. Recent demonstration that HUCB-NSC grafting may promote endogenous neurogenesis proves the relevance of somatic cell – dependent alternative therapeutic mechanism. Whether the newly ascended precursors differentiate into mature neurons and establish a functional cross-talk with the different cells of brain microenvironment is so far unknown.

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Systemic inflammation impairs object recognition but not spatial memory in male mice. The role of redox state and PARP-1

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Our previous data demonstrated that systemic inflammation evoked by intraperitoneal injection of lipopolysaccharide (LPS; 1 mg/kg b.w) induces alterations of expression of several genes, as *Tnfa* or *Ptgs2*, and changes activity of many enzymes including nitric oxide synthase (NOS) and poly(ADP-ribose) polymerase-1 (PARP-1). In the present study, time-dependent alterations of glutathione system were evaluated. Significantly lower concentration of reduced form of glutathione (GSH) and lower activity of glutathione reductase was observed 48h after LPS injection. Concomitantly oxidized form of glutathione (GSSG) was elevated, and the ratio

GSH/GSSG was significantly lower, comparing to control value. The observed enhanced activity of DNA-bound enzyme PARP-1, the nuclear target for free radical cascade and DNA damage, leads to accumulation of poly(ADP-ribose). These all alterations may affect object recognition memory that is significantly impaired 4 days after LPS injection. LPS-evoked systemic inflammation has no effect on spatial memory. Inhibitor of PARP-1 has ameliorating effect, and enhances significantly recognition function.

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Modulation of potassium (rubidium) fluxes and cell volume changes by Kir4.1 channel in HEKT293 cells exposed to ammonia and/or hypotonia

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Ammonia and hyponatremia contribute to astrocytic swelling and cerebral edema associated with hepatic encephalopathy (HE). The inward rectifying potassium channel Kir4.1 abounds in astrocytes where it has been implicated in ion movement across the cell membrane and cell volume regulation. However, the functional role of this channel in ammonia- and/or hypotonia-exposed cells has not been examined.

We measured fluxes of ⁸⁶Rb (tracer for K⁺) and cell volume (³H-O-methyl-glucose method) in control ("Kir4.1^{-/-}") human embryonic kidney (HEKT293)

cells and cells transfected with Kir4.1 pcDNA ("Kir4.1^{+/+}"), and assessed modulation of these parameters by exposure to 5 mM ammonium chloride (ammonia) for 72 h and/or hypoosmotic media for additional 20 min. Kir4.1 transfection did not alter ⁸⁶Rb efflux from the cells in isotonic (122 mM Na) medium, while in media with reduced osmolarity (100 mM or 60 mM Na) the efflux was substantially lower in Kir4.1⁺ than in Kir4.1⁻ cells, both in the presence and absence of the sodium pump inhibitor, ouabain. Both the ouabain-sensitive and -resistant com-

ponent of ^{86}Rb uptake was slightly elevated in Kir4.1⁺ cells. Treatment with ammonia did not affect ^{86}Rb efflux or uptake in Kir4.1⁺ or Kir4.1⁻ cells, under any incubation conditions. Incubation in 60 mM Na increased the volume of Kir4.1⁺ or Kir4.1⁻ cells to a similar degree. While ammonia alone produced no cell volume change in Kir4.1⁺ or Kir4.1⁻ cells, ammonia potentiated the cell volume-increasing effect of

hypotonic treatment in Kir4.1⁻ but not Kir4.1⁺ cells, indicating that in the brain *in vivo*, Kir4.1 may act towards preventing exacerbation of ammonia-induced cell swelling by hyponatremia, the latter frequently occurring in patients with overt HE.

