Opening lecture

Mechanism of endothelial dysfunction and its pharmacological correction

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The phenotype of healthy endothelium may be switched on to the dysfunctional one due to a variety of endogenous pro-inflammatory stimuli including cytokines (IL-6, IL-8), chemokines (MCP-1), C reactive protein (CRP), adhesive molecules (VCAM-1, ICAM-1), free radicals (O$_2^-$), peroxynitrite (ONOO$^-$), regulatory peptides, e.g. ET-1, Ang 2, PAI-1, as well as due to endothelial enzymic aberrations, e.g. splitting endothelial NOS-3 homodimer to monomers or overexpression of ACE/kininase 2. Then endothelium loses its position of “maestro of the blood circulation” as Sir John Vane put it nicely [Vane, Phil Transactions of Royal Soc, 1994] and the vascular wall becomes the territory that cannot defend itself against a pathological invasion. Atheromatic plaques may be formed, hypertension, atherothrombosis and various diabetic angiopathies develop, remodelling of vascular and cardiac wall may appear. There exists a vast number of direct triggers which initiate that endothelial dysfunction: high blood levels of oxidized LDL, lipid peroxides, hyperglycemia, advanced glycated endproducts (AGE), nicotinism, hyperhomocysteinemia, and folate avitaminosis. The tissue-penetrating ACE inhibitors (ACE-I, perindopril, quinapril, ramipril) constitute the first line pharmacological correctors. We proved that ACE-Is acted via the bradykinin-mediated release of endothelial prostacyclin (PGI$_2$). PGI$_2$ acts as a major protector of endothelial function. Similar final beneficial effects are being achieved (although through different mechanisms) by statins (atorvastatin, simvastatin) and by β-adrenergic receptor antagonists (carvedilol, nebivolol). In case of these drugs except for the PGI$_2$ release, the endothelial generation of nitric oxide (NO), tissue plasminogen activator (t-PA), endothelium-derived hyperpolarizing factor (EDHF) or thrombomodulin (TM) are also involved. The newly born pharmacology of endothelium constitutes a new approach to the treatment of cardiovascular diseases.
Neuroprotective signaling in neurodegeneration and neurorepair

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Synaptic activity modulates the formation of lipid messengers through phospholipase-mediated cleavage of specific phospholipids from membrane reservoirs. Multiple effectors are involved, including neurotransmitters, membrane depolarization, ion channels, cytokines, and neurotrophic growth factors. Lipid messengers in turn, modulate and interact with other signaling cascades, contributing to the development, differentiation, function (e.g., LTP and memory), protection, and repair of cells of the nervous system. Overall, bioactive lipids participate in the regulation of synaptic function and dysfunction. Signaling mediated by platelet-activating factor (PAF), phospholipase A2, and COX-2-synthesized PGE2 modulates synaptic plasticity and memory [Clark et al., Neuron, 1992; Kato et al., Nature, 1994; Izquierdo et al., Proc Natl Acad Sci USA, 1995; Teather et al., Learning and Memory, 2002; Chen et al., J Neurophysiol, 2002]. Oxidative stress disrupts lipid signaling, lipid peroxidation, and neurodegeneration. Lipid messengers are necessary in the extensive interactions among neurons, astrocytes, oligodendrocytes, microglia, cells of the microvasculature, and other cells. This conglomerate of interrelated cells is referred to as the neurovascular unit. Signaling at the neurovascular unit is clearly altered in early stages of cerebrovascular disease as well as in neurodegenerations. Here we will describe the elucidation of critical events essential for neuronal survival and the integrity/repair of the nervous system. Deficiencies in docosahexaenoic acid (DHA), a major membrane phospholipid and essential omega-3 fatty acid required for brain and retinal development, are associated with cognitive decline and retinal dysfunction. Earlier results have shown that the DHA content in Alzheimer’s disease (AD) brains is decreased, and that lipid peroxidation targets phospholipids containing this highly unsaturated fatty acid. Using tandem LC-PDA-ESI-MS-MS-based lipidomic analysis in combination with mouse brain ischemia-reperfusion, we have demonstrated that free docosahexaenoic acid released in the brain leads to the synthesis of stereospecific messengers through oxygenation pathways. Aspirin, used prophylactically for cerebrovascular diseases, activates an additional docosahexaenoic acid pathway. The newly discovered messenger, 10,17S-docosatriene (neuroprotectin D1), counteracts leukocyte infiltration and pro-inflammatory gene expression in brain ischemia-reperfusion [Marcheselli et al., J Biol Chem, 2003]. We have also studied neuroprotection in the retina, where photoreceptor survival depends on retina pigment epithelial (RPE) cell integrity. The pathophysiology of several retinal degenerations (e.g. age-related macular degenerations) involves oxidative stress-mediated injury and RPE cell death mediated by A2E and its epoxides. Also, using human retinal pigment epithelial cells, we demonstrate that the synthesis of neuroprotectin D1 (NPD1) was enhanced by the calcium ionophore A-23187, by IL-1β, or by supplying DHA. Acting as an apoptostatic mediator, NPD1 potently counteracted oxidative stress-induced apoptosis. NPD1 inhibited oxidative stress-induced caspase-3 activation. NPD1 also inhibited IL-1β-stimulated expression of COX-2 [Mukherjee et al., Proc Natl Acad Sci, USA, 2004]. Furthermore, induction of RPE cell apoptosis by A2E-triggered oxidative stress was also attenuated by...
NPD1. In addition, very recently we found that DHA attenuated amyloid beta (Aβ) peptide secretion from aged and cytokine-stressed human neural (HN) cells. This effect is accompanied by enhanced biosynthesis of neuroprotectin D1 (NPD1). DHA and NPD1 levels are reduced in CA1 hippocampal regions from AD patients. Activation of pro-apoptotic and pro-inflammatory genes in Aβ42-stressed HN cells was repressed by both DHA and NPD1, with concomitant up-regulation of the Bcl-2 anti-apoptotic proteins Bcl-2, Bcl-xl, and Bfl-1(A1). sAPPα, a neurotrophic derivative of β-amylloid precursor protein, was found to stimulate NPD1 production. NPD1 inhibits Aβ42-induced HN cell apoptosis, and endogenous content of NPD1 was dramatically reduced in the hippocampal CA1 of AD brain. These results indicate that NPD1 mediates induction of both anti-apoptotic and neuroprotective gene-expression programs that down-regulate Aβ secretion, and modulate intrinsic anti-inflammatory signals that promote neural cell survival. These findings have several implications in the understanding of how the nervous system counteracts disturbances in redox homeostasis, mitochondrial dysfunction, oxidative stress, and pro-inflammatory conditions. These newly discovered pathways will allow for the design of novel therapeutic strategies for neurodegenerative diseases. Moreover, the new docosahexaenoic acid-signaling pathways may lead to the clarification of clinically important issues relevant to stroke, age-related macular degeneration, spinal cord injury, aging, and other diseases that include neuroinflammatory components. The specificity and potency of this novel docosanoid (neuroprotectin D1) indicate an important target for therapeutic intervention.

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Phospholipase A₂ (PLA₂) catalyzes the hydrolysis of the ester bond of fatty acids at the sn-2 position of membrane glycerophospholipids and, together with other lipolytic enzymes, it contributes to their turnover. Beside this function, PLA₂S produce polyunsaturated free fatty acids and lysoglycerophospholipids that are lipid mediators themselves or their precursors. Secretory PLA₂S (sPLA₂) have a low molecular weight (13–18 kDa), show a low specificity for fatty acids and require mM Ca²⁺ for full activity. Rat brain contains enzymes shearing biochemical properties with sPLA₂S isolated from other tissues which, on the basis of their immunological properties, have been identified as group IIA (GIIA) and group V (GV) [Macchioni et al., J Biol Chem, 2004]. mRNAs for both isoforms were found in all rat brain regions [Molloy et al., Neurosci Lett, 1998]. GIIA is present in cytosol and mitochondria of neural cells and in vitro studies have shown that the enzyme is released from mitochondria under reduced membrane potential [Macchioni et al., J Biol Chem, 2004]. GV sPLA₂ is cytosolic but it is also largely present in the nuclei of neural cells. Enzymes belonging to these groups are synthesized as pro-enzymes with an N-terminal signal peptide and are secreted from inflammatory cells upon their activation. In cultured astrocytes, GIIA is induced by inflammatory factors [Oka and Arita, J Biol Chem, 1991; Li et al., J Interferon Cytokine Res, 1999], indicating a role in the production of lipid mediators in pathological conditions. In addition, GIIA sPLA₂ plays a role in neurotransmission since it is released from synaptosomes and binds to specific neuronal receptors [Matsuzawa et al., Biochem J, 1996; Kolko et al., J Biol Chem, 1996]. Since GIIA and GV sPLA₂ are present in intracellular organelles, they should have intracellular functions that are currently unknown. The release of GIIA sPLA₂ from energy-depleted mitochondria might indicate that it could be involved in neurodegeneration because it induces neuronal cell death via apoptosis [Yagami et al., Mol Pharmacol, 2002].
Reactive oxygen species (ROS) resulting from oxidative stress may provide a link between seemingly diverse abnormalities occurring in AD including enzymes of the tricarboxylic acid (TCA) cycle and calcium homeostasis.

Oxidants can lead to AD-like changes in the TCA cycle. α-Ketoglutarate dehydrogenase (KGDHC), a key enzyme of the TCA cycle, is diminished in brains from AD patients and the reduction is highly correlated to a clinical dementia rating before the patients die. KGDHC is sensitive to numerous oxidants including those altered in AD. Reductions in KGDHC make the brain more sensitive to other insults that promote neurodegeneration. The sensitivity to oxidants and the central role of KGDHC in normal brain metabolism may promote a viscous cycle that could promote neurodegeneration.

