Oral communications

The role of alpha-synuclein in dopaminergic system function and in molecular mechanisms of neurodegeneration

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Alpha-Synuclein (ASN) play a key role in pathogenesis of Parkinson's disease and is involved in the other age-related neurodegenerative disorders including Alzheimer's disease and dementia with Lewy bodies. However, its relevance in brain aging is unknown and it is far from clear how ASN contributes to cell death and neurodegeneration. Our study indicated age-related decrease of ASN expression and the level of soluble form of this protein with enhancement of its oligomerisation in the brain. This ASN conformational changes could affect synaptic function and promote initiation and progression of alpha-synucleinopathy. Concomitantly, is liberated from the nerve endings into extracellular space during oxidative/nitrosative stress that accompanied brain aging and neurodegenerative processes. The study showed the significant role of extracellular ASN in activation of free radicals cascade and in disturbances of voltage-operated calcium channels (VOCC) function in the brain. ASN-evoked Ca^{2+} influx through VOCC and N-methyl-D-aspartic acid (NMDA) receptor leads to activation of neuronal nitric oxide synthase (nNOS). NO-dependent inhibition of dopamine transporter (DAT) causes dopaminergic system dysfunction. In addition, the study carried out on dopaminergic PC12 cells presented ASN-dependent mitochondria failure and indicated that NO pool liberated by ASN activates caspase-3 and poly (ADP-ribose) polymerase-1 (PARP-1) cleavage. Inhibitor of NOS (NNLA), caspase-3 (Z-DEVD-FMK) and a mitochondrial permeability transition pore blocker, cyclosporine A protected cells against ASN evoked cells death, indicating these compounds as a potential therapeutic agent for slowing the progression of neurodegeneration. In summary, our results indicate that oligomeric form of ASN evokes NO-mediated dopaminergic system dysfunction and caspase-dependent programmed cell death.

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Dementia from mild form till Alzheimer disease

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Mutations in MAPT and PGRN in polish patients with frontotemporal lobar degeneration

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Frontotemporal lobar degeneration (FTLD), the second most common form of presenile dementia, is a heterogeneous disorder on clinical, pathological, and genetic levels. Previous studies have shown that mutations in two genes, microtubule-associated protein Tau (MAPT) and progranulin (PGRN) are major cause of FTLD. Mutations in MAPT are associated with tau-positive neuropathology and occur in familial FTLD in frequencies that vary in the range 5–50% between populations. Mutations in PGRN are major cause of FTLD with ubiquitin-positive brain inclusions (FTLD-U). PGRN mutation frequency ranged from 2% to 11% of FTLD cases worldwide, and from 13% to 25 % in a subpopulation of patients with familial FTLD.

The aim of the study was to screen genes for mutations connected with FTLD in a Polish cohort.

DNA sequence analysis of MAPT revealed a known, pathogenic mutation (P301L) in one patient. Screening for PGRN mutations revealed two different pathogenic mutations in two patients. A novel 2bp deletion was found in exon 11 (2988_2989delCA) in a patient with clinically diagnosed frontotemporal dementia (FTD) in the sixth decade of life. The mutation introduces a premature stop codon in position 444. The second mutation (Arg418X) diagnosed in a patient with FTD is a nonsense one, and was previously reported in connection with the same clinical phenotype.

Cell cycle regulation in immortalized lymphocytes differentiate sporadic from familial Alzheimer's disease patients

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Reactivation of the cell cycle (CC) in brain neurons seems to underlie development of Alzheimer's disease (AD). The aim of this study was to determine whether CC alterations can be detected in lymphocytes from patients in both sporadic and familial form of AD (SAD and FAD). Immortalized B-lymphocytes from 18 SAD patients, 8 FAD patients with mutations in presenilin1, and from 26 non-demented subjects, proliferated under standard conditions and their CC regulation was assessed by immunoblotting, real-time PCR-arrays, and flow cytometry. We found differences in the regulation of G1/S CC phases between SAD, FAD, and control cells. SAD cells compared to FAD and control cells showed high increase in the level of p21 protein, which promotes G1-arrest. Increase in cyclinE level occurred only in FAD cells. Expression profiles of the 92 CC genes also differentiated lymphocytes from sporadic versus familial patients, for example higher level of cyclinD1 mRNA was found only in SAD cells. CC phases were af-