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Neuroprotective activity of ClABT and 4BrABT, the new 2-amino-1,3,4-thiadiazole derivatives. *In vitro* study

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The N-substituted 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole are well known as compounds of interesting biological activity. In our previous publications we have described synthesis and anticancer activity of the new synthesized aminothiadiazole derivatives. We have shown that tested compounds inhibited growth and motility of tumor cells derived from cancers of peripheral and nervous system origin. Interestingly, in anticancer concentrations they had no influence on normal cell viability. Therefore, the aim of the study was an *in vitro* evaluation of neuroprotective properties of the two new aminothiadiazole derivatives 2-(3-chlorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (ClABT) and 2-(4-bromophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (4BrABT). The applied cell cultures were mouse neurons (differentiated P19 cell line), primary rat astro-

cytes and rat oligodendrocytes (OLN-93 cell line). Cells were exposed to aminothiadiazole derivatives alone and combined with glutamate and serum deprivation (SD). Cell viability was assessed by means of MTT and LDH method. Obtained results indicate that tested compounds were not toxic to neurons, astrocytes and oligodendrocytes. Moreover, a prominent neuroprotective effect was observed in neuronal cultures exposed to trophic stress and glutamate. A putative glutamate antagonist activity of tested aminothiadiazole derivatives is suggested.

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Effect of CDP-choline and erythropoietin on ultrastructural characteristics of neurodegeneration in a model of slow glutamate excitotoxicity *in vitro*

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Glutamate excitotoxicity is a well-documented pathogenic factor of neurodegeneration that is considered to contribute to the development of amyotrophic lateral sclerosis (ALS).

The aim of this study was to determine the potential neuroprotective effects of CDP-choline and erythropoietin (ER) on the glutamate-mediated injury of motor neurons (MNs) *in vitro*. The study was performed on organotypic cultures of the rat lumbar spinal cord. The well-differentiated cultures were incubated with 100 μM of specific glutamate uptake blocker, DL-threo-beta-hydroxyaspartate (THA) or were pretreated with one of neuroprotective agents, i.e. 100 μM of CDP-choline or 5U/ml of ER and then were subjected to 100 μM of THA. After 24 hours, 3, 5, 7, 9 and 14 days post expositions the cultures were processed for electron microscope and examined in JEOL 1200EX.

Ultrastructural study evidenced that THA exposition induces selective degeneration of MNs character-

ized by various types of morphological changes, including necrosis, apoptosis and autophagocytosis. The cultures pretreated with CDP-choline or ER exhibited less severe neuronal injury. Pretreatment with CDP-choline induced inhibition of the development of late apoptotic neuronal changes, whereas the early necrotic THA-induced injury as well as autophagic degeneration of MNs was still observed. The cultures exposed to ER + THA showed inhibition of early MNs degeneration, including various mode of degenerative changes typical for THA effect. However, in later period (7–9 days) of observation the delayed necrotic changes of MNs, typical for excitotoxicity, were seen. The results of this study indicate that both CDP-choline and ER exert neuroprotective effect in the investigated model of chronic excitotoxicity mainly through prevention of apoptotic neuronal changes.

Genetic impact on the etiology of Tourette's syndrome

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Tourette syndrome (TS) is a neurological disorder characterized by chronic motor and vocal tics. Genetics of TS remains still unclear. So far, there is no drug for the efficient therapy. The genetic background of TS seems to be rather complex and multiple genes are believed to be implicated in causing the disorder. Different SNPs within candidate genes in dopaminergic and serotonergic pathways (MAO-A, DAT1 and TPH2)

can influence TS etiology. The inversion defect on chromosome 13 and pathogenic mutations of SLITRK1 gene are probably responsible for rare familial cases of TS. Recently, age-related changes in gene expression in NK cells and T-lymphocytes were observed in TS patients suggesting that immune abnormalities may be also important in the etiology of the disease. Probably the majority of patients are determined by

still poor known DNA sequence heterogeneity (e.g.: copy number variations and microdeletions) in such genes like: CNTNAP2 or IMMP2L.

We selected 78 children with Tourette syndrome diagnosed according to DSM-IV-TR criteria during their hospitalization at the Dept. of Developmental Neurology, University of Medical Sciences in

Poznań, in a range of age from 6 to 18 years. The first step of our studies includes a variability testing of MAPT and BDNF genes. Genetic candidate gene association analysis has been planned to perform in cohorts of patients and healthy controls. Both a preliminary data and a possible role of the genes in TS etiology will be presented and discussed.