Oxidants can also lead to AD-like changes in calcium homeostasis that accompany AD. Bombesin-releasable endoplasmic reticulum Ca²⁺ stores (BRCS) are exaggerated in fibroblasts from patients with AD as well as in neurons from transgenic mice bearing a mutant presenilin-1 (PS-1) gene. To determine which ROS might lead to the AD-like changes in BRCS, a variety of oxidants were tested for their effects on BRCS. Multiple oxidants were added outside the cells and selective induction of ROS was deduced from interaction with selective fluorescent probes. These oxidants selectively modified basal [Ca²⁺]i and BRCS. Among the oxidants tested, t-BHP and H₂O₂ were the most specific for exaggerating BRCS without affecting basal [Ca²⁺]i. Furthermore, ROS induced by t-BHP were higher in fibroblasts from AD patients than in those from controls. α-Keto-β-methyl-n-valeric acid (KMV) selectively diminished DCF-detectable ROS. KMV diminished BRCS either with or without exogenous oxidants and reduced the AD-related exaggeration in the BRCS. On the other hand, DAF-detectable NO⁺ was not scavenged by KMV and it did not exaggerate BRCS.

The results suggest that appropriate antioxidants may alter specific oxidant-induced changes in cell systems and that an understanding of these interactions will be important in the treatment of AD.

Phospholipases A₂ in neurodegenerative diseases
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The high consumption of oxygen, the enrichment of polyunsaturated fatty acids in neuron and glial membranes and the relatively low level of oxidant defense enzymes in the brain are important factors rendering this organ highly susceptible to oxidative insults. Increase in oxidative stress has been implicated in the pathophysiology of a number of neurodegenerative diseases including Alzheimer’s disease (AD), Parkinson’s disease and stroke. Under pathological conditions, excessive production of reactive oxygen species (ROS) is a prerequisite to lipid peroxidation and altered Ca²⁺ homeostasis, which trigger Ca²⁺-dependent enzymes, e.g. proteases, phospholipases, and nucleases, and subsequently the apoptotic pathways. Phospholipases A₂ (PLA₂) are ubiquitous enzymes responsible not only for maintenance of membrane phospholipids but also for providing lipid mediators and precursor for eicosanoid production. Increase in PLA₂ has been implicated in the oxidative and inflammatory responses associated with many neurodegenerative diseases. This presentation is to provide evidence indicating the involvement of both the cytosolic cPLA₂ and the secretory sPLA₂ in the pathogenesis of Alzheimer’s disease (AD). Studies with rat primary cortical neurons indicated the ability of oligomeric β amyloid (Aβ42), excitatory neurotransmitters and oxidant compounds to stimulate arachidonic acid (AA) release and the involvement of oxidative stress and cPLA₂ in the AA release pathway. Studies with rat astrocytes indicated the ability of pro-inflammatory cytokines (TNFα, IL-1β and IFNγ) to induce sPLA₂-IIA and COX-2 mRNA, and subsequently an increased production of prostaglandin E2. In support for the inflammatory role of sPLA₂, studies with primary astrocytes from postmortem human brain further indicated upregulation of sPLA₂-IIA mRNA by Aβ42 as well as
pro-inflammatory cytokines. Taken together, these results demonstrate a role for both cPLA$_2$ and sPLA$_2$ in the oxidative and inflammatory responses in AD. Understanding mechanisms for ROS production and the signal transduction pathways leading to activation of these PLA$_2$s will aid the development of new tools to target intervention strategies for retarding the progression of these neurodegenerative diseases.

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α-Synuclein in neurodegeneration
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α-Synuclein, originally discovered in an insoluble fraction of Alzheimer’s disease brains and identified by cDNA cloning [Uéda et al., Proc Natl Acad Sci, USA, 1993], is a major constituent of pathological inclusion bodies, a common feature of several neurodegenerative diseases. Three missense mutations in the α-synuclein gene have been identified in confirmed autosomal-dominant familial Parkinson’s disease and dementia with Lewy bodies, which segregate with the illness. However, the physiological function of α-synuclein remains unknown. To understand the pathological role of α-synuclein, we have performed affinity chromatography using α-synuclein column and human brain proteins and revealed tubulin to be an α-synuclein binding protein. Microtubule assembly was performed using purified tubulin and wild-type and mutant forms of α-synuclein. Deletion mutants of α-synuclein were made to identify active domain for microtubule assembly. Immuno-EM was used to confirm microstructures. α-Synuclein induced polymerization of purified tubulin into microtubules. This activity was as high as that of tau. Mutant forms of α-synuclein lost this potential. The binding site of α-synuclein to tubulin was identified. This is the first demonstration of microtubule-polymerizing activity of α-synuclein. Now we can see a striking resemblance between α-synuclein and tau: both have the same physiological function and pathological features, making abnormal structures in diseased brains known as synucleinopathies and tauopathies. The discovery of a physiological role for α-synuclein should provide a new dimension in researches into the mechanisms of α-synuclein-associated neurodegenerative diseases.

Neuroprotective and neurotoxic potential of tetrahydroisoquinolines
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1,2,3,4-Tetrahydroisoquinolines (TIQ’s) are substances present in the brain either as endogenous compounds, synthesized by enzymatic or nonenzymatic condensation of catecholamines, or ingested with various foods. At least some of them may be easily converted to quaternary ions that may generate neurotoxic free radicals. This property attracted the attention of neuroscientists after discovery of strong neurotoxic properties of TIQ analog, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that may cause “an instant parkinsonism” in man. Most of TIQs are regarded as proneurotoxic substances, causing, in particular, degeneration of dopamine neurons, and were suspected to be involved in etiology of Parkinson’s disease. The compounds, however, have interesting psychopharmacological properties that allow to classify them as
potential atypical neuroleptics. Our studies indicate that they specifically block the active conformation of dopamine receptors, thus inhibiting the phasic, but not tonic activity of dopaminergic system. Related to this property may be their action alleviating signs of opiate abstinence, inhibiting development of opiate tolerance, and preventing the reinstatement of cocaine self-administration after a prompting dose of cocaine administered to cocaine-experienced rats. We have also reported that the compounds may potentiate opiate analgesia. All those potentially useful properties cannot be put to use therapeutically if the compounds are neurotoxic. It has been found, however, that TIQs, though similar in respect of their antidopaminergic properties, vary among them in the degree of their neurotoxicity, and some of them, as shown for 1-methyl-TIQ, may even exert neuroprotective action, protecting against behavioral and biochemical effects of such neurodegenerative compounds as MPTP or rotenone. The effects of TIQs on neuronal survival depend on their influence on enzymes involved in the catecholamine metabolism. While several TIQs shift the metabolism of dopamine toward the oxidative pathway that generates free radicals and makes the compounds inhibitors of complex I, others may inhibit monoamine oxidase and, therefore, reduce the generation of oxygen reactive species. The combination of neuroprotective and partial-antidopaminergic properties of compounds such as 1-methyl-TIQ, make them interesting as potential psychopharmaceuticals.

Gene profiling of the postischemic brain reveals novel pathways for recovery of function following stroke

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Approximately 5 million persons are afflicted by stroke every year. Out of these patients, those will be treated with thrombolysis if the onset of stroke is witnessed and within 3 h the treatment is commenced. Today there is no stroke treatment available that prevents development of cell death in the brain or that enhances functional recovery after stroke. There is a need for new knowledge related to the brain tissue response to ischemia that can be translated into novel treatment paradigms. In order to study the complex response of the brain to cerebral ischemia, we performed a massive analysis of gene expression in the rat brain following experimental stroke. In one set of experiments, rats were subjected to transient occlusion of the middle cerebral artery with eight time points of reperfusion up to 24 h. In another set of experiments, rats were subjected to enriched environment during 30 days after experimental stroke, and compared to rats in standard laboratory housing conditions. mRNA was extracted from the brain of sham operated and ischemic animals, and subtractive cDNA libraries were constructed. These libraries contained an enriched pool of clones corresponding to overexpressed genes in the ischemic brain. cDNAs were printed and hybridized with labelled targets from three peri-infarct brain regions at the eight time points of reperfusion. Using cluster analysis, genes with particular activation profiles were identified. A biphasic expression cluster was identified, with an initial induction at 0–3 h after the end of occlusion and then a second increase in gene expression after 9–15 h, the last one restricted to surviving tissue. The clusters contained 17 immediate early genes, and other 44 were related to transcriptional regulation, the signaling pathways and proliferation. Another set of four clusters showed a progressive increase in expression over time of genes related to gliosis or regenerative events, particularly lipid and myelin synthesis. We conclude that the genomic response to experimental stroke is complex, highly dynamic and dependent on the intensity of the ischemic insult. Using this approach will allow us to identify novel pathways and individual genes involved in cell survival and tissue repair and recovery.
Age-related alteration of alpha-synuclein and its role in dopaminergic system in relation to Parkinson’s disease

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α-synuclein (ASN) belongs to a larger family of molecules, including β, γ-synucleins and synoretin. This protein accumulates in dopaminergic neurons as intraneuronal inclusions, Lewy bodies (LB) in Parkinson’s disease, subtype of Alzheimer’s disease (AD) with LB, as well as in other neurodegenerative disorders. ASN was originally identified in human brain as the precursor protein of the non-amyloid beta component of AD amyloid (NAC). This 35 amino acid central domain (residues 61–95) within the ASN molecule is probably responsible for its toxicity. The aim of present study was to evaluate the expression of ASN in the different brain parts of the adult (4-month-old) and aged (24-month-old) rats. Moreover, the role of brain aging, ASN and NAC in striatal dopamine transporter (DAT) function was determined. ASN mRNA expression was assayed by RT-PCR and protein level by Western blot technique. For [3H]-dopamine (DA) uptake radiochemical method was used. The results indicated that gene expression of ASN significantly decreased in aged striatum and cerebellum comparing to adult by 39%, 24% and 65%, respectively. Moreover, brain aging decreased specific [3H]-DA uptake by about 25%. In addition, ASN and NAC peptide at 10 μM concentration inhibited DAT activity by about 50% and 30%, respectively. These peptides stimulated by 57% intrasynaptosomal generation of reactive oxygen species (ROS) measured by using fluorogenic probe, 2’7’-dichlorofluorescein diacetate. Oxidative stress evoked by FeCl₂ (25 μM) in the presence of ascorbic acid (250 μM) and by nitric oxide (NO) donor, sodium nitroprusside (SNP) (10 μM) significantly decreased DA uptake. The alteration of ASN expression could significantly affect synaptic endings function and could promote the aged brain for α-synucleinopathy and neurodegeneration. On the other hand, we suggest that ROS generated during brain aging and by toxic peptides accumulated at high concentration in PD may affect DAT function and dopaminergic neurotransmission.