fected accordingly: SAD, but not FAD cells, had longer G1-phase. These data show that SAD involves G1 prolongation pathway linked to p21, which is not activated in FAD lines. The inhibition of gamma-secretase did not change the CC profiles of any of the cell lines indicating that the aberrations observed in AD cells are induced by factors different than proteolytic products of this enzyme. Altogether, the data show that mechanism of SAD differs from FAD linked to PS1 mutations, and disturbances of CC pathways in lymphocytes might have a diagnostics value.

FTDP – 17 without parkinsonism in family with the tau gene (MAPT) mutation

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Mutations in the tau gene (MAPT) cause frontotemporal dementia with parkinsonism linked to chromosome 17(FTDP-17). We present results of two members of FTDP-17 family with behavior and cognitive disturbances without parkinsonism.

Two brothers, one aged 50 with suspicion of dementia and other, aged 48 with no evident clinical complaints underwent neurological, neuropsychological and MRI examination. We obtained consent and DNA was isolated from blood leukocytes using standard procedure. All MAPT exons with flanking intronic regions were sequenced. Patients' father, and his two brothers presented behavioral and cognitive deficits about their mid fifties, but were never diagnosed.

Neurological examination showed no deficits. In the neuropsychological evaluation cognitive disorders were found, suggesting underlying frontal and temporal pathology. Elder brother presented general dysfunction more apparent in his lack of control and insight, distractibility, executive and language functions deficits, with relatively spared delayed memory. He needs a supervision on activity of daily living. Younger brother showed only discrete, however significant deficits in immediate memory and verbal fluency. Neuroimaging results of elder brother showed asymmetric general cortico-subcortical atrophy, more dominant in the left hippocampus. Younger brother MRI revealed discrete frontal atrophy. Both brothers' DNA sequence analysis of MAPT revealed a C to T missense mutation in exon 10 causing a proline to leucine change at codon 301 (P301L) in the microtubule binding domain.

Further observation of all members of the family is needed. Molecular, neuropsychological and neuroimaging testing is essential in familial neurodegenerative diseases research.

The role of AIF and PARP-1 in Amyloid β cytotoxicity

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Our previous results indicated that Alzheimer's disease related Amyloid β peptides induced toxicity by nitric oxide (NO) and Phospholipase A_2 signaling leading to oxidative stress, DNA damage, activation of poly(ADP-ribose) polymerase-1 (PARP-1), mitochondrial failure and release of apoptosis inducing factor (AIF).

The role of AIF and PARP-1 in Aβ cytotoxicity mediated by cyclooxygenases (COX) and lipoxygenases (LOX), was investigated in PC12 cells expressing Aβ precursor protein, wild-type (APPwt) or bearing Swedish mutation (APPsw).

We found the close relationship between Aβ level and COX-2/12-LOX expression/activity, nuclear translocation of p65/NF-κB and enhancement of mitochondrial AIF level in APPsw cells. However, in this cells PARP-1 inhibition was observed.

To better understand the role of NO in Aβ toxicity the effect of sodium nitroprusside (SNP) was investigated. SNP (0.5 mM), caused 80% of all cell types died after 24h without changes in mitochondrial AIF level and PARP-1 activity. COX and LOX inhibitors ameliorated SNP-evoked cell death.

We conclude that AIF accumulation may be responsible for the survival of APPsw cells under oxidative stress (Aβ and 0,1 mM SNP). Inhibition of PARP-1 activity may affect DNA-repair. COX and LOX inhibitors protected the cells against nitrosative stress-induced death.

