# Posters

## Mutations of the SNCA gene and alpha-synuclein level in the patients with diseases of the extrapyramidal system

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Parkinson's disease (PD) is one of the most common degenerative diseases of the extrapyramidal system and the incidence of this disease increases several times with age. It is believed currently that the cause of PD are environmental and genetics factors. An important genetic factors associated with disclosure of PD are SNCA gene mutations and polymorphisms of the region NACP-Rep1 promoter of SNCA, which may affect on the level of alpha-synuclein (ASN).

The aim of the study was analysis of G88C mutation and the NACP-Rep1 region in the promoter of SNCA gene and the study of ASN level.

The study was conducted on 78 individuals of the Polish population: 40 patients with diagnosed PD (18W and 22M, the average age 65 years), 4 patients with diagnosed multiple system atrophy (3W and 1M, the average age 59 years), 9 patients with diagnosed parkinsonism (5W and 4M, the average age 55 years) and 25 control volunteers without neurological symptoms and dementia (18W and 7M, the average age 57 years).

G88C mutation in the SNCA gene was analyzed by performing PCR-RFLP reaction, polymorphism of the NACP-Rep1 promoter region of SNCA gene was evaluated using PCR reaction and capillary electrophoresis. The ASN level in blood plasma was determined by ELISA.

The result of the conducted studies was the exclusion of the presence of the familiar grounds of PD, which are connected with the G88C mutation in the SNCA gene, both among 53 patients with diseases of the extrapyramidal system and 38 control volunteers. Moreover, it was shown that in the Polish population the allele 0, +1, +2, +3 and the haplotypes 0/+1, 0/+2, +1/+1, +1/+2, +2/+2 and +2/+3 of the SNCA promoter region can appear with a various frequency among patients with analyzed disorders of the extrapyramidal system. It was also shown that both the allele 0 and +1 and the haplotypes +2/+2 and +2/+3 of the promoter region SNCA gene can influence differently the disclosure of PD. It was also proved that level of ASN both among the patients with PD and with parkinsonism depends from the haplotype of the promoter region SNCA gene. The advancement and duration of the disease among patients with PD can have an impact on the level of ASN.

It seems that polymorphism of the NACP-Rep1 in the promoter region of SNCA gene influence on the level of ASN among patients with diseases of the extrapyramidal system.

# No association of the TNF alpha gene polymorphism in position -308 with susceptibility to MS

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TNF alpha may play a decisive role un the pathomechanism of multiple sclerosis (MS). Genes coding components of the immune system, as TNF alpha, are candidate genes in MS. The association between MS and polymorphism of the TNF alpha has been analyzed in several studies, but no definite conclusions have been reported. Our study material included 96 MS patients diagnosed according to the criteria of Mc Donald et. al. and 43 healthy control. The patients were in the relapsing-remitting phase of MS with clear-cut relapses of the disease. Genomic DNA was extracted from peripheral blood cells using a Blood Mini Kit. The frequency of polymorphism of the TNF alpha gene was determined by two stage polymerase chain reaction (nested PCR). In first stage PCR was to amplify a 519 bp of the TNF alpha promoter and the products were used in the second stage with specific primers (length products 443 bp). In our material the frequency of the genotypes GG, GA and AA were almost identical in MS cohort and in the control material. Comparing our results with those obtained in other geographical regions and ethnic groups it should be stressed the great similarity to those obtained in Bulgaria, Serbia, Sweden and Italy. Only in the Balcan countries (Croatia and Slovenia) the greater frequency of genotype GA in MS group was reported. Critical evaluation of above studies lead to the conclusion that there is no association between MS and the -308 GA polymorphism.

# PARP-1 inhibitors, tetracycline and docosahexaenoic acid protect hippocampal neurons against genotoxic stress

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The human genome is exposed to potentially deleterious genotoxic events during every cell division cycle. Cellular exposure to exogenous genotoxic agents leads to a variety of nucleotide modification and DNA damage which is thought to contribute to death. Poly (ADP-ribose) polymerase-1 (PARP-1) is a nuclear enzyme strongly activated in response to single and double-strand DNA breaks. The overactivation of the enzyme under different stress conditions leads to the depletion of its substrate, $NAD^+$ and in a consequence to the neuronal death. However, the last data show that the enzymatic product of PARP-1, Poly(ADP-ribose) (PAR) can play a significant and complex role in cell death signaling. PAR translocated to mitochondria acts as a signaling molecule and induces release of apoptosis inducing factor (AIF) and cell death. The exact mechanism of this novel pathway as well as the overall role of PAR in cell death has not been fully understood.