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MAPKs mediate activation of PLA₂ by prooxidants (oxLDL and amyloid-β) in microvascular cells

Mario Alberghina

The hypothesis that oxidized LDL (oxLDL), administered in sublethal doses to the culture medium of immortalized rat brain endothelial cells (EC, GP8.39), functions as a prooxidant signal of peroxidation processes and membrane phospholipid hydrolysis was tested. EC were grown at confluency in a medium with or without native LDL or oxidized LDL (1.5 mg/dish; up to 350–450 nmol hydroperoxides/mg protein) for two temporally distinct phases (1 and 24 h). OxLDL (100 μM hydroperoxides) markedly increased lipid peroxidation, cytosolic phospholipase A₂ (cPLA₂) activity and arachidonic acid (AA) release in a dose-dependent manner. Caspase-3 activity and LCFM analyses indicated that oxLDL had no effect on triggering an apoptotic process. The results suggest that (i) EC may be the target of oxidative damage; (ii) activation of cPLA₂ mediates AA liberation; (iii) Ca²⁺-independent phospholipase A₂ (iPLA₂) activity was also stimulated by oxLDL [Lupo et al., BBA, 2002]; (iv) phosphorylation of ERK1/2, p38 and JNKs and their nuclear translocation was significantly enhanced in a dose-dependent manner. Caspase-3 activity and LCFM analyses indicated that oxLDL had no effect on triggering an apoptotic process. The results suggest that (i) EC may be the target of oxidative damage; (ii) activation of cPLA₂ mediates AA liberation; (iii) Ca²⁺-independent phospholipase A₂ (iPLA₂) activity was also stimulated by oxLDL [Lupo et al., BBA, 2002]; (iv) phosphorylation of ERK1/2, p38 and JNKs and their nuclear translocation was significantly enhanced in a dose-dependent manner. Caspase-3 activity and LCFM analyses indicated that oxLDL had no effect on triggering an apoptotic process. 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cose utilization in anterior cingulate cortex in patients with chronic liver failure. Proton Magnetic Resonance Spectroscopy (MRS) confirms increased brain glutamine in chronic liver failure but no changes in high energy phosphates. 13C- MRS in acute liver failure, on the other hand, reveals increased brain lactate consistent with impending cerebral energy failure. Molecular techniques reveal alterations in expression of genes coding for essential brain proteins that have been reported in both experimental and human HE. Such proteins include the astrocytic structural protein GFAP, the glial glutamate and glycine transporters EAAT-2 and GLYT-1 and the “peripheral-type” benzodiazepine receptor (PTBR). Exposure of primary astrocyte cultures to liver-derived toxins such as ammonia results in a similar pattern of alterations of gene expression. “Knock-down” of EAAT-2 gene leads to brain edema and hyperexcitability. Loss of expression of EAAT-2 and GLYT-1 in experimental liver failure leads to increased extracellular brain concentrations of the neuroactive amino acids glutamate and glycine. Given that these amino acids are potent agonists at distinct sites on the NMDA receptor, these findings are consistent with activation of the NMDA receptor-mediated NO-cGMP signal transduction pathway. NO production via this pathway results in nitration of glutamine synthetase resulting in a further impairment in capacity of brain to remove ammonia. Increased PTBR expression is a consistent feature of HE. PTBR sites are increased in human HE resulting in stimulation of the synthesis of neurosteroids such as allopregnanolone, a potent GABA-A receptor agonist.

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A possible role of NO/cGMP pathway in pathomechanism of Parkinson’s disease

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The cause of the neuronal degeneration in Parkinson’s disease (PD) is unknown. Intense interest is now focused on whether free radicals may play a role in the pathogenesis of PD. Since it is known that nitric oxide (NO) may be one of major reactive radicals involved in pathomechanism of PD, we investigated a possible role of NO/cGMP pathway in animal model of PD induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The expression and the activity of neuronal nitric oxide synthase, soluble guanylyl cyclase (GC) and cGMP level in the striatum and midbrain in Parkinsonism evoked by MPTP injections (3 inj. ip in saline at 2 h intervals at a total dose of 40 mg/kg) were measured in C57/BL mice. Control mice received saline only. Animals were killed after 3, 7 and 14 days after MPTP treatment. We observed the increase in NOS and GC activities in striatum and midbrain after MPTP injection. 7-Nitroindazole inhibited MPTP-induced NOS activity by about 63–70% and 1400 W by about 10–15% as compared to total activity detected in all investigated experimental groups. It was accompanied by an enhancement of both nNOS and GCβ1 mRNA expression and GCβ1 subunit protein level. Moreover, nNOS and GCβ1 immunoreactivity enhancement was found in substantia nigra pars compacta (SNpc) 7 days after MPTP treatment. MPTP induced GCβ1 mRNA expression and increased protein level of GCβ1 in the striatum and midbrain up to 14 days after its injection which was accompanied by marked enhancement of cGMP formation. Furthermore, it appeared that the activation of GC occurs through change in maximal enzyme activity (V_max). Simultaneously, no change in PDE activity has been detected in all investigated regions of the brain. Efficacy of MPTP treatment was evaluated by immunohistochemical study which demonstrated about 75%
and 65% decrease in tyrosine hydroxylase (TH) positive neurons in SNpc and striatum, respectively.

The view that NO/cGMP pathway may be involved in pathomechanism of PD was further explored in vitro on PC12 cells. Our results show that PKG inhibitor (KT5823) prevented MPP⁺-induced enhancement of free radical production (assayed by DCF method) and increased PC12 cells viability measured by 3-[4,5-dimethylthiazol-2]-2,5-diphenyl tetrazolium bromide (MTT) assay. Summarizing, the obtained data give a support to the view that NO/cGMP signaling pathway may at least partially contribute to dopaminergic neuron degeneration in the SNpc and striatum, the damage attributed to PD.

Does synuclein play a central role in dopaminergic neuronal degeneration?

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Abstract not submitted.

Role of cytokines in pathogenesis of Parkinson’s disease – gender difference

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The incidence of Parkinson’s disease (PD) changes with gender and age. Higher prevalence in men suggests a link between gonadal hormone levels, such as estrogens and PD. Neurodegenerative processes in this disease are associated with an inflammatory response regulated by specific signaling molecules – cytokines. Estrogens could modulate the cytokines expression.

Age is an important risk factor for PD. Advanced age is associated with increased expression levels of various proinflammatory cytokines which may enhance the brain’s susceptibility to neurodegeneration.

The aim of the present study was to determine how aging and gender influence cytokine gene expression in a model of PD induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP). The levels of mRNA for: TNFα, IFNγ, IL-1β, IL-6 and TGFβ were measured by RT-PCR method in the striatum of male and female C57BL/6 mice (3 and 12 months old) after 6 h; 1, 3, 7, 14 and 21 days post MPTP intoxication.

MPTP intoxication caused increases in expression of all cytokines, but the patterns of expression for many were different in young and aged male versus young and aged female mice. As for IFNγ and TGFβ, in aged and young male mice, we noticed maximal increase in TGFβ and IFNγ mRNA after 1-day intoxication. In contrast, in young and aged female mice, TGFβ and IFNγ were elevated at later time points. We also noticed that induction of TNFα and IL-1β mRNA in males preceded the induction of the expression of these cytokines in females. MPTP caused an increase in IL-6 mRNA in the striatum of males and females, but the increase was significantly higher in females.
Our study also demonstrated that the increase in levels of mRNA for all investigated cytokines were more pronounced in both aged male and female than in young male and female mice.

The data indicate that there is an age- and gender-dependent difference in the TNF gene expression profiles in the striatum after MPTP injection. These observations may help in better understanding of the age- and gender-related differences which exist in PD.

The role of nitric oxide in the mechanism of inflammation-related mitochondria failure in the brain

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Nitric oxide (NO) plays an important role in a number of physiological processes in the brain. However, excessive formation of NO has been implicated in the pathogenesis of many diseases of the central nervous system. The most important is reaction with superoxide radical leading to formation of peroxynitrite, involved in mitochondrial damage. Moreover, NO can also directly inhibit the mitochondrial respiratory chain, leading to energetic disturbances. In inflammation, an increased production of NO by inducible nitric oxide synthase (iNOS) occurs, but little is known about the role of the constitutive isoforms, eNOS, in mitochondrial dysfunction.