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Genetical and biochemical alterations in the brain during systemic inflammation

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Many neurodegenerative disorders of the Central Nervous System (CNS), including ischemia, Parkinson and Alzheimer's diseases involve mechanisms of local inflammatory reaction. Activation of glial cells may be beneficial, however, in many pathological conditions, improper activation of inflammation in CNS may occur, what has detrimental consequences. Recent studies indicated that also systemic inflammatory response (SIR) may significantly affect brain function. SIR induces release of many active molecules in the brain, alters cerebral blood flow and impairs cerebral metabolism, leading to activation of cell death pathways. Our previous studies indicated that during lipopolysaccharide (LPS)-evoked SIR, free radical-evoked oxidative damage of lipids and proteins occurs that impairs function of membranes and several enzymes. DNA damage leads to activation of Poly(ADP-ribose)Polymerase-1 (PARP-1) that subsequently may influence expression of a panel of genes. The profile of gene expression was analyzed for identification of genes involved in brain dysfunction and for finding the most important signaling

pathways activated in the brain by SIR. Then, the overactivation of PARP-1-related PAR formation and release of Apoptosis Inducing Factor (AIF) was found to be responsible for observed by us activation of apoptosis in midbrain and hippocampus. Moreover, Cathepsin B-related autophagy was also activated. These biochemical alterations were accompanied by disturbances of cell ultrastructure, including formation of autophagolysosomes, swelling of synapses and mitochondria. Subsequently, these all changes may be responsible for impairment of cognitive function.

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Effect of adenosine A_{2A} receptor antagonists on hydroxyl radical generation in reserpinized rats

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Although the cause of DA neurons neurodegeneration is still unknown, oxidative stress is paramount in the pathogenesis of Parkinson's disease. An accumulation of cytosolic DA has been shown to be neurotoxic through the generation of free radicals (FR). Searching for FR scavengers, we studied the effect of selective A2A adenosine receptor antagonists, shown to have neuroprotective properties, on hydroxyl radical (•OH) production in rat striatum with reserpine impaired DA storage. We found an increase in extracellular glutamate and •OH levels in DA-depleted striatum. CSC (1 mg/kg), ZM 241385 (3 mg/kg) and L-DOPA (25 mg/kg) normalized glutamate release and combination of A2A antagonists and L-DOPA showed similar effect. CSC increased DA and •OH levels but ZM 241385 given alone did not affect DA nor •OH levels. L-DOPA enhanced DA extracellular level but did not change the production of •OH. Combination of L-DOPA and CSC further elevated DA extracellular level and markedly increased •OH production while combination of L-DOPA and ZM 241385 attenuated DA level enhanced by L-DOPA and had no effect on •OH production. This data suggests that disrupted balance between DA and glutamate in DA depleted nigrostriatal neurons results in generation of neurotoxic •OH. Both A2A antagonists, like L-DOPA, redress the DA/glutamate balance. However, A2A antagonists in combination with L-DOPA show different pharmacological profile in their effect on DA release and subsequent generation of •OH.

Parkinson disease – only dopaminergic insufficiency?

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Since mid 70-ties of the last century Parkinson disease was perceived, after Hornykiewicz, as nigrostriatal dopaminergic insufficiency due to atrophy of nervous cells in substantia nigra. For a long time whole therapy was aimed at the stimulation of dopaminergic system. This concept, however, gradually

began to evolve as destruction of other brain areas and other neurotransmitters became obvious. Soon it was realized that beside symptoms responding well to dopaminergic treatment (to the biggest extend bradykinesia), there are symptoms which are such treatment non-responsive. The whole spectrum of the symptoms of Parkinson disease will be shown, with a division into dopaminergic and non-dopaminergic ones. Also therapeutic possibilities concerning dopaminergic symptoms in the late stages of the disease, when the treatment becomes ineffective as well the treatment of non-dopaminergic symptoms will be discussed.

Brain genomic response to stress and perspectives for pharmacological preconditioning

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The term "stress" is used in so many different meanings (eg. hypo- or hyperoxic, hypo- or hyperglycemic, cellular, endoplasmic reticulum, proteotoxic, oxiadtive) and is really difficult to define. The broadest and simplest definition describes "stress" as "a bearing of pressure". Consequently, life always involves a certain level of stress and organisms mastered ways to cope with it. Yet, when a stress is excessive, organism or its parts (organs, cells) are not able to compensate. Compared to other body organs, brain enjoys a kind of "splendid isolation" behind bones of skull and blood-brain barrier shield. Nevertheless, several types of stress do reach brain, which otherwise is particularly sensistive to some of them. Widely known is unique vulnerability of brain to hypoxia, ischemia and hypoglycemia frequently inflicting its irreversible damage and death. Cellular responses to stress may be classified as "acute" ("immediate") which do not involve, and more "chronic" which do involve changes in gene expression. Whereas lethal stress induce expression of both pro- and anti-survival genes, brief exposure to sublethal stress (known as "preconditioning") induces tolerance related to induction of several pro-survival genes. Preconditioning by exposure to brief sublethal stress is the most efficacious neuroprotective procedure, and there is some experimental evidence that it may be mimicked by pharmacological induction of hypoxia-inducible genes, heat shock proteins, antioxidant enzymes, etc. However, benefits of pharmacological preconditioning in clinical neurology remain to be shown.