The aim of our study was to explore the relationship between AIF and PARP/PAR in death signaling in HT22 hippocampal neuronal cell line subjected to DNA genotoxic stress evoked by alkylating agent, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The

effect of PARP-1 inhibitors (3-aminobenzamide and PJ-34) on mitochondria function, AIF level and cell death were investigated. Moreover, the protective role of tetracycline third generation and docosahexaenoic acid was evaluated.

The genotoxic stress evoked by 100 uM MNNG in time dependent manner enhanced the level of PAR. Concomitantly, the protein level of AIF significantly decreased in mitochondria. Moreover, MNNG at high concentration causes PARP/PAR-dependent translocation of AIF from mitochondria into nucleus. In these conditions massive cell death was observed. Both PARP-1 inhibitors: PJ34 and 3AB as well as tetracyclines: doxoycycline and minocycline and also docosahexaenoic acid (DHA) protected most of cells against MNNG-induced death.

Conclusion: Our data show that PARP-1 inhibitors, third-generation of tetracycline and docosahexaenoic acid have positive effect on the mitochondrial integrity and function in genotoxic stress.

Supported by MS&HE grant NN401139938

# Role of bioactive lipids and PARP-1 in neuronal cells death evoked by glucose deprivation stress

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The mechanism of neuronal cell death evoked by hypoglycemia is not fully understood. Recent data suggest that activation of neuronal glutamate receptors, production of reactive oxygen (ROS) and nitrogen species, neuronal zinc release and mitochondria failure are involved in this pathology.

The aim of this study was to examine the role of lipoxygenases (5-LOX, 12/15-LOX) and poly(ADP-ribose) polymerase-1 (PARP-1) in ROS signalling and cell death induced by glucose deprivation (GD)/glucose reload (GR) in immortalized mouse hippocampal cell line (HT22). We determined the effect of LOXs, PARP-1 and NADPH oxidase inhibitors on HT22 cell survival. Our data indicated that 6 h GD caused HT22 cells death by about 20–60% in a time dependent manner. About 30% of HT22 cells died after 6 h GD followed by 24 h GR. Lipoxygenases inhibitors such as AA-861 (5-LOX and 12/15-LOX inhibitor) and zileuton (5-LOX inhibitor) protected 15% of cells exposed to 6 h GD. In additional experiments an NADPH oxidase inhibitor, apocynin exerted its ameliorating effect on cells survival. Also the inhibitor of PARP-1(PJ-34) significantly enhanced cell survival by about 15%.

Moreover, our data indicated, that third class generation of tetracycline antibiotics such as minocycline and doxycycline had significant cytoprotective effect against metabolic oxidative stress.

All investigated inhibitors have relatively similar effect on cell protection which indicates that several molecular events might be concomitantly involved in mechanisms of GD/GR cell death.

# The level of cytokines and features of inflammation in rat brain after oral exposure to polychlorinated biphenyls and brominated flame retardants

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Epidemiological studies have demonstrated that human exposure to polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs) during development causes long-lasting changes in nervous system functioning, and these findings are supported by experimental studies in several animal models. Tetrabromobisphenol-A (TBBPA) is a main representative of a large group of brominated flame retardants (BFRs), which are additives to plastics, textiles and electronic equipment to reduce their combustibility. Aroclor 1254, one of the PCBs, has been extensively used and dispersed from industrial applications worldwide and it is ubiquitous in the environment. Our study was designed to assess the effects of developmental exposure to PCBs and BFRs on the profile of cytokines in immature rats brain. Aroclor 1254 and TBBP-A were administered to the rats by oral gavage for two weeks. Exposure to Aroclor 1254 induced inflammatory response in rats, significantly elevating proinflammatory cytokines IL-6 (25%), IL-1beta (50%) and TNF-alpha (45%) over control values. We also observed increase in the level of these cytokines in rats exposed to TBBP-A (IL-6 – 60%; IL-1beta – 30% and TNF-alpha – 30%). These changes were associated with microglial activation. Light and electron-microscopic studies revealed morphological features in microglial cells connected with the activated state which were characteristic for both groups of exposed animals. Obtained results indicate that activation of microglia with coexisting inflammation is a common mechanism of PCBs and BFRs neurotoxicity in immature rat brain.

This study was supported by grant no. NN401024635 from Polish Ministry of Science and Higher Education.

# Sphingosine kinase(s) and their role in alpha-synuclein secretion and toxicity

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Sphingosine kinases (SK1 and SK2) are conserved lipid kinases that catalyze formation of sphingosine-1-phosphate (S1P). These enzymes are involved in regulation of wide spectrum of biological processes including calcium mobilization, cell growth, survival, differentiation, motility, cytoskeletal organization and cell death. It is strongly suggested that SK1 could play a critical role in Aβ-induced neuronal cell death. However, little is known about the disturbances of sphingolipids homeostasis in Parkinson's disease (PD) and in α-synuclein (ASN) toxicity.