The aim of the present study was to analyze the role of inducible and constitutive isoforms of NOS in lipopolysaccharide (LPS)-evoked mitochondrial disturbances.

C57BL6 mice were injected with LPS (1 mg/kg ip) alone or together with NOS inhibitors: 7-NI (25 mg/kg), NNLA (30 mg/kg) and 1400 W (5 mg/kg). The studies were carried out by using RT-PCR, radio-, immunohistochemical, fluometric and spectrophotometric methods. Ultrastructural analysis was performed with electron microscope.

Our results indicated that LPS enhanced exclusively iNOS expression and activity in the substantia nigra. However, application of specific inhibitors indicated that eNOS was also involved in LPS-evoked molecular alterations leading to apoptosis. NNLA and 7-NI, beside 1400 W, prevented LPS-evoked lipid peroxidation, β-NAD⁺ depletion and apoptosis inducing factor (AIF) translocation from mitochondria to nucleus. However, NOS inhibitors did not have any effect on poly(ADP-ribose)polymerase (PARP) activity, suggesting that energetic disturbances and AIF release may be directly dependent on NO-evoked mitochondrial alterations. Electron microscopic examination and immunocytochemical analysis of neurons in the substantia nigra revealed swelling, unusual configuration of mitochondrial cristae, and AIF release.

Our results indicated that both iNOS and eNOS were involved in LPS-evoked mitochondrial failure and in induction of apoptosis.

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Neurotrophic and neurotoxic effects of the Ca\textsuperscript{2+}-modulated protein, S100B: mechanisms of action

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The Ca\textsuperscript{2+}-modulated protein, S100B, is expressed in high abundance in astrocytes and released by them [Donato, Int J Biochem Cell Biol, 2001]. Besides exerting regulatory roles in astrocytes, S100B can regulate astrocyte, neuronal and microglia activities once released into the brain extracellular space. At low levels normally found extracellularly in the brain, S100B is trophic to neurons, protecting them against stress-induced apoptosis and stimulating neurite outgrowth, which might be important during brain development and in the initial phases of brain damage. S100B protects neurons against apoptosis by binding to RAGE (receptor for advanced glycation end products) and activating the Ras-MEK-ERK1/2-NF-κB pathway and upregulating the anti-apoptotic factor, Bcl-2 [Huttunen et al., J Biol Chem, 2000]. Also, at low doses S100B counteracts the toxic effects of β-amyloid (Aβ) peptide on neurons RAGE dependently by the same mechanism [Businaro et al., submitted for publication]. However, high levels of S100B are found in the brain of Alzheimer’s disease patients and in Down’s syndrome, and Aβ peptide upregulates S100B expression. At high levels (as expected due to astrocyte death or leakage from damaged astrocytes) S100B is toxic to astrocytes and neurons and activates microglia. At high doses S100B is toxic to neurons directly, causing excessive reactive oxygen species production again via RAGE binding [Huttunen et al., J Biol Chem, 2000], and indirectly, stimulating NO production in astrocytes [Hu et al., J Biol Chem, 1996] and microglia [Petrova et al., Brain Res, 2000; Adami et al., Glia, 2001]. However, although microglia express RAGE [Hofmann et al., Cell, 1999], S100B’s ability to stimulate NO production depends on the density of RAGE molecules on the microglial cell surface rather than on RAGE transducing activity [Adami et al., Biochim Biophys Acta, 2004]. By contrast, S100B upregulates the expression of the pro-inflammatory COX-2 in microglia by stimulating RAGE transducing activity and activating a Ras-Cdc42-Rac-JNK pathway and NF-κB. Thus, extracellular S100B might play a dual role in brain trophism depending on the levels attained, being trophic at low levels and participating in the pathophysiology of neurodegenerative disorders and/or brain inflammatory processes at high levels.

Pivotal role of mitochondria in amyloid beta-induced cell death

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Alzheimer’s disease (AD) is characterized by two major histopathological hallmarks, extracellular plaques of fibrillar β-amyloid (Aβ) peptides and intracellular neurofibrillary tangles (NFT) composed of hyperphosphorylated tau protein. Importantly, current data indicate a complex relation between the amyloid pathology and pathology involving microtubule-associated protein tau during disease. The aim of the present study was to further elucidate the pathophysiological relation between the two defining neuropathological Alzheimer’s proteins, Aβ and tau, with regard to oxidative damage and mitochondrial func-
tion as potential common final target within cell death cascade. Thus, we investigated effects of acute and chronic exposure to increasing concentrations of Aβ on mitochondrial function and nitric oxide (NO) production in vitro and in vivo. Our data demonstrate that PC12 cells and HEK cells bearing the Swedish double mutation in the amyloid precursor protein gene (APPsw), exhibiting substantial Aβ levels, have increased NO levels, reduced activity of cytochrome c oxidase, reduced ATP levels, and decreased Bcl-xl/Bax ratio. The inhibition of intracellular Aβ production by a functional γ-secretase inhibitor normalizes NO and ATP levels indicating a direct involvement of Aβ in these processes. Extracellular treatment of PC12 cells with comparable Aβ concentrations only leads to weak changes, demonstrating the important role of intracellular Aβ. In 3-month-old APP tg mice, which exhibit no plaques but already detectable Aβ levels in the brain, reduced ATP levels and decreased Bcl-xl/Bax can also be observed showing the in vivo relevance of our findings [Keil et al., J Biol Chem, 2004].

Mutations in tau have been identified in a related neurodegenerative disorder called frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) with neurofibrillary tangles (NFT) formation in the absence of plaque formation. Transgenic mice overexpressing the P301L mutant human tau show an accumulation of hyperphosphorylated tau and NFT formation similar to those in FTDP-17 and AD. However, little is known about the distinct intracellular mechanisms underlying the consequences of tau pathology. Functional analysis showed a mitochondrial dysfunction in P301L tau mice together with reduced NADH-ubiquinone oxidoreductase activity and, with age, impaired mitochondrial respiration and ATP synthesis. Furthermore, P301L tau mitochondria showed increased vulnerability towards Aβ peptide insult, suggesting a synergistic action of tau and Aβ pathology on the mitochondria. Taken together, we conclude that tau pathology involves a mitochondrial and oxidative stress disorder distinct from that caused by Aβ [David et al., J Biol Chem, 2005].

Neuroprotective/anti-apoptotic effects of the nitric oxide/cGMP/protein kinase G signaling pathway: BAD, CREB and IAPs as downstream target proteins

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Activation of cGMP/protein kinase G (PKG) signaling pathway in PC12 cells, stimulated by nitric oxide (NO) at low physiological concentrations [Kim et al., J Neurosci, 1999] or natriuretic peptides (ANP or BNP) [Fiscus et al., Neuroreport, 2001], results in anti-apoptotic effects. Even basal levels of cGMP/PKG activation (in cultured neural cells exposed to serum factors and (likely) brain neurons exposed to physiological levels of NO) appear sufficient to partially activate this protective mechanism, thus helping prevent spontaneous onset of apoptosis [Fiscus, Neurorsignals, 2002]. We have also shown that pretreatment of NG108-15 cells with ANP (24 h) protects against pro-apoptotic effects of high (pathological) levels of NO [Cheng Chew et al., Histochem Cell Biol, 2003]. Recently, our laboratory has shown that PKG directly phosphorylates the apoptosis-regulating protein BAD at serine 112 and 155, which (in concert with PI3-kinase/Akt-mediated phosphorylation of serine 136 of BAD) would remove pro-apoptotic effects of BAD. PKG also directly phosphorylates the transcription factor CREB, potentially stimulating expression of specific anti-apoptotic proteins. Two such proteins may be c-IAP-2 and LIVIN, members of the inhibitor of apoptosis protein (IAP) family. We have shown that depletion of cGMP with ODQ downregu-
lates whereas cGMP/PKG stimulation with ANP, BNP or cGMP analogs upregulates c-IAP-2 and LIVIN expression in NG108-15 cells. Because IAPs are endogenous inhibitors of caspase-3 activity (keeping in check the pro-apoptotic effects of basal caspase-3 activity), cGMP/PKG-mediated regulation of IAPs may represent a novel mechanism protecting neural cells against apoptosis. Overall, the data suggest that cGMP/PKG-mediated neuroprotection may involve BAD and CREB phosphorylation and c-IAP-2 and LIVIN upregulation. This protective mechanism may serve an important function to help prevent neuronal apoptosis caused by high-level NO (and other neurotoxins), thus helping to protect against the damage that could lead to Alzheimer’s disease and Parkinson’s disease.

Novel therapeutic approaches to treatment of degenerative diseases using CDK inhibitors

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Cyclin dependent kinases (CDKs) are ubiquitously distributed in different mammalian cells. The family of CDKs consists of at least 11 well characterized proteins. There is a reason to believe that additional CDKs are yet to be discovered since several Ser/Thr protein kinases possess the PLSTAIRE homology motif that is characteristic of the the cyclin binding domain of known CDKs. CDKs form complexes with specific cyclins. Through successive cell cycle-dependent expression of distinct cyclins, their association with specific CDKs and finally, the appropriate activation of complexes, the progression of the cell cycle is regulated. The proper regulation of the cell cycle protects cells against cancer. Some of these functions are tissue specific, including among others the specialized role of CDKs in the development and maintenance of the nervous system.