Effects of hypoglycemia and thiamine deficit of viability and function of SN56 cholinergic neuroblastoma cells

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Common feature of glucose and thiamine deficits is inadequate supply of acetyl-CoA for energy production, resulting in impairment of brain metabolism and function. The aim of this work was to investigate how these neurodegenerative factors affect acetyl-CoA metabolism in cholinergic neurons. Nondifferentiated (NC) and cAMP/retinoic acid differentiated cells (DC) were used to discriminate cytotoxic sensitivity of neurons with low and high level of acetylcholine metabolism. DC with high level of cholinergic metabolism were more sensitive to hypoglycemia than NC. In DC decrease of [glucose] in the growth medium from 25 to 1 mmol/l caused no significant decrease of pyruvate dehydrogenase and acetyl-CoA levels but suppressed their cholinergic parameters and viability by 65 and 11% respectively. Same conditions caused none or minimal alterations in NC. Culturing of DC and NC with amprolium, resulted in concentration-dependent increase of their mortality that at 5 mmol/L concentration reached 43 and 10%, decrease of acetylcholine content 40 and 0%, respectively at similar 40% decrease in acetyl-CoA content.

These data indicate that limitation of acetyl-CoA provision is more harmful for cholinergic neurons of high level of transmitter functions due to greater utilization of this intermediate for acetylcholine synthesis. Thus functional suppression of neurotransmission could paradoxically rescue cholinergic neurons in neurodegenerative conditions.

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Exploring and manipulating functions of microglia to foster neuroprotection/neurorepair

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Microglia are multifunctional immune cells of the brain executing various functions and rapidly responding to pathological insults. The extrinsic signals command microglia activation under pathological conditions towards a detrimental phenotype. Mechanisms responsible and controlling the shift from beneficial to detrimental microglial phenotype are poorly known. Identification of signalling pathways contributing to discrete phenotypes of microglia and discovery of transcription regulators which may serve as "master switches" for induction of an inflammatory phenotype, will allow to target specific functions of microglia. Therapeutic approaches targeting signal transduction in microglia will be discussed. Recent studies demonstrated that neuroprotective or neurodestructive actions of microglia are related to the acquisition by these cells of distinct phenotypes: proinflammatory (M1) or cell protective phenotype (M2) related to differential expression of genes and production of specific proteins. We have found that rat glioma cells release soluble factors which enhance cytoprotective phenotype of microglia, enhance microglial migration and significantly stimulate phagocytosis of fluorescent beads by rat primary microglial cultures.

HPLC fractionation of glioma conditioned medium and proteomic analysis helped to identify two soluble proteins which stimulate phagocytosis without induction of proinflammatory responses. Phagocytosis is an activity primarily performed by cells of the immune system, in the brain mostly by microglial cells. It is known that the balance between production and clearance/degradation of Aβ is disturbed in Alzheimer's disease (AD), the most common form of ageing-related dementia, characterised clinically by progressive dementia and neuropathologically by extracellular amyloid deposits, intracellular neurofibrillary tangles, reduced synaptic density and neuronal loss. Stimulation of cellular uptake and phagocytosis of Aβ is a promising strategy for treatment. In collaboration with prof. M. Schulzberg team we performed a preliminary study to determine if factors released by glioma or recombinant proteins will be able to modulate phagocytosis of fluorescent beads or $A\alpha_{1\text{-}42}$ by human microglial cells to test if phagocytosis of Aα can be stimulated. Understanding of how microglial cells operate in healthy and diseased brain coupled with recent advances in immunology of innate immunity, pharmacology and gene therapy techniques strongly support the idea of developing functionally manipulated microglial cells which can be employed to convey neuroprotection/neurorepair.