Ultrastructural alterations of A β PPsw-transfected PC12sw cells; correlation to an intracellular A β deposition and the level of GSK-3 β -P(Y216) phosphorylated form

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Herein we demonstrate that PC12 cells, which over-express human wild-type amyloid- β precursor protein (A β PPwt) or A β PP bearing double Swedish mutation (A β PPsw), reveal phenotype characteristic for Alzheimer's disease (AD). The examination of cell ultrastructure showed the presence of peptide aggregates within the cells, activation of endosomal-lysosomal system and extensive exocytosis. Furthermore, the autophagy induction was also characteristic hallmark of amyloid- β -induced cytotoxicity. Morphological changes were positively correlated with the extent of phosphorylated glycogen synthase kinase-3 β (phospho-Tyr²¹⁶-GSK-3 β , GSK-3 β -P(Y216)). The activity

of GSK-3 β is believed to cause tau protein hyper-phosphorylation, increased amyloid- β production and local plaque-associated microglial-mediated inflammatory responses. All of them are symptomatic for AD. In our studies, the highly significant Y216 phosphorylation and over-expression of total GSK-3 β were observed in A β PPsw-transfected PC12 cells. In addition, the immunocytochemical analysis showed co-localization of GSK-3 β -P(Y216) and amyloid- β deposits. Thus, our data support a functional role of GSK-3 β in A β PP processing, further implicating this kinase in the amyloid- β -dependent pathogenesis.

Ultrastructural evidences of amyloid β -induced autophagy in A β PPsw-transfected PC12 cells

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Herein we demonstrate that PC12 cells overexpress human amyloid β precursor protein bearing double Swedish mutation (A β PPsw), show the phenotype characteristic for Alzheimer's disease (AD). Examination of cells at ultrastructural level revealed the intracellular presence of peptide aggregates. Further-

more, the autophagy induction was found to be a hallmark of amyloid β -induced cytotoxicity. Importantly, autophagic vacuoles were co-localized within amyloid β (A β) deposits. It suggests the involvement of autophagy process in amyloid β -elicited cell degeneration.

Functional effects of neural cord blood stem/progenitor cell systemic transplantation after focal brain damage in rats

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The aim of the study was to compare therapeutic effectiveness of intra-arterial infusion of human umbilical cord-blood derived mononuclear (HUCB-MNs) cells at different stages of their neural conversion *in vitro*. Freshly isolated HUCB-MNs (D-0) neurally directed progenitors (D-3) obtained during 3 days culture of HUCB-MNs and neural stem cells (NSC) line derived from HUCB-MNs were assessed. Focal brain damage of dorsolateral striatum was induced in Wistar rats by stereotactic injection of previously established low dose of ouabain (1ul or 1,5ul 5mmol). Three days later 10⁷ HUCB cells were infused into carotid artery. Following surgery rats were housed in large enriched environment cages, in groups of 7–8 animals per cage, for 30 days observation period. Behavioral assessing consisted of tests for sensorimotor

deficits (walking beam task, rotarod, vibrissae elicited forelimb placing), cognitive impairments (habit learning task and object recognition test), exploratory behavior (open field test) and apomorphine induced rotations. Functional effects of different subsets of HUCB cells shared substantial diversity in various behavioral tests. This makes difficult to conclude which stage of neural conversion of cord blood cells is the most effective in functional recovery. Thus the scores concerning positive effects of cells treatment visible in all parameters were calculated. The sum of scores revealed that the most effective in functional restoration and reduction of lesion volume were freshly isolated D-0 HUCB cells.

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Analysis of the 5' upstream regulatory sequence of the PMP22 gene in a group of Charcot-Marie-Tooth type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP) affected patients

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The gene dosage effect has been previously reported in some patients with Down syndrome, Rett syndrome or in some cases of Alzheimer's disease or Parkinson's disease. In CMT disease gene dosage mechanism is a key regulator of clinical variability observed in CMT1A/HNPP patients. Duplication (CMT1A) or deletion (HNPP) of the PMP22 gene is associated with increased or decreased expression of the PMP22 gene product.

The gene dosage in CMT disease may be regulated by the sequence variants in regulatory sequence of PMP22 gene.