The aim of the present study was to evaluate the role of SKs in expression, secretion and toxicity of ASN. Also the involvement of SKs in PC12 cell sur-

vival differentiation was analyzed. Moreover, the effect of exogenous ASN on the expression of SKs and S1P receptors was determined.

Our data indicate that inhibition of SKs significantly decreases PC12 cells survival, induces apoptotic cell death and suppresses NGF-dependent differentiation. In addition, inhibition of SKs abolishes the protective effect of S1P receptor agonist (FTY720) on cell viability. Our study demonstrates that inhibition of SKs induces secretion of ASN from synaptoneurosomes into extracellular space and enhances ASN liberation evoked by oxidative stress. Furthermore, ASN in oligomeric form decreases PC12 cells survival in concentration dependent manner. In addition, extracellular ASN decreases expression of SK-1 but not SK-2 and affects S1P receptor (R1) expression. These data present the relationship between ASN and sphingolipids signalling which leads to the vicious circle of events and to cells degeneration and death.

Supported by MS&HE grant NN401587040

# Cerebral microvessels and microglia in rat subjected to EAE over-express P2X7 purinergic receptor

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Blood-brain barrier (BBB) is a structure built by tight junctions between endothelial cells of microvessels that maintains central nervous system homeostasis by isolating it from external factors. One of the important proteins, Claudin5, is often used as a marker of BBB state. In pathological conditions (i.e., inflammation) the structure of BBB is loosened and he immune cells have a free access to the brain and the spinal cord. That is the main mechanism of pathogenesis in multiple sclerosis (MS) and its rodent model – experimental autoimmune encephalomyelitis (EAE). Activated T lymphocytes produce proinflammatory cytokines and degrade myelin together with activated microglia. Purinergic receptor P2X7R is suspected to be a part of neurodegeneration mechanisms present in MS/EAE. It participates in maturation and release of proinflammatory cytokines and may create transmembrane pore which drive to cell death.

In this study we present results of P2X7R expression analysis in microvessels and microglia in rat brain and the status of blood-brain barrier during development of EAE.

Western blot analysis performed with brain homogenates revealed the increased level of proinflammatory cytokine IL-6 starting from day 6 post immunization (p.i.) with the maximum level at day 10 p.i. Expression of Claudin5 significantly decreased only at day 4 p.i. comparing to the control level. However, in brain microvessels fraction expression of this protein decreased significantly in all examined time points, reaching the minimum level at days 2 p.i. and 4 p.i. The tendency towards increased P2X7R expression in early stage after immunization and significant increase in the stage of neurological symptoms (10 d.p.i.) was observed. Microscopic analyses of microvessels smears show decreased level of Claudin5, especially at days 4 and 6 p.i., confirming the western blotting results.

Microscopic analysis of brain sections obtained from control and EAE rats revealed morphological changes in microglia characteristic for activated state which correlated well with overexpression of P2X7R and increased level of proinflammatory cytokines.

The overall results show early overexpression of P2X7R connected mainly with activated microglial cells and to a smaller extent with brain microvessels where it is negatively correlated with Claudin5 expression. The pattern of observed changes suggests contribution of P2X7R into pathological mechanisms connected with development of EAE.

# An involvement of G-protein-coupled receptor 30 in neuroprotective effects of daidzein

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Daidzein is a plant-derived isoflavone which binds to estrogen receptors with selective estrogen receptor modulator (SERM) properties. SERMs represent a class with a growing number of compounds that act as either estrogen receptor agonists or antagonists in a tissue-specific manner distinct from this of estradiol. Phytoestrogens prevent neuronal damage, however, mechanism of their neuroprotective action has not been fully elucidated. This study aimed to evaluate the role of newly identified G-protein-coupled receptor 30 (GPR30) in daidzein effects on glutamate-induced apoptosis in mouse primary neuronal cell cultures. Glutamate (1 mM) enhanced caspase-3 activity and lactate dehydrogenase-release in the cerebellar neurons in a time-dependent manner, and these data were confirmed at the cellular level with Hoechst 33342 and calcein AM staining. Daidzein (0.1–10 microM) inhibited glutamate-induced apoptosis and neurotoxicity. Specific estrogen receptor and GPR30 antagonists: MPP, PHTPP, and G-15, reversed daidzein effects. However, a high-affinity estrogen receptor antagonist, ICI 182,780, and GPR30 specific agonist, G-1, potentiated daidzein neuroprotection. These data point to neuroprotective effects of daidzein at low micromolar concentrations against glutamate-evoked apoptosis and provide evidence for involvement of GPR30-mediated signaling pathway in anti-apoptotic action of daidzein.