CDK5 is widely distributed in different tissues, however, the highest concentration of the active form is found primarily in neuronal cells due to the selective localization of its activator proteins p39–p25 in the nervous system. However, abnormal expression and deregulated activity of CDK5 have been observed in some neurodegenerative diseases, such as Alzheimer’s disease. The upregulation of CDK5 activity is associated with an increased apoptosis rate in certain cells but the mechanism by which CDK5 may facilitate apoptosis has not yet been elucidated. Recently, the activation of the p53 tumor suppressor by CDK5 was reported. The CDK5 mediated elevation of the apoptotic rate suggests an involvement of CDK5 in the pathological progression of these diseases. Therefore, the inhibition of CDK5 activity is a rationale for establishing novel therapeutic approaches. Indeed, discovery of new and potentially selective CDK inhibitors as well as progress in the clinical development of candidate drugs are necessary.
Cyclic GMP-dependent pathways in protection of mononuclear cells

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Intracellular level of cGMP is controlled by guanylyl cyclases (GCs), which synthesize the nucleotide from GTP, and phosphodiesterases (PDEs) hydrolyzing it to GMP. Guanylyl cyclases exist as cytosolic (soluble, sGC) or membrane-attached (particulate, pGC) enzymes. Soluble GCs are activated by nitric oxide, while different isoforms of particulate cyclases by peptide ligands (natriuretic peptides, guanylin and bacterial termostable enterotoxins) or Ca$^{2+}$-binding proteins. PDEs belonging to seven families hydrolyze either cGMP or both cGMP and cAMP. Once synthesized, cGMP may regulate activities of effector proteins including cGMP-dependent PDEs (PDE2,3,5,10 and 11) and protein kinases (PKG1 and PKG2). Cyclic GMP has been shown to affect several important functions of cells belonging to a monocyte/macrophage lineage. Inflammatory response mediated by these cells depends on the activity of nuclear factor-κB (NF-κB), a common transcription factor regulating expression of multiple genes relevant for immune reactions. Its constitutive activity was also reported to prevent spontaneous apoptosis of circulating leukocytes. A number of data have indicated that a relationship exists between NF-κB activity and intracellular concentration of cGMP. The nucleotide has been shown to affect expression of TNFα, IL-1, IL-2 and NOS2. In peripheral blood mononuclear cells (PBMC), cGMP is able to increase the activity of NF-κB only when PKG1 is present. Moreover, in PBMC nitric oxide (NO) stimulates at low and inhibits at high doses the NF-κB activity and in the stimulatory effect of NO, the PKG1 is involved. Since antiapoptotic effects of low concentrations of NO have been reported for several cell types, these observations suggest that NO/cGMP/PKG1 signaling pathway could be, at least in part, responsible for the antiapoptotic effect of NF-κB activity described for blood mononuclear cells. However, it has been noted that expression profiles of GCs and PDEs change under influence of environmental conditions and/or during maturation of monocytes to macrophages. Therefore, one can expect that the role of cGMP will also change in these cells.

Pharmacological activation of heat-shock proteins and hypoxia-inducible genes

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Heat-shock proteins (Hsps) comprise a distinct population of proteins, mostly molecular chaperones, which are overexpressed in the cells in response to heat and other stressors. Cellular regulations of the protein folding and association are disturbed in many neurological diseases, acute and chronic, and in brain senescence. Moreover, both the ability of Hsp70 to suppress several types of brain cell death and the role of increased Hsps expression in the establishment of brain tolerance have been experimentally shown. Properties and functions of Hsps make them attractive as potential targets of pharmacological manipulations aimed at establishing neuroprotection, and the search continues for non-toxic compounds which could stimulate Hsps expression. Sodium salicylate and some other NSAIDs activate the heat shock transcription
factor 1 (HSF-1), but it remains to be shown whether this is neuroprotective. Nontoxic synthetic hydrazine derivatives bimoclomol and arimoclomol increase Hsps response to heat shock. Arimoclomol prolonged survival in transgenic murine model of amyotrophic lateral sclerosis (ALS) and is currently under development as a drug for human ALS. A synthetic anti-ulcer drug geranylgeranylacetone (GGA) both induces Hsp in the brain and produces acute neuroprotection in animal models. Other low-molecular weight, nontoxic compounds able to induce Hsps, paeoniflorin and celerystrol, were recently isolated from natural sources following the leads from traditional Chinese medicine; their neuroprotective properties remain to be investigated. The other family of proteins which may be of therapeutic significance for treatment of neurologic diseases is that coded by hypoxia-inducible genes and upregulated in response to hypoxia. It comprises: > 80 proteins upregulated by hypoxia, including erythropoetin (Epo), Epo receptors, VEGF, glucose transporters and several glycolytic enzymes. Recombinant Epo as well as Epo variants devoid of erythropoietic activity display neuroprotective properties mediated, at least in part, by tissue-protective heteroreceptors. Epo and non-erythropoietic Epo variants are currently subject of clinical development as treatments of various neurodegenerative conditions including stroke and glaucoma.

Cerebral microcirculation after experimental subarachnoid hemorrhage

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Increased intracranial pressure (ICP), decreased perfusion pressure and severe ischemia which are observed in humans in the acute phase following intracranial aneurysm rupture represent also characteristic features of experimental models of subarachnoid hemorrhage (SAH). Although a decrease in cerebral perfusion pressure below the lower limit of autoregulation will inevitably result in a passive decrease in cerebral blood flow at regional or local level. The data obtained by us and also by others suggest that active vasoconstriction contributes directly to acute ischemia after experimental SAH. This acute vasoconstriction, ischemia and subsequent changes in microvascular reactivity may predispose blood vessels to develop late vasospasm which is the most serious consequence of SAH. Studying the reactivity of cerebral microflow to vasodilators in a course of 96 h following perforation of the bifurcation of the intracranial portion of the internal carotid artery in the rat [Veenken et al., Stroke, 1995; Bederson et al., Stroke, 1995] we have found that reactivity to direct as well as endothelium-dependent vasodilators in the ipsilateral cortex was severely impaired up to 48 h after SAH. At 96 h slight recovery of LDF responses was noted. On the contralateral side, a lack of the reactivity to all tested vasodilators was observed only at 24 h after SAH which correlated with a massive cerebral edema. Interestingly, the response to L-NAME was well preserved on both sides at all times. It seemed, however, that in the ipsilateral cortex vasoconstrictor metabolites of arachidonic acid participated to various extent in microflow decrease following L-NAME administration at different times after SAH. Electron microscopy revealed damage of endothelial cells and rearrangement of the contractile elements in ipsilateral MCA and in BA. This study demonstrates that vasodilatory capacity of microcirculation is severely impaired following SAH. Structural changes observed in MCA and BA suggest remodeling of the vessel wall.

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Anti-inflammatory therapy in neurodegeneration

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The inflammatory reaction plays an important role during neurodegenerative processes in the central nervous system. Activation of microglia and secretion of an array of pro-inflammatory cytokines, complement proteins, cytotoxic molecules may accelerate nervous cell death. Microglia is involved in the pathology of senile plaque formation in Alzheimer’s disease and in production and polymerization of amyloid beta. Many experimental studies showed that depression of microglia activation might lessen the damage. Similar observations were made when an inhibition of pro-inflammatory cytokine production or action, led to less injury. That comes to the point that the inflammatory reaction evoked by injury may be detrimental to some extent to the nervous system, and that the regulatory mechanisms of such inflammatory reaction work unsatisfactorily in the central nervous system.

Use of anti-inflammatory drugs in the experimental models of neurodegeneration showed also that diminishing the extent of inflammation may have protective effect. In a mouse model of Parkinson’s disease evoked by MPTP, we showed that use of dexamethasone, and selective or non-selective non-steroidal anti-inflammatory drugs (indometacin, rofecoxib or ibuprofen) diminished the extent of injury. However, the mechanism of such protective properties of these drugs is not resolved, but one of the most possible is based on their wide anti-inflammatory action. Study of McGeer et al. [Neurology, 2000] and Chen et al. [Arch Neurol, 2003] showed that the use of non-steroidal anti-inflammatory drugs might diminish risk of Alzheimer’s disease and might delay or prevent the onset of Parkinson’s disease. However, the first clinical trials with anti-inflammatory treatment were unsuccessful in AD, but the promising observation still give us hope for finding the treatment for neurodegenerative disorders.

Inflammatory signaling and oxidative stress in experimental models of Alzheimer’s disease

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Whole genome expression profiling studies have identified brain genes that are significantly mis-regulated in Alzheimer’s disease (AD) when compared to age-matched controls. Alterations in these gene expression patterns correlate with the development and progression of AD. Increases in the expression of specific brain genes are in contrast to the majority of expressed RNA populations (55%–67%) which are found to be down-regulated in AD. Our data show increases in RNA abundance for an oxidative stress-responsive, pro-inflammatory, pro-apoptotic and pro-angiogenic gene family that occurs during the transition from fetal to aged, and again during the transformation from aged to AD brain. Significantly up-regulated RNAs include those encoding stress-induced factors and molecular chaperones, transcriptional repressors, pentraxins, pro-apoptosis factors, and several inflammatory and angiogenic markers. The findings support the hypothesis of a continuum of stress-related gene expression as the brain ages, and an advancement of inflammatory, apoptotic and angiogenic gene signaling that correlates with the transition to AD. A dysfunctional cerebral vasculature appears to play an accessory role in several of these gene expression changes, and may be an important contributory factor to the etiopathology of the AD
process. Cytokine-, amyloid peptide-, hypoxia-, and neurotoxic metal-stressed human neural (HN) cells, a primary co-culture of neurons and glia, are providing a useful *in vitro* model system to study altered gene expression patterns in both aging brain cells and in AD brain. Our most recent results show that physiologically relevant amounts of iron and aluminum, when added to HN cells, induce Fenton chemistry and the production of reactive oxygen intermediates, inducing both oxidative stress and pro-inflammatory gene expression.