To test this hypothesis, we have sequenced a 5' upstream regulatory sequence of PMP22 in which potential binding sites for transcription factors (TF), which are known to play a role in myelination, have previously been defined.

Sequence analysis were performed on 50 CMT1A/HNPP patients harboring duplication/deletion of the PMP22 gene and 50 ethnical matched controls.

The genetic analysis has revealed a sequence variant -612 C > T in 22 patients and in 24 healthy individuals from control group. No sequence variants have been found in the TF binding sites.

We conclude that the sequence variants in the TF binding sites in the PMP22 regulatory sequence are not associated with PMP22 gene dosage.

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Ammonia interferes with permeability and proliferation of rat brain endothelial cells in a RBE-4 cell line

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Ammonia is a neurotoxin involved in the pathomechanism of hyperammonemic encephalopathies (HA) including hepatic encephalopathy (HE). While the effects of ammonia on the metabolism and function of astrocytes and neurons are well documented, the response of cerebrovascular endothelial cells forming the blood-brain barrier (BBB) has not been examined in detail. In this study we tested the effects of ammonia on cell permeability and proliferation in a rat brain endothelial cell line (RBE-4), an *in vitro* BBB model. Treatment of the cells with 5mM ammonium chloride for 24h significantly increased the permeability of the cells to FITC-labeled dextran, and decreased their

proliferation as assessed in a Coulter counter. The ammonia-induced permeability of the cells, but not proliferation, was attenuated by C-type natriuretic peptide (CNP) and the effect appeared to be partly mimicked by a cGMP analogue (8-Br-cGMP), indicating that CNP may affect the former process by activating the natriuretic peptide receptor B (NPR-B)/cGMP/PKG pathway. The results document the potential of ammonia to interfere with cerebrovascular endothelial cell physiology and indicate that HE may be related to altered BBB function.

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The possible involvement of extracellular matrix (ECM) components and MMPs in the development of neural stem cells from human umbilical cord blood

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Matrix metalloproteinases are recognized as proteolytic enzymes that are involved in the remodelling of extracellular environment during stem cells development. The aim of our study was to investigate if the application of extracellular matrix components like: laminin, fibronectin, and collagen promote MMPs activity (MMP-9 and MMP-2) which might be correlated with enhanced cell proliferation and differentiation. Methods. The cells of HUCB-NSC were cultured without serum for two weeks on extracellular matrix components-coated plates. On 4th, 8th, and 14th day we performed proliferation assay, and determination of MMPs activity (in situ zymography) followed by immunocytochemistry with specific neural markers. Our results show that among all of the investigated ECM components fibronectin stimulated most intensively cell proliferation and differentiation, espe-

cially toward neurons. We also observed the increase of MMPs activity (~20% increase in the 2-weeks culture) in the presence of fibronectin. To confirm the influence of MMPs on developmental processes of neural stem cells we used inhibitors of MMPs – GM6001, and doxycycline. We found that the inhibition of MMPs by GM6001 decreased cell proliferation (~30%) and differentiation into neurons (~20%). As it was shown in the present study the fibronectin occurred to be the potent factor in promoting cell proliferation and differentiation and support the idea that MMPs may contribute to the mechanism involved in the development of neural stem cells.

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Effects of adenosine A_{2A} antagonist, KW6002 on the markers of neurodegeneration in rats chronically infused with MPP+ into the cerebral ventricle through Alzet osmotic minipumps

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by a loss of dopaminergic neurons in the substantia nigra (SN), and a drop in dopamine (DA) level in the striatum (CP). The current pharmacotherapy (L-DOPA) constitute "the DA replacement" strategy, however, it does not halt progression of the degeneration process. Recently antago-

nists of adenosine A_{2A} receptors have been shown to improve motor features of PD and counteract the loss of dopaminergic neurons in the SN in an acute/sub-chronic animal models.