This work was supported by the Polish Ministry of Education and Science grant No. N N401 572138.

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

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Multiple Sclerosis (MS) is human autoimmunological disease that cause neurodegeneration. Experimental Allergic Encephalomyelitis (EAE) is the animal model of MS. The autoimmune base of the disease caused treatment searching in immunological mechanisms. For now most MS treatments are based on general immunosupression. This method is not very effective and costs number of health complications. For that reason, it is needed to find more specific and effective method for MS treatment. Few years ago we proposed application of animal spinal cord hydrolysate for inducing oral tolerance. We presented the effectiveness of this type of treatment in EAE rat model. The success of oral tolerance with mixture of peptides stimulates us to development bacteria that may express active peptide related to myelin fragment. We used three strains of *Lactococcus lactis*, normal, laboratory strain (L), strain which is autolis-

ing in the alimentary duct (A), and strain which is colonising the alimentary duct (C). Each of them is expressing one of the myelin peptides: Myelin Basic Protein (MBP aa85-97), Proteolipid Protein (PLP aa 139-151) and Myelin Oligodendrocyte Protein (MOG 35-55).

The aim of our study was to investigate, if feeding animals with *Lactococcus lactis* expressing myelin peptides is sufficient treatment for EAE due to evoking oral tolerance.

Supported by N302 009 32/1139 grant..

# Mitofusin deficiency affects cellular energy metabolism

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Mitofusin 2 (Mfn2) is a nucleus-encoded protein located in the outer mitochondrial membrane, which is crucial for mitochondrial fusion. In recent years Mfn2 involvement in pathophysiology of diabetes and vascular proliferative disorders has been revealed. However, most attention is paid to Mfn2 role in inherited neuromyopathy known as Charcot-Marie-Tooth 2A disease (CMT2A). MFN2 gene mutations are considered as a main cause of axonal loss observed in CMT2A. Finding the molecular mechanism of cell damage in absence of functional MFN2 would be crucial for CMT2A diagnosis and therapeutic strategies. Disturbances in mitochondrial fusion result in irregular mitochondria localization, alteration in mtDNA exchange between mitochondria and might lead to accumulation of mtDNA mutations. We assume that Mfn2 can influence mitochondria energetic metabolism, thus bioenergetics failure is considered to play a role in cell death in CMT2A. In this study we investigated effects of Mfn2 absence on cell respiration and mitochondrial and actin network architecture. Experiments were performed using three lines of Mouse Embryonic Fibroblasts: wild-type (MEFwt), cells lacking Mfn2 ($MEF^{Mfn2-/-}$) and cells lacking both isoforms of mitofusin ($MEF^{Mfn1-/-Mfn2-/-}$). Oxygen consumption was measured polarographically with the use of OROBOROS-2k oxygraph respirometer. It was found that the respiration rate of $MEF^{Mfn1-/-Mfn2-/-}$ cells was substantially lower than that observed in MEFwt. This observation was in line with the results of western blot analysis of selected respiratory chain proteins and mitochondrial ATP synthase subunits (MitoProfile® Total OXPHOS Rodent WB Antibody Cocktail, MitoScience). Effects of Mfn2 deficiency ($MEF^{Mfn2-/-}$) on oxygen consumption and oxidative phosphorylation proteins profile were less obvious and need further investigation. Confocal microscopy analysis of the mitochondrial network organization shown that lack of both Mfn isoforms correlates with exclusively perinuclear localization of mitochondria while in the wild-type cells mitochondrial network is spread throughout the cell. In $MEF^{Mfn2-/-}$ cells mitochondria almost exclusively localize to the cellular body but do not penetrate more distal cellular endings. Interestingly, $MEF^{Mfn1-/-Mfn2-/-}$ cells have affected actin cytoskeleton architecture, however any explanation of this observation has not been proposed so far.

This project is supported by MSHE grant N N402 474640.

# Depressive-like behavior in animal models of Parkinson's-disease

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Depression is a frequent comorbid disturbance in course of Parkinson's disease (PD) which may precede appearance of its motor symptoms for several years. Pathomechanisms underlying PD could be also partially responsible for the PD-related depression. We injected different doses of selective toxin 6-OHDA into the striatum creating distinct sizes of dopaminergic terminals lesions reflecting early stages of PD and examined their influence on the "depressive-like" behavior of rats in the forced swimming (FS) test. After two different doses (3.75 mg and 7.50 mg per 2.5 ml of 0.2% ascorbic acid) we observed dose-dependent changes: showing increased immobility times and reduced swimming activities in FS test while no motor deficits in automated general locomotor activity analysis. Those "depressive-like" symptoms observed 2 weeks after the treatment disappeared after 4 weeks suggesting active compensation of the lesions.