The novel mechanism of non-Aβ component (NAC) of Alzheimer's diseaseamyloid neurotoxicity: the interplay between p53 protein and Cdk5

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Recent studies on the extracellular α-synuclein suggest that exocytosis of this protein and its cleavage to non-A component of Alzheimer's disease amyloid (NAC) may be an important mechanism for amplifying and spreading degenerative changes in the brain. However the molecular mechanism of extracellular -synuclein and NAC toxicity is not fully understood. Our previous study presented that intact ASN and its neurotoxic fragment, peptide NAC induced oxidative stress and dopaminergic system impairment. Moreover it was shown, that NAC increased the translocation of nuclear factor kappa B (NFkB) and its activity, that subsequently may influence expression of several genes, including p53. Our present study focused on the role of p53 protein in mechanism of PC12 cells death evoked by NAC peptide using immunochemical, spectrophotometrical and spectrofluorometrical methods. We here found that exposure of PC12 cells to extracellular NAC peptide enhanced free radicals, induced mitochondria dysfunction and apoptosis. We also observed time-depended enhancement of p53 expression after NAC treatment. The inhibition of p53 by pifithrin significantly protected PC12 cells against NAC peptide evoked mitochondria failure and death. Our data also show that exposure to NAC peptide resulted in the higher expression of cyclin-dependent kinase 5 (Cdk5), one of the enzymes responsible for p53 phosphorylation and activation. Concomitantly, we observed the increase of expression of p35 and p39 peptides, that are essential co-factors in regulation of Cdk5 activity. Moreover the specific Cdk5 inhibitor (BML-259, 10 μM) prevented large population of cells against NAC-evoked cell death. Our findings indicate that NAC peptide exerts toxic effect by activation of p53/Cdk5 dependent apoptotic signaling pathway.

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Genetic defects accelerating brain aging

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The conversion of what has been interpreted as „normal brain aging" to Alzheimer's disease (AD) *via* a transition state, i.e. mild cognitive impairment, appears to be a continuous process caused primarily by aging-dependent accumulation of amyloid β-peptide β) in the brain. Alzheimer's disease can be considered as an integral part of the aging process. Aβ is constantly produced/ released from its precursor (app) and immediately catabolized under normal conditions, whereas dysmetabolism of Aβ seems to cause its pathological deposition upon aging. An increase in an anabolic activity of Aβ (in familial AD cases) or a decrease in a catabolic activity of Aβ (in sporadic AD cases and normal aged individuals) is a triggering event initiating Aβ accumulation. An oligomerization of Aβ42-43 alloforms into protofibrils leads to the pathological cascade of AD.

Several genes involved into Aβ clearance from neurons may influence an efficiency of the catabolic pathway of Aβ metabolism. Many DNA polymorphisms within the genes can be considered as genetic factors important for a rate of brain aging process. Data from the DNA sequence variation analysis of the *APOE*, *Neprilysin* and *IGF-1* genes will be presented and the impact of the genetic heterogeneity on aging process of human brain will be discussed.

Vascular abnormalities after experimental subarachnoid hemorrhage

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Subarachnoid hemorrhage (SAH) results in acute ischemia which is fatal for about 30% of patients. In about 30% of survivors late persistent vasospasm of large arteries at the base of the brain develops resulting in delayed ischemic deficits. Majority of experimental studies on SAH focus on the mechanisms of vasospasm. Our study addressed the question of the impact of acute post-SAH ischemia on the microcirculation in cerebral cortex. SAH was induced in adult, anaesthetized male Sprague-Dawley rats. Microflow (LDF) in cerebral cortex was measured bilaterally before, during, and for, at least, 30 min after SAH using laser Doppler flowmeter. Reactivity of microcirculation to CO_2, intravenous papaverine, acetylcholine, or thromboxane receptor antagonist (SQ 29,548) was assessed at 3, 24, 48, and 72 after SAH. At the same time points middle cerebral artery and basilar artery were harvested and processed for electron microscopy and histology.