We searched for the neuroprotective effects of 28-days administration of KW6002 (3 mg/kg once a day, po), an adenosine A_{2A} antagonist, in the chronic

model of early stages of PD in which rats received 28-days, constant infusion of MPP⁺ iodide (0.284 mg/kg/day) into the cerebral ventricle using an ALZET osmotic minipump. The KW6002 reversed partially the loss of DA and its metabolites (DOPAC, HVA) in the CP, ipsilateral to the infusion side. This compound reversed also the decrease in the number and density of TH-ir neurons in the SN on the lesioned side estimated stereologically. Additionally the effect of chronic po injection of KW6002 on mRNA expression for adenosine A_{2A}, dopamine D₂ receptors

in the CP as well as BDNF mRNA in the hippocampus will be presented.

The present results confirmed the putative neuroprotective effects of A_{2A} receptor antagonist, KW6002 in the chronic model of MPP⁺ infusion, in which a mild degeneration in the dopaminergic nigrostriatal system is seen and when pathological processes are in progress.

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The involvement of signal transduction from extracellular matrix in ischemia-induced neurogenesis

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Recent *in vitro* studies indicate the involvement of metalloproteinases (MMPs) in the regulation of proliferation and differentiation of neural progenitor cells by providing an environment that is instructive and/or permissive to stem cells activation. To elucidate if MMPs participate in neurogenesis-associated processes after transient ischemic insult *in vivo*, we aimed to establish spatial and temporal relationships between neural stem-cell development and the expression of MMPs on the level of enzymatic activity in the adult hippocampal dentate gyrus as well as in the ischemia damaged CA1 sector. We also checked if MMPs activation may be involved in the modulation of intracellular signaling pathway. To approach this problem we aimed to quantify the activity of non-receptor protein kinases: FAK and PYK-2. Our results show that post-ischemic acceleration in the proliferation of progenitors in the dentate gyrus is accompanied by increased activity of MMPs. The most pro-

nounced activation occurs in the nucleus of newborn neurons and might be associated with proteolysis of transcription factors. This indicate that MMP-2 and -9 might be involved in neurogenesis-associated processes. In contrary, the endogenous neurogenesis in the damaged CA1 pyramidal layer seems to be rather elusive. Despite the appearance of BrdU/NF200-positive cells, the newborn neurons did not attain maturity. It is worthy to point that in this structure the activity of MMPs decreases below the control level. Simultaneously, the marked activation of MMPs appears in astrocytes in neighbouring structures (stratum oriens and stratum radiatum). The fluorescence signal is visible in the cytoplasm as well as outside the cell bodies and is probably associated with tissue remodeling and delayed repair processes. In conclusion, our results show that MMPs may, at least in part, contribute to ischemia-induced neurogenesis in the dentate gyrus of the adult brain.

Receptor mediated versus non-specific interactions of HUCB-NSC with biomaterials: effect on crucial developmental processes

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The proper interactions of the stem cells with the components of their microenvironment are crucial for the harmonized growth and development. To study the mechanisms underlying this interactions and their effect on such developmental processes as, adhesion, migration and proliferation, human cord blood-derived neural stem cells (HUCB-NSC) were grown on diverse biofunctional domains. The growth platforms with bioengineered surfaces were obtained by the microcontact printing technique, which allows creating spatial arrangement of functional domains of either polyaminoacids or ECM proteins responsible for un-specific (electrostatic) or specific (receptor-mediated) types of cell adhesion, respectively. We have found that HUCB-NSCs were immobilized to the surface upon the biomolecule dependent manner. While poly-L-lysine allowed the maintenance of non differentiated cells, fibronectin promoted their spreading

and stronger adhesion. The migration observed in the time-laps experiment was extensive and easy to follow on poly-L-lysine array, while on fibronectin the cells were immobile. To test cell proliferation the different serum and plating conditions were investigated. In any case the proliferation of HUCB-NSCs was dependent upon the type of substrate. It was higher (number of Ki67-positive cells) on PLL than on fibronectin. We assume that above cell behavior may be due to the non-stable anionic-cationic interactions between cells and polyaminoacids, as compared to their stronger anchoring to fibronectin RGD binding sites of integrin receptors. These results indicate that the type of interactions between neural stem cells and biomaterials has to be considered in the case of applying biomaterials in the stem-cell based biomedical and toxicological studies.