The levels of dopamine were decreased in the striatum and nucleus accumbens, and the density of dopaminergic neurons counted stereologically were lowered in the substantia nigra.

Densitometric analysis of the dopaminergic terminals in the striatum stained for tyrosine hydroxylase showed the lesion localized mostly in the ventrolateral part.

The presented results may indicate that a relatively small lesion of dopaminergic terminals in the ventral striatum, which does not produce any motor disturbances may induce "depressive-like" symptoms and involve compensatory mechanisms. This also corroborates the thesis that depression could be the preclinical symptom of PD.

The study was supported by the Project "Depression-Mechanisms-Therapy", co-financed by EU from the European Regional Development Fund as a part of the Operational Programme "Innovative Economy 2007-2013".

# The effects of INF-β1a and INFβ1b on proinflammatory cytokines concentration and demyelination process in brain cortex of experimental autoimmune encephalomyelitis

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Amelioration of experimental autoimmune encephalomyelitis (EAE) by treatment of interferon beta has been recently demonstrate. However, the mechanism underlying regulation of cytokines concentration in EAE treated with INF-β are unclear. The aim of this study was to investigate the effects of INFβ-1a and INFβ-1b treatment on inflammatory factors as IL1β, IL-6, TNFα, nitric oxide concentration and protein

level of myelin in brain cortex of the Levis rat EAE that is an animal model of multiple sclerosis (MS). To induce EAE, rat were immunized with inoculums containing spinal cord guinea pig homogenized in PBS and emulsified in Freund's complete adjuvant (CFA) containing 110 μg of the appropriate antigen in 100 μl of an emulsion and additionally 4 mg/ml *Mycobacterium tuberculosis* (H37Ra). The animals were observed daily for monitoring of neurological deficits and weight. The rats were treated three times per week with subcutaneous applications of 300,000 units INFβ-1a or INFβ-1b. The treatments were started 8 days prior to immunization and continued until day 14 after immunization. The rats were sacrificed on the 14$^{th}$ day of the experiment. EAE induced dramatic increased IL1β, IL-6 and TNF-α concentration and iNOS expression in brain which corresponded closely with the course of neurological symptoms and loss of weight. These changes in cytokines concentration and iNOS expression were suppressed by treatment with both isoforms of interferon β. In EAE rats, treatment with INFβ-1a and INFβ-1b also attenuated EAE neurological symptoms and inhibited demelinization process. These results suggest that INFβ-1a and INFβ-1b prevent of inflammation processes which might play a critical role in pathological changes and neuronal dysfunction in EAE.

Supported by the MS&HE Grant No. NN401620038.

# The levels of apoptotic factors in epilepsy patients treated with antiepileptic

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Treatment of epilepsy, one of the most frequent neurological disease, requires a long-term antiepileptic drug (AED) therapy. A number of studies have shown that these drugs may induce plenty of plasma molecular changes (level of homocysteine, asymmetric dimethylarginine, lipids, lipoproteins, vitamins and apoptotic factors).

Apoptosis is a programmed death of the cell. Caspases, proteolytic enzymes play the crucial role in apoptosis. One of them, active caspase-3 being the end stage of caspase transformation is directly responsible for DNA fragmentation thus being a good cell apoptosis indicator. Some of the research have indicated that valproic acid (VPA) may bring about apoptosis in many cancer cell lines by activation proteins such as: p53 and Bcl-2 family.

The aim of the study was to analyze the expression of the apoptotic proteins in peripheral lymphocytes (p53, Bax, Bcl-2) and the level of apoptotic cells in epileptic patients, before and during AED treatment [VPA, carbamazepine (CBZ) and lamotrigine (LTG) in monotherapy and polytherapy] and in controls.

In the study group were 23 epileptic patients at the age of 18 to 69, 20 of them were treated with AEDs, 3 before initiating the treatment of AEDs. Control group consisted of 22 individuals at the age of 22 to 61. The levels of apoptotic proteins in peripheral lymphocytes were analyzed by western blotting method and the level of lymphocyte apoptotic cells by detection of active caspase -3 by flow cytometry method.

The studies revealed elevated level of apoptotic cells in patients treated with AEDs, especially with CBZ and polytherapy, comparing to the control group. Simultaneously, lower level of p53 protein was observed in all epileptic patients. Significant differences were disclosed in apoptotic proteins expression depending on specific AED. In patients treated with LTG and in polytherapy higher Bcl-2 protein level

was accompanied by decreased Bax/Bcl-2 ratio. However, in patients with epilepsy treated with VPA increased Bax/Bcl-2 ratio was observed.