Following SAH reactivity of microcirculation to vasodilators was impaired. The severity of this impairment correlated with the severity of ischemic insult. In addition, in the animals with profound ischemia in the early phase after SAH, at 72 hours after the bleeding cerebrovascular resistance decreased after blocking of TXA2/PGH2 receptor. This study shows that critical underperfusion in the acute phase after SAH results in progressive functional deterioration of microcirculation leading to the increase of arachidonic acid metabolites-dependent tone of microvessels in the late phase after SAH.

Our results suggest that delayed microcirculatory dysfunction may develop after SAH independent of the late vasospasm of large arteries.

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Glutathione in Parkinson's Disease: Application of thiols as a new therapeutic strategy

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Parkinson's disease (PD) is a slowly progressing neurodegenerative disease characterized by rigidity, bradykinesia, resting tremor and postural instability. Its main neuropathological feature is a loss of dopaminergic neurons of the substantia nigra pars compacta (SNc), which leads to a deficit of dopamine (DA) in the caudate-putamen. One of the earlier biochemical changes observed already in the presymptomatic stage of PD is a decreased nigral level of reduced glutathione (GSH), the most abundant cellular non-protein thiol in mammalian brain that plays a key role in the protection of cells against the deleterious effects of free radicals. Parallel to GSH loss, in the SN of parkinsonian patients there was also observed a marked increase in the activity of -glutamyl transpeptidase (-GT), a membrane-bound enzyme responsible for the extracellular degradation of GSH molecule. GSH is regarded as a major regulator of the intracellular redox state, acting as either an antioxidant by scavenging reactive oxygen species, or a substrate in various enzymatic antioxidant defense mechanisms. The reduction of GSH level in PD is thought to be a consequence of enhanced oxidative stress, a process which is supposedly a major mechanism involved in the death of dopamine neurons in the SNc. However, a growing body of evidence suggests that GSH depletion may itself play an active role in the progress of PD. The present lecture is aimed at reviewing the most recent studies into the contribution of GSH depletion to the pathogenesis of PD, and into the application of certain thiols as a new therapeutic approach.

Role of environmental toxins in pathomechanisms of Parkinson's disease

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Pathomechanisms responsible for a drammatic loss of dopaminergic neurons in Parkinson's disease are unknown, so far. However, several processes e.g. oxidative stress, mitochondrial dysfunctions, disturbances of protein degradation, neuroinflammation, glutamate excitotoxicity and others, seem to be involved. Recent studies have suggested some contribution of the glycogen synthase kinase 3 (GSK3) to degeneration of dopaminergic neurons. Since an epidemiological association has been found between the use of a potent herbicide – paraquat in agriculture and incidence of Parkinson's disease, the long-term exposure to this agent seems to be a risk factor for the development of this disease. The toxic effects of paraquat have been suggested to be related to the production of oxygen radicals and to an energy crisis, which leads to apoptosis. Our recent data obtained in rats have shown a moderate toxic effect of paraquat on dopaminergic neurons when administered acutely or subchronically. Moreover, the long-term paraquat administration (up to 9 months) produced a slowly progressing degeneration of dopaminergic neurons of the substantia nigra pars compacta and ventral tegmental area, loss of noradrenergic neurons of the locus coeruleus and

moderate deficits of dopaminergic and noradrenergic transmissions, in the striatum. We found additionally that the long-term paraquat administration influenced the levels of GSK-3 in several brain structures. Among others this herbicide increased the levels of total and active (pY216) forms of GSK-3 in the midbrain and pons, whereas decreased them in the striatum.

In conclusion, recent experimental data seem to suggest a contribution of GSK-3 to the paraquat-induced toxic influence on dopaminergic neurons and may support the view on a potential etiological role of this herbicide in development of Parkinson's disease.