Are age-related neurodegenerative changes triggered by an imbalance between proinflammatory and antiinflammatory influences?

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It has been consistently reported that synaptic plasticity, specifically long-term potentiation (LTP) in the hippocampus, is negatively affected by age. The evidence indicates that neuroinflammatory changes, typified by an increase in hippocampal concentration of the proinflammatory cytokine IL-1β, contribute to the age-related deficit. However, recent evidence has indicated that inflammatory changes extend beyond this, with significant age-related increases in other inflammatory cytokines, as well as increased NO production. At least 2 anti-inflammatory cytokines, IL-4 and IL-10, have been shown to antagonize the effects of IL-1β and the finding that hippocampal concentrations of both is decreased with age has led us to propose that the age-related deficit in LTP probably results from an imbalance between proinflammatory and anti-inflammatory influences. Among the treatment strategies which appears to have the capacity of redressing this imbalance is the polyunsaturated fatty acid eicosapentaenoic acid (EPA). Treatment of aged rats with EPA attenuates the age-related changes in hippocampal cytokines concentrations and also reverses the age-related impairment in LTP. Data will be presented which indicate that the activation state of microglia is critically important in determining the ability of aged rats to sustain LTP and the data will also provide evidence suggesting that the primary action of EPA may be to downregulate microglial activation.

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The role of nitric oxide in the primary proprioceptive sensory signaling

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In this study NOS immunohistochemistry supported by NADPHd histochemistry was used to demonstrate the NOS immunoreactivity in the monosynaptic Ia-motoneuron pathway exemplified by structural components of the soleus H-reflex in the dog. A noticeable number of medium-sized intensely NOS-IR somata (1,000–2,000 μm² square area) and large intraganglionic NOS immunoreactive fibers, presumed to
be Ia axons, was found in the L7 and S1 DRG. The existence of NOS-IR fibers (6–8 μm in diameter, not counting the myelin sheath) was confirmed in L7 and S1 dorsal roots and in the medial bundle of both dorsal roots before entering the dorsal root entry zone. By virtue of the funicular organization of NOS-IR fibers in dorsal funiculus (DF), the largest NOS-IR fibers represent stem Ia axons located in the deep portion of DF close to the dorsomedial margin of the dorsal horn (DH). Upon entering the gray matter of L7 and S1 segments and passing through the medial half of DH, tapered NOS-IR collaterals of the stem Ia fibers pass through the deep layers of DH and intermediate zone, and terminate in the group of homonymous motoneurons in L7 and S1 segments innervating the gastrocnemius-soleus muscles. Terminal fibers issued in the ventral horn intensely NOS-IR terminals with long axis ranging from 0.7 to < 15.1 μm are presumed to be Ia bNOS-IR boutons. This finding is unique in that it focuses directly on contribution of NOS-IR fibers to the signalling transmitted by proprioceptive Ia neurons. NOS-IR boutons were found in the neuropil of Clarke’s column of L4 segment, varying greatly in size from 0.7 to < 15.1 μm in length × 0.7 to 4.8 μm wide. Subsequent to identification of the afferent NOS-IR limb of the monosynaptic Ia-motoneuron pathway on control sections, intramuscular injections of the retrograde tracer Fluorogold into the gastrocnemius-soleus muscles, combined with NOS immunohistochemistry of L7 and S1 DRG, confirmed the existence of a number of medium-sized NOS-IR somata (1.000–2.000 μm² square area) in the dorsolateral part of both DRG, presumed to be proprioceptive Ia neurons. Concurrently, large NOS-IR fibers were detected at the input and output side of both DRG. S1 and S2 dorsal rhizotomy caused a marked depletion of NOS-IR in the medial bundle of S1 and S2 dorsal roots and in the DF of S1, S2. In addition, anterograde degeneration of large NOS-IR Ia fibers in DF of L7-S2 segments produces direct evidence that the afferent limb of the soleus H-reflex is NOS-IR and presents new immunohistochemical characteristics of the monosynaptic Ia-motoneuron pathway, inseparably coupled with the performance of the stretch reflex.

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**Calcium regulation in brain aging and Alzheimer’s disease**

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Calcium plays fundamental roles in learning and memory (synaptic plasticity) and is also involved in neuron survival and death. Dysregulation of intracellular calcium signaling has been implicated in the pathogenesis of brain aging and Alzheimer’s disease (AD) [LaFerla, Nat Rev Neurosci, 2002]. Most genes known to increase susceptibility to AD also modulate at least some aspects of calcium signaling.

Data from our group about peripheral cells (lymphocytes) from aged humans or from patients with sporadic AD have demonstrated abnormalities in calcium signaling reminiscent of those in neurons [Müller, Ann NY Acad Sci, 1996]. These findings were confirmed in PS1 transgenic mice [Eckert, Neurobiol Dis, 2001]. Presently, we are characterizing calcium signaling in specific transgenic neuronal cell culture models, reflecting diverse aspects of the disorder (mutated APP- or neurofibrillary tangle-bearing cells). After additional stress (Aβ1–42, endoplasmic reticulum- or oxidative stress) we found typically altered calcium responses of those cells, while baseline characteristics were usually less affected.

While the data clearly show that many risk factors of AD may have alterations of calcium signaling as common final pathway, it is not yet finally known if altered calcium signaling is cause or consequence of AD relevant neurodegeneration [Mattson, J Mol Neurosci, 2001]. Our data using PC12 cells stably transfected with mutant human APP rather suggests the first mechanism.
Sphingolipid metabolism and neuronal function

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The lipid second messenger sphingosine 1-phosphate (SPP) has been implicated in the regulation of a variety of important mammalian cell processes including proliferation, differentiation, and apoptosis. Interest in SPP has focused recently on two distinct cellular actions of this lipid, namely, the function of SPP as an extracellular ligand activating specific G protein-coupled receptors and the role of SPP as an intracellular second messenger. The mechanism of intracellular action of SPP, however, remains unclear.

Sphingosine kinase (SPHK), the enzyme that catalyzes the phosphorylation of sphingosine, plays a central role in the regulation of intracellular levels of SPP. Two isoforms of mammalian SPHK (SPHK1 and SPHK2) have been cloned and characterized. Overexpression of SPHK1 induces cell proliferation by promoting the G1 to S transition of the cell cycle as well as by inhibiting the apoptotic response to serum deprivation or ceramide treatment. On the other hand, SPHK2 is a nuclear protein and inhibits DNA synthesis when overexpressed in mammalian cells. However, the physiological role of SPHK especially in the central nervous system remains largely unknown.

In the present studies, we have identified a brain specific protein, delta-catenin/neural plakophilin-related armadillo repeat protein (NPRAP) as a potential binding partner for SPHK1 by yeast-two hybrid screening. From co-immunoprecipitation analyses carboxyl-terminal portion of delta-catenin/NPRAP containing the 7th to the 10th armadillo repeats was found to be required for interaction with SPHK1. Endogenous delta-catenin/NPRAP was co-localized with endogenous SPHK1 and transfected delta-catenin/NPRAP was co-localized with transfected SPHK1 in dissociated rat hippocampal neurons. Madin-Darby canine kidney (MDCK) cells stably expressing delta-catenin/NPRAP contained elevated levels of intracellular SPP. In a purified system, delta-catenin/NPRAP stimulated SPHK1 in a dose-dependent manner. Furthermore, delta-catenin/NPRAP-induced increase in cell motility in MDCK cells was completely inhibited by dimethylsphingosine, a specific inhibitor of SPHK1. These results strongly suggest that at least some of delta-catenin/NPRAP functions including increased cell motility are mediated by an SPHK/SPP signaling pathway.

Design of novel selective PARP inhibitors in search for new neuroprotective agents

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Poly(ADP-ribosylation) is a transient post-translational modification which takes place in eukariotes in response to exposure to DNA-damaging agents, and is involved in fundamental processes related to the preservation of genomic integrity such as DNA repair, chromatin decondensation, and, under certain circumstances, cell necrosis, and apoptosis. Potent and selective PARP inhibitors have been shown to be endowed with neuroprotective properties in experimental models of brain ischemia and thus represent a promising new class of anti-ischemic agents. Poly(ADP-ribosylation) is accomplished by a family of enzymes, among which PARP-1 and PARP-2 are the most characterized. The selectivity between the two PARP enzymes is turning out to be a particularly relevant issue and the quest for selective PARP-1/PARP-2 inhibitors.
is motivated by the need of further clarifying the pathophysiological role of individual enzyme isoforms. In this communication, we present an in silico approach aimed at identifying the molecular basis for enzyme isoform’s selectivity. The thus obtained molecular models are instrumental for virtual screening which allows the identification of novel chemical entities representing the basis for novel neuroprotective agents.