Our study indicated that in epileptic patients treatment AEDs increased the level of apoptotic cells in peripheral lymphocytes. The role of apoptotic properties of anticonvulsants needs further investigations.

# Simultaneous analysis of selected mutations in SNCA and PARK2 genes in patients with Parkinsons disease

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Early diagnosis of Parkinson's disease (PD) is often problematic. It is known, that PD is caused either by environmental and genetic factors, associated primarily with SNCA and PARK2 mutations. Until now in PD it have analyzed mutations of genes PARK2 (mostly in 2, 4, 7, 8 and 11 exons) and SNCA as well its promoter separate. In the literature there is no conclusive data concerning the interaction between SNCA and PARK2 genes and the proteins encoded by SNCA and PARK2.

The aim of the study was to analyze mutations of PARK2 gene and polymorphism of the SNCA promoter together, in patients with PD (37 subjects, average age 58 years) and in controls (25 persons, average age 60 years).

DNA was isolated from peripheral blood of subjects using a kit of Novazym. Genetic testing was performed by PCR, capillary electrophoresis, HRM real-time PCR and sequencing.

Analysis of exons 2 and 4 of PARK2 gene detected no deletions both in PD patients, as well as in the control group, while the point mutations of PARK2 (G601A, C924T, G1281A) were identified in 7 (19%) PD patients compared to 1 (4%) control subject. Moreover, it was showed that allele +1 of SNCA gene promoter occurs frequently in healthy individuals and generally seems to have a protective role, while the variant 0 and haplotypes +2/+2 and +2/+3 occurred more frequently in PD patients and it is probably that predispose to PD.

Simultaneously, first time in this studies was demonstrated the coexistence of C924T PARK2 mutation and the haplotype +2/+2 of the SNCA promoter. It seems that coexistence of PARK2 mutations and SNCA promoter polymorphism may be associated with increased risk of PD and provide an alternative pathway in the manifestation of PD probably through enhanced alpha-synuclein (ASN) aggregation due to increased expression of ASN while parkin dysfunction. It seems that simultaneous analysis of SNCA and PARK2 genes mutation may be an additional diagnostic and prognostic factor in PD patients.

# Differences in blood-brain barrier structure as possible cause of distinct tumor susceptibility in Sadowski HA/LA mice

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In this study we examine distinct tumor susceptibility in Sadowski HA/LA mice. Specimens of HA line (HA – high analgesia), in comparison to mice of the LA (LA – low analgesia) line, display pathologies in blood – brain barrier (BBB) structure that may affect tumor growth rate trough infiltration of brain derived growth factors like BDNF and NGF into circular system.

Due to prolonged delivery in HA there is possibility of oxidative stress occurence that affect BBB structure.

In HA mice subcutaneous inoculation with B16-T0 melanoma cells into the plantar region of unilateral hind paw caused very fast tumor growth (after 2–4 days of inoculation). In LA mice, the tumor appeared only in 10% of examined mice and was considerably delayed in comparison to the HA line (after 2–3 weeks). Moreover, in the most of LA mice, the paw tumor displayed tendency to spontaneous regression.

Putting mice into chronic stress condition effected with similar results, but in both lines tumor appeared faster. Forced BBB opening in LA by mannitol administration also effected by rapid tumor growth.

# The isoleucine92valine variant in the LITAF/SIMPLE – gene modifier of clinical variability of Charcot-Marie-Tooth disease or a harmless polymorphism?

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Charcot-Marie-Tooth disease (CMT) is clinically and genetically heterogeneous disorder of the peripherial nervous system. Alterations of several genes have been associated with the disease and are thought to cause a variety of CMT phenotypes. Mutations in LITAF/SIMPLE gene have been associated with CMT disease. The exact function of the LITAF/SIMPLE protein is still under investigation, however, it is thought to play an important role in the regulation of expression of the TNF alpha gene and in ubiquitin-mediated protein degradation of PMP22. It has been suggested that the Ile92Val sequence variant in LITAF/SIMPLE gene may modify CMT phenotypeTo determinate the influence of the c.274A>G (Ile92Val) sequence variant on clinical variability of CMT affected patients we have sequenced the LITAF/SIMPLE gene in a group of 115 patients with familial and sporadic CMT disease and in control group consistsing of 50 unaffected individuals.