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Short term effects of Zn in cholinergic SN56 neuroblastoma cells

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Zinc accumulates in extra and intracellular compartments of the brain in course of various cholinergic encephalopaties. We investigated whether zinc-evoked toxicity may result from its direct inhibitory interactions with enzymes of energy metabolism. In SN56 cell homogenates zinc caused concentration dependent, inhibition of pyruvate dehydrogenase (PDH), aconitase, isocitrate dehydrogenase and ketoglutarate dehydrogenase (KDH) activities, with Ki values equal to 0.058, 0.010, 0,004, 0.0015 mM, respectively. Zn-evoked mortality in differentiated cells was almost two times higher than in non differentiated ones. Zn also decreased cytoplasmic levels of acetyl-CoA and ACh and inhibited ACh release. It increased cytoplasmic and decreased mitochondrial Ca levels. Presented findings indicate that short-term inhibitory effects of Zn on PDH and KDH activities may be caused by its interaction with their lipoamide binding sites and/or by formation of Zn-lipoamide complexes.

ChAT activity was not directly inhibited by Zn. However, the decrease of cytoplasmic acetyl-CoA could evoke secondary, adaptative suppression of cell cholinergic phenotype. Such shortage of acetyl-CoA in cytoplasmi could acutely inhibit acetylcholine synthesis/accumulation and release. In long-term such deficit suppresses cholinergic phenotype of these cells. Therefore chronic inhibition of energy metabolism and acetylcholine synthesis/release by Zn could be responsible both for primary and secondary chronic cytotoxic and cholinosuppresive effects in encephalopathic brains.

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Ageing and age-related diseases – the common denominator?

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Stroke genetics

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Family-based and twin studies show that genetics affects stroke risk and prognosis. Stroke genetics can be considered in two aspects: stroke in the course of inherited disorders, and genetic risk factors of stroke. Although less than 1% of stroke cases are inherited, the ability to establish genetic diagnosis prevents such cases from exposure to unnecessary and potentially harmful therapeutic agents and diagnostic tests, allows to introduce specific effective treatment and allows planning rational family counseling.

The candidate gene approach is the most common way to study the significance of chosen genetic variants as risk factors of stroke. Unfortunately, only few genetic variants were shown to affect stroke risk in the independent replication studies. A novel approach, genome wide association studies, use the markers evenly spaced throughout genome without regard to their function or location and allows to find all genetic variants related to the disease. Because available data suggest that the effect on stroke risk is related to many genetic variants with small effect size, large number of cases and controls are required to find such risk variants. International effort is being made to collect enough DNA samples to find out all genetic variants related to stroke risk.

Genetic factors can contribute to conventional risk factors or intermediate phenotypes; they may interact with another genetic or environmental factors; they may affect the course of the disease and outcome and they may determine response to treatment.

The perspective for the future of stroke genetics is the era of personalized prevention and therapy, where specific biochips will help stroke clinicians to decide on the best individual prevention program and treatment.

Mitochondria, reactive oxygen species (ROS) generation and cell life

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Aerobic metabolism allows animal cells to efficiently produce ATP at the expense of energy supplied by respiratory substrates oxidized in mitochondria. However, this undoubtedly advantageous mechanism is coupled with continuous formation of reactive oxygen species. Under normal conditions mitochondria seem to convert as much as 2% of the total oxygen consumed to the superoxide radical and hydrogen peroxide. It is commonly agreed that the major site of mitochondrial ROS generation is the respiratory chain, particularly its complex I and III. However, other mitochondrial enzymes may also be involved. Despite various mitochondrial and extramitochondrial antioxidative systems involved in neutralizing reactive oxygen species, these are inevitably damaging to mitochondrial constituents, particularly mitochondrial DNA. This results in a progressive mitochondrial impairment, gradual increase of ROS formation and eventually to an irreversible cell dysfunction. Such a scenario is considered as an important mechanism of cellular aging. Moreover, under various pathological conditions such as ischemia-reperfusion diabetes and mitochondrial diseases related to aberrant mitochondrial metabolism, these organelles and, in consequence, the whole cell are challenged by increased amount of mitochondrially-produced ROS. Apart from apparently harmful oxidative effects caused by the mitochondrially produced reactive oxygen species

on numerous cellular constituents, these compounds are also recognized as cellular mediators of mitochondria-dependent apoptosis. Interestingly, a protective action of ROS on cell survival upon ischemia-reperfusion insult is also considered. Mechanisms responsible for the mitochondrial ROS production as well as implications of unbalanced ROS formation/neutralization reactions under pathological conditions in death or survival of affected cell will be discussed.