Mitochondrial calcium regulation and reactive oxygen species generation: sources for neurodegeneration after brain ischemia and targets for neuroprotective mechanisms

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The brain depends more on continuous energy supply than other tissues. The disturbance of energy supply during ischemia is fatal for brain tissue. Partial energy depletion triggers a broad range of mechanisms leading to progressive cell death in the penumbra. The delay in the induction of cell death in brain tissue opens the possibility for medical interventions. A prerequisite for interventions is the understanding of cellular mechanisms induced by energy deprivation. Mitochondria play a central role not only in energy production of the brain but also in induction of cell death. Generation of reactive oxygen species (ROS) due to disturbed oxidative phosphorylation in brain tissue is a major cause of neurodegeneration. We used defined mixed hippocampal cell cultures [Kahlert and Reiser, Cell Calcium, 2004; Kahlert et al., Neurosci Res, 2005], to study cytosolic Ca2+ level, mitochondrial potential and ROS generation. Both neurons and astrocytes, showed similar Ca2+ responses to excitotoxic glutamate challenge and recovery of cytosolic Ca2+. The latter was delayed in neurons in comparison to astrocytes. Ca2+ in astrocytes was mainly released from internal stores. In contrast, neuronal Ca2+ depends strongly on the presence of extracellular Ca2+ and the opening of NMDA/glycine-sensitive channels. The increase in cytosolic Ca2+ mediated a small, reversible mitochondrial depolarization in both astrocytes and neurons, measured by Rh123. However, prolonged glutamate challenge increased mitochondrial potential in both astrocytes and neurons, determined by application of FCCP and oligomycin subsequent to the glutamate challenge. In contrast, neuronal ROS burden measured under identical conditions was ten-times higher than that in astrocytes and was reduced in Ca2+ -free conditions. The results indicate a central role of ROS in the glutamate toxicity of neurons and a coupling to extracellular Ca2+ influx. This suggests that the regulatory mechanisms differ in the various cell types in the brain, such as astrocytes, neurons and oligodendrocytes in excitotoxicity of glutamate.
Intracellular signaling pathways in dopaminergic specification of mesencephalic neural stem cells

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In previous studies, we demonstrated expansion and functional dopaminergic differentiation of human and rodent mesencephalic neural stem cells (NSCs). The major factor for orienting the progeny of these stem cells towards the dopaminergic phenotype is interleukin-1 (IL-1). Thus, the combination of IL-1 and forskolin leads to approximately 5 to 10% of tyrosine hydroxylase-immunoreactive (TH-ir) cells. Here we investigate the mechanisms of dopaminergic differentiation by measuring glial, neuronal and dopaminergic gene expression in rat fetal mesencephalic NSCs exposed to IL-1 for up to 72 h. We performed quantitative real-time-PCR and immunocytochemistry. Gross microscopic examination showed rapid attachment of NSCs with formation of cell extensions (neurites) after 2 to 3 h. The NSC marker nestin displayed high expression at both the mRNA and protein level in undifferentiated cells, but dropped dramatically down during the first three hours after incubation with IL-1 and forskolin. In contrast, neuronal and dopaminergic genes (β-tubulin-III and Nurr1, respectively) showed transiently increased mRNA levels after stimulation with IL-1 with a maximum after 3 to 6 h. Immunocytochemistry of GFAP, GalC, MAP2, TH and Nurr1 revealed that 100% of Nurr1-ir cells were also positive for TH, but GFAP-ir and GalC-ir cells did not stain for Nurr1. Extensive analyses of intracellular signaling pathways and pharmacologic inhibition of various key molecules suggest that the NFκB pathways did not contribute to the dopaminergic differentiation process, but the MAP kinase pathways were crucially involved in both neuronal and dopaminergic differentiation of mesencephalic NSCs. These data demonstrate that IL-1 induces the expression of specific dopaminergic transcription and differentiation factors and subsequent dopaminergic differentiation of mesencephalic NSCs. Furthermore, this initiation of dopaminergic specification depends on activation of MAP kinase pathways and AP-1.

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Nitric oxide – the molecular switch for cell life and death in brain aging and neurodegeneration

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Nitric oxide (NO) is a very important inter- and intracellular second messenger. Moreover, NO is also the retrograde messenger involved in mechanism of learning and memory. However, the excessive synthesis of NO and its interaction with superoxide radical leads to cells degeneration and death. Our last data indicated that NO was liberated at excessive amount during brain aging, inflammation and by amyloid beta peptides accumulated intracellularly. Our data indicated that in brain aging covalent modification of neuronal isofrom of NO synthase (nNOS) but not alteration of gene expression is responsible for higher NO
release that subsequently influences turnover of arachidonic acid (AA). The mechanism of NO-evoked alteration of AA metabolism and the role of NO in activation of apoptosis was investigated. It was observed that NO enhanced AA release from synaptoneurosomal phospholipids by stimulation of cytosolic phospholipase A2 (cPLA2) and inhibited its incorporation into phospholipids. NO exerts this effect through cGMP and protein kinase G. Protein kinases ERK1/2 and PKC are also involved in regulation of basal and NO-induced cPLA2 activation. NO donors, b-cGMP and H2O2 inhibit AA incorporation into phospholipids by suppression of arachidonyl-CoA transferase (AA-CoAT) activity. The activity of AA-CoA synthase is not changed by NO. Specific inhibitors of protein kinases ERK1/2, PKC and PKG have no effect on NO-dependent lowering of AA incorporation into phospholipids. However, AA (10 μM), itself markedly inhibited AA incorporation by about 50% into synaptoneurosomal lipids. The lowering of AA incorporation evoked by NO/cGMP/PKG is caused by the increased release of AA and its metabolites. These results suggest that NO, through alteration of cPLA2 and AA-CoA acyltransferase activities, may affect the intracellular level of AA and may have important implication in alteration of nerve endings properties and function. Moreover, excessive NO release may also participate in energy depletion, DNA damage and in activation of apoptotic pathway.

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Inhibition of poly(ADP-ribosyl)ation protects brain against ischemia-reperfusion injury

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Poly(ADP-ribose) polymerase (PARP-1 EC 2.4.2.30) is the key enzyme in DNA repair. However, massive DNA damage leads to overactivation of PARP-1, depletion of its substrate NAD+ and to cell death. The last data suggest that PARP-1 is involved not only in necrotic but also in apoptotic cell death. Recently, we indicated that PARP-1 inhibitor, 3 aminobenzamide (3-AB), protected significantly hippocampal neurons against death after 3-min of transient forebrain ischemia. In additional studies, the effect of 3-AB on the ischemia-evoked morphological and ultrastructural alterations in intracellular organelles was investigated. Administration of 3-AB markedly decreased swelling of astrocytes and neuronal cells following 1 and 7 days of reperfusion after 3 min forebrain ischemia. Moreover, PARP inhibitor protected mitochondria and Golgi apparatus against damage. Nuclear membranes were better preserved and most of the neuronal fibres contained normal inner structure. 3-AB significantly enhanced the amount of surviving neurons and eliminated the cytoplasmic dark condensates, the signs of ongoing necrotic neuronal death. The immunostaining of glial fibrillary acidic protein (GFAP) enhanced by ischemia in the stratum: oriens, radiatum and lacunosum-molecular was decreased by 3-AB. Inhibition of PARP prevented also the release of apoptosis inducing factor (AIF) from the mitochondria. However, 3-AB had no protective effect on neuronal survival in CA1 layer of hippocampus after prolonged 10-min ischemia-reperfusion injury. In conclusion, the data clearly show that 3-AB had an ameliorating effect on neuronal survival and on neuronal ultrastructure after short forebrain ischemia.

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Polyphenols protect cerebral ischemia-induced mitochondrial dysfunction and neuronal apoptosis

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Increased oxidative stress has been regarded as an important underlying cause for neural damage by cerebral ischemia/reperfusion (I/R) insult. The possible neuroprotective effects of curcumin, a polyphenol enriched in turmeric, and a known potent antioxidant were investigated. Bioavailability study indicated a rapid increase in curcumin concentration in plasma. It reached the brain within 1 h after treatment. Global cerebral ischemia was induced in Mongolian gerbils by transient occlusion of the common carotid arteries and histochemical analysis indicated extensive neuronal cell death together with the activation of astrocytes and microglial cells in the hippocampus CA1 area 4 days after I/R. These ischemic injuries were preceded by a rapid increase in lipid peroxidation, changes in membrane potential, increased cytochrome c release, and subsequently caspase-3 activation and neuronal apoptosis. Dietary supplementation of curcumin (2.0 g/kg diet) in AIN 76 diet for 2 months significantly attenuated ischemia-induced neuronal cell death as well as glial activation. Interestingly, administration of curcumin by ip injection (30 mg/kg) at five min after ischemic insult, and again at 24 h after I/R also protected the gerbil brain from neuronal cell death and glial activation. Curcumin administration also decreased lipid peroxidation, mitochondrial dysfunction and apoptosis. In addition, curcumin also ameliorated the increase in locomotor activity observed 24 h after ischemic insult. Similar results were obtained with other polyphenol compounds, such as resveratrol, and mixture of polyphenols in grape extract. Taken together, these observations indicated that the neuroprotective effects of polyphenols against I/R-induced neural damages were due to their antioxidant capacity in reducing oxidative injury and their ability to inhibit signaling cascade leading to apoptotic cell death. The data also suggest the possible therapeutic value of polyphenols for prevention or treatment of stroke and other neurodegenerative disorders.

Phenotype-dependent vulnerability of cholinergic neurons to neurodegenerative signals

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Preferential loss of cholinergic neurons in brains of Alzheimer’s disease victims is accompanied by a proportional impairment of acetyl-CoA and energy metabolism. Remarkable susceptibility of cholinergic neurons to neurotoxic signals might result from relative shortage of acetyl-CoA due to its increased utilization for acetylcholine synthesis to maintain its pool during sustained neuronal depolarization. Differentiation of SN56 cells by cAMP and retinoic acid increased choline acetyltransferase (ChAT) activity, acetylcholine content/release, p-75 receptor expression and calcium accumulation. It was accompanied by three-fold decrease in mitochondrial and respective increase in cytoplasmic acetyl-CoA levels. In differentiated cells (DC) AI caused similar inhibition of pyruvate utilization but much greater increase in Ca accumulation and decrease in acetyl-CoA level than in nondifferentiated ones (NC). NO generators and amyloid-beta caused much greater suppression of cholinergic phenotype, cell survival and acetyl-CoA
level in DC than in NC. Joint addition of these neurotoxins exerted partially additive effect on cell mortality, suppression of the cholinergic phenotype and acetyl-CoA levels. These changes were more prominent in DC. In NC nerve growth factor (NGF) caused upregulation of cholinergic phenotype and shift of acetyl-CoA from cell mitochondria to cytoplasm. On the contrary, in DC NGF suppressed cholinergic phenotype through p-75 receptor activation and sensitized cells to detrimental effects of amyloid-beta and NO. These cytotoxic effects were alleviated by anti-p75 receptor antibodies. L-acetyl-carnitine reversed suppressive effects of neurotoxins on acetyl-CoA content and acetylcholine metabolism, but did not improve rate of DC survival. Inverse correlation was found between mitochondrial levels of acetyl-CoA and cholinergic activity as well as the direct correlation between viability and acetyl-CoA content in cholinergic cells. These data indicate that susceptibility of cholinergic neurons to neurotoxins depends on availability of acetyl-CoA in their mitochondria, whereas yield of cholinergic transmission on acetyl-CoA level in their cytoplasm.

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Poly(ADP-ribose) polymerase-1 activation and mitochondrial impairment in in vitro model of cerebral ischemia

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Poly(ADP-ribose) polymerase-1 (PARP-1) [also called poly(ADP-ribose) synthetase] is a nuclear enzyme that catalyzes formation of (ADP-ribose)$_n$ chains from NAD$^+$ on acceptor proteins, including histones and PARP-1 itself. Poly(ADP-ribose) is known to be involved in various physiological and pathological events such as DNA repair, cell differentiation, cell cycle, malignant transformation, cell aging, and cell death. The excessive activation of PARP-1 leads to depletion of cellular NAD$^+$. The depletion of NAD$^+$, a co-enzyme in energy metabolism, results in reduction of ATP generation, while ATP is used for replenishment of NAD$^+$. This “energy crisis” is considered to be causative of necrotic cell death.

PARP-1 (113 kDa) consists of three functional domains; the amino-terminal DNA-binding domain, the central automodification domain, and the carboxy-terminal catalytic domain. In apoptosis, PARP-1 is cleaved by caspase 3 (or 7) at a site within the nuclear localization signal into a 24-kDa amino-terminal fragment and an 89-kDa carboxyl-terminal fragment. The cleavage is accompanied by inactivation of the enzyme and, probably, contributes to NAD$^+$/ATP saving for apoptosis. In this study, we investigated the molecular mechanism of neuronal apoptosis after cerebral ischemia and reperfusion by focusing on the roles of PARP-1 and mitochondria. We used rat cortical neurons in culture exposed to oxygen-glucose deprivation (OGD) and reoxygenation as an in vitro model of cerebral ischemia and reperfusion. A 2-h OGD induced apoptosis in rat cortical neurons. PARP-1 was activated in the period from 12-h to 48-h reoxygenation, resulting in a decrease in intracellular NAD$^+$ contents. A 2-h OGD induced mitochondrial impairment, including membrane depolarization and a release of cytochrome c (cyt c) and AIF. The cytosolic cyt c activated a caspase cascade, and led to a cleavage of PARP-1 by caspase 3. AIF, once released from mitochondria, translocated itself to the nucleus and induced apoptosis in a caspase-independent manner. These apoptotic changes of mitochondria and the nucleus were attenuated by membrane depolarization and a release of cytochrome c (cyt c) and AIF. The cytosolic cyt c activated a caspase cascade, and led to a cleavage of PARP-1 by caspase 3. AIF, once released from mitochondria, translocated itself to the nucleus and induced apoptosis in a caspase-independent manner. These apoptotic changes of mitochondria and the nucleus were attenuated by PARP-1 inhibitors, 1,5-dihydroxyisoquinoline and benzamide, and also by small interfering RNA specific for PARP-1. These results indicated that PARP-1 played a principal role in inducing mitochondrial impairment that ultimately led to apoptosis of neurons after cerebral ischemia.
Lipid signaling mechanisms of spinal cord trauma: *in vitro* and *in vivo* models

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Spinal cord injury (SCI) is associated with release of free fatty acids, in particular arachidonic acid (AA), from cell membranes. Our data indicated that increased levels of AA can lead to induction of cellular oxidative stress and apoptosis as well as diminished expression of BDNF, FGF-2, and NGF in cultured spinal cord neurons. In contrast, exposure to AA increased mRNA levels and activity of choline acetyltransferase (ChAT) in dose- and time-dependent manners. A series of experiments with specific signal transduction inhibitors indicated that AA-induced stimulation of ChAT was mediated primarily at transcriptional levels by activation of protein kinase C (PKC). In addition, our research focused on the effects of agonists of neuronal nicotinic receptors, including nicotine in protection against pathophysiological events associated with *in vitro* and *in vivo* models of SCI. In spinal cord neurons, treatment with nicotine activated the extracellular regulated kinase 1 and 2 (ERK1/2) pathway, upregulated expression of neurotrophic factors, and exerted antiapoptotic effects. In addition, nicotine administration following the experimental compressive SCI markedly attenuated oxidative stress, activation of NF-κB, AP-1 and CREB, as well as overexpression of MCP-1 and TNF-α. These effects were especially pronounced in the lumbar region of injured spinal cords. Administration of nicotine also was effective in sparing tissue of the injured spinal cord. Neuroprotective effects of nicotine were fully reversed by inhibition of neuronal nicotinic receptors, such as mecamylamine (a non-specific antagonist of neuronal nicotinic receptors) and α-bungarotoxin (a specific antagonist of the α7 receptor). These results indicate that nicotine administration can attenuate the oxidative injury to spinal cord and suggest that neuronal nicotinic receptors can be attractive targets for neuroprotective therapy of traumatic SCI.

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Amyloid A-beta (1-40) action: friendly and stressful consequences experienced in cell culture and *in vivo*

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The dualistic activities of the amyloid beta (Aβ) peptide as pro-oxidant and constituent of amyloid deposits in Alzheimer’s disease (AD) and as antioxidant of purported physiological function are far from being understood. In an attempt to identify the two-sided heads of the Aβ, neuronal cells were treated with Fe²⁺ and Aβ₁–40 peptide and oxidative stress (OS) parameters and activation of pro-apoptotic and anti-apoptotic signaling kinases were measured. A combination of Fe²⁺ and Aβ₁–40 caused delayed ERK activation, reduced PIP3/Akt activity, enhanced both p38 MAPK and caspase 9 and 3 activities and ultimately resulted in cell death. In contrast, addition of Aβ₁–40 without Fe²⁺ enhanced PKC levels, stimulated Akt and enhanced Ser-136 BAD phosphorylation suggesting a potential anti-apoptotic function of the peptide.
Intraperitoneal injection of Aβ1-40 into rat fetuses subjected to global ischemia in vivo enhanced levels of antioxidant activities and stimulated the levels of pro-survival signals. Pretreatment with Aβ1-40 reversed the consequences of a transient hypovolemic/hypotensive OS episode by restoring glutathione levels and lowering production of lipid peroxides presumably by activating the aforementioned pro-survival signaling cascades. These data suggest that pre-exposure to Aβ1-40 stimulates fetal tolerance to ischemia via regulation of glutathione metabolism and as such may be considered as neuroprotective.

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**Ischemia modulates signal transduction from extracellular matrix**

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Cell adhesion to extracellular matrix (ECM) functions as a survival factor for many cell types, including neurons. Cell attachment via the association of integrins with ECM proteins generates intracellular signals which lead to the specific tyrosine phosphorylation cascade of a limited number of protein substrates, and these participate in the regulation of cytoskeletal organization and gene expression. This pathway involves a non-receptor tyrosine kinase called focal adhesion kinase (pp125FAK), a key component responsible for the flow of information from the ECM to the cell interior. Phosphorylated FAK may interact directly with other non-receptor kinases, adaptor molecules and cytoskeletal proteins, perhaps providing a pathway by which ECM may regulate cell viability. Disruption of cell-ECM interaction via proteolytic degradation of the matrix components may be expected to affect the linkage between ECM and signaling cascade to which it is connected with profound effect on cell survival. In the present study, the temporal relation between activation of extracellular metalloproteinases (MMP2 and MMP9), degradation of extracellular matrix protein laminin and the expression of pp125FAK were investigated after 5 min of global ischemia in gerbil hippocampus. While significant activation of both investigated metalloproteinases occurred in the course of reperfusion, only changes in MMP9 activity were correlated with degradation of laminin. These ischemia-induced extracellular events coincided temporarily with proteolytic modification of FAK protein and diminished level of its phosphorylated form, to about 50% of the initial value. Down-regulation of FAK activity was followed by the loss of enzyme capacity to associate with its molecular partners – Src kinase and adaptor protein p130Cas. These results are indicative of an involvement of ECM-pp125FAK signaling pathway in ischemia-induced neuronal degeneration.
Changes in cellular localization of cytoskeletal tau protein in aged cholinergic neurons

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We hypothesize that the age-related degeneration of cytoskeleton in basal forebrain cholinergic neurons (BFCNs) renders the NGF-TrkA signaling system non-functional and thereby impairs trophic support. Comparing young (4-month) and aged (28-month) rat brain, we examined immunohistochemically the compartmentalization of phosphorylated Tau protein using antibodies against phospho-Tau404 and -Tau231, the GSK3β kinase known to phosphorylate Tau, the neurotrophin NGF and its receptor phospho-TrkA. We also characterized the efficiency of retrograde axonal transport of BFCNs. Retrograde labeling of basal forebrain cholinergic cells after injection of fluorogold into multiple sites in the cortex and