The genetic analysis has revealed the sequence variant c.274A>G (Ile92Val) in families in which we have aggregation of inherited form of neuropathy – CMT and some cases of acquired neuropathy i.e., chronic inflammatory demyelinating polyneuropathy (CIDP) or Guillain-Barre syndrome (GBS). We also detect the substitution Ile92Val in CMT1A affected

patients with duplication of the PMP22 gene and component of inflammatory neuropathy. In patients with hereditary neuropathy with liability to pressure palsies (HNPP) with deletion of PMP22 gene some cases with the variant Ile92Val seem to be atypical. The sequence variant c.274A>G has also been detected in control group with a frequency of 0.3.

In agreement with previously published date we suggest that the amino acid substitution Ile92Val in LITAF/SIMPLE may affect the clinical course of CMT.

# Mechanism of Glycogen Synthase Kinase-3β alterations by Alzheimer's disease amyloid β peptides

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The Glycogen Synthase Kinase-3β (GSK-3β) a multitasking serine/threonine kinase may play important roles in protein synthesis, cells proliferation, differentiation, microtubule dynamics and cell motility. The regulation of GSK-3β is a complex process that till now is not fully understood. Phosphorylation of GSK-3β on tyrosine 216 (Tyr216) is necessary for its activity but enhancement of phosphorylation on serine 9 (Ser9) leads to enzyme inactivation. The aim of this study was to investigate the short time effects of amyloid β peptide (Aβ1-42) oligomers on the phosphorylation of GSK-3β in PC12 cells line. This short term effect of extracellular Aβ1-42 was compared with long term action of endogenously liberated Aβ peptides in PC12 control cells and in stably transfected with human gene for Aβ precursor protein (APP) bearing double Swedish mutation (APPsw).

Our results demonstrated that Aβ1-42 (1 μM) added into PC12 cells for 24 h enhanced GSK-3β phosphorylation on Ser9 what could be responsible for lowering of its activity. This phosphorylation was decreased by the inhibitor of Cyclin Dependent Kinase 5 (CDK5) BML-259, indicating that CDK5 activity participates in mechanism of GSK-3β regulation. In APPsw cells long term liberation of Aβ peptides leads to lowering of GSK-3β phosphorylation on Ser9, enhancement of its activity and higher MAP tau Ser396 phosphorylation. This alteration of GSK-3β phosphorylation on Ser9 is connected with the lower activity of CDK5 in APPsw transfected cells.

Our data presented that CDK5 plays a crucial but opposite roles in regulation GSK-3β activity in PC12 cells during short and long term action of Aβ peptides. Moreover, our data indicated that independently of time and of exogenous or endogenous pool of Aβ, the prosurvival/adaptive processes and proapoptotic pathways are activated, and the proportion between them decided on the pool of cells that survive during different stage of Aβ toxicity.

# Combined blockade of mGluR1, AMPA and NMDA receptors effectively eliminates neurological dysfunctions in rats subjected to EAE

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Experimental autoimmune encephalomyelitis (EAE) is the main animal model used for the investigation of multiple sclerosis (MS) mechanisms. So far the etiology of MS is unknown. Recent studies suggest that glutamate neurotoxicity is involved in the pathogenesis of MS. The disturbance in glutamate level and changes in expression of mGluR GI receptors were observed in brains MS patients. It was suggested that glutamate production by macrophages might be involved in axonal damage and oligodendrocyte pathology in MS lesions. There is the possibility that activation of glutamate receptors contributes to the process of cell death in chronic neurodegenerative disorders. The aim of our investigation was to study the role of NMDA receptors and group I mGluRs in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), an animal model of MS. We tested the effect of MPEP (2-methyl-6-(phenylethynyl)-pyridine), the mGluR5 antagonist, in dose of 5 mg/kg b.w./day, and amantadine (the uncompetitive NMDA receptor antagonist) in dose of 100 mg/kg b.w./day on development of neurological deficits in EAE rats. The neurological symptoms of EAE started at day 10–11 post immunization (p.i.) and peaked at day 12–13. We noted the changes in body weight during the course of EAE. Starting from day 8 p.i. rats in all groups showed a progressive weight loss by about 20–30% until day 14 p.i. Application of amantadine was found to be effective and significantly reduced neurological symptoms of EAE. We did not observed neuroprotective effects of MPEP. The level of mGluR5 protein did not increase in early phase of EAE (4 day p.i.). However, starting from day 8 p.i. until day 25 p.i. we observed its significant elevation. The difference between control and examined group reached 20% at day 25 p.i. Our results confirm the involvement of glutamate into pathogenesis of EAE. Although we noted changes in the expression of mGluR5 during the course of EAE, MPEP was ineffective in reducing the symptoms of the disease. Results suggest the main role of NMDA glutamate receptors in the pathogenesis of EAE.

This work was supported by the Polish MNiSW grant N N401 620038.

# Cardio-respiratory effects of systemic neurotensin injection are mediated through activation of neurotensin NT1 receptors

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Neurotensin (NT) acts in the mammalian brain as a primary neurotransmitter or neuromodulator of the classical neurotransmitters. In the central nervous system neurotensin modulates dopamine signalling, is involved in opioid-independent antinociception, hypothermia, muscle relaxation, pituitary hormone secretion and is also engaged in controlling respiration. Neurotensin-immunoreactive cell bodies, fibers and nerve terminals have been found in the rat brainstem areas involved in the control of breathing i.e., nucleus

tractus solitarii, nucleus ambiguous, nucleus parabrachialis lateralis and Kolliker-Fuse nucleus. NT injected intracerebroventricularly in rats produced respiratory depression and apnea. In cats this neuropeptide applied on ventrolateral nucleus tractus solitarii evoked an apneustic pattern of the phrenic nerve discharges.

The objective of the present study was to determine the cardio-respiratory pattern exerted by the systemic injection of neurotensin. Further we tried to evaluate the role of the infra- and supra-nodose vagus nerve in the effects of neurotensin. Finally, the involvement of NT1 neurotensin receptors was examined.

Anesthetized, spontaneously breathing rats were used. Tidal volume was measured at tracheostomy. The timing components of the breathing pattern and arterial blood pressure were recorded. Intravenous injection of neurotensin at a dose of 10 μg/kg in the intact rats provoked an increase in tidal volume and short-lived acceleration of breathing, followed by the long-lasting slowing down. Changes in respiration were accompanied by a biphasic response in the blood pressure: prompt increase appeared at the end of drug injection being ensued by extended hypotension. Section of the midcervical lung vagi abrogated only the response of the respiratory rate. Following supranodose vagotomy neither tidal volume increase, nor blood pressure effects were precluded. Blockade of NT1 receptors with an intravenous dose of 500 μg/kg of SR 142948, significantly diminished post-NT cardio-respiratory effects.

This study shows that neurotensin acting through NT1 receptors augments the tidal component of the breathing pattern independent of the vagal afferentation. This latter mediates the respiratory timing responses to NT. Blood pressure effects evoked by an intravenous injection of neurotensin occur besides the vagal pathway and might result from activation of the peripheral vascular NT1 receptors.

# Cardiorespiratory effects of opioid and tachykinin pharmacophores chemically linked or separate in anesthetized rats

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Opioid drugs are the most effective analgesics but they induce respiratory depression and hypotension.

Substance P (SP) stands as a neuropeptide involved in pain transmission and is thought to be an excitatory mediator in the control of respiration.

The new drug termed AWL3106, recently synthesized, consists of two pharmacophores of antagonistic activities: analgesic – mu-opioid receptor agonist (dermorphin) and anti-analgesic – tachykinin NK1 receptor agonist (substance P(7–11)).

The aim of this study was to compare the cardiorespiratory pattern induced by an intravenous challenge with the chimeric peptide to that evoked by a mixture of two pharmacophores comprised in this drug.

We measured cardiovascular and respiratory parameters in 17 anesthetized Wistar rats that breathed spontaneously room air, and were subdued to an intravenous (*iv*) injection of 0.3 μmol/kg of AWL3106 or to a mixture of 0.3 μmol/kg of dermorphin and substance P(7-11) (D+SP(7-11)).

*Iv* injection of AWL3106: (i) evoked an apnea of mean duration of 5 s followed by 19% inhibition of breathing (f) and (ii) 23% augmentation of tidal volume (VT).

The mixture of 0.3 μmol/kg of dermorphin and SP(7-11) collated with peptide chimera induced (i) an insignificantly longer apnea (8 s). Post apneic breathing was of significantly less diminished frequency (13%) and VT showed no change.

Minute ventilation remained unaffected in both experimental conditions as a result of compensating increase in VT after AWL3106 or due to the markedly weaker bradypnea after SP(7-11) plus dermorphin.

Immediate fall in blood pressure induced by either compound and short-lived rise at 30 s was followed by hypotension reaching 40 percent of the baseline level.

AWL3106 had no effect on heart rate whereas D+SP(7-11) induced long lasting bradycardia.

This study has shown that pharmacophores comprised in AWL3106 applied as a mixture inhibited less the timing component of the respiratory pattern and had no benefit on tidal volume.

It seems possible that chemical binding of two compounds modifies the strength of their biological activity presumably as a result of change of receptor affinity.

This work has been partially supported by EC Grant "Normolife" (LSHC-CT-2006-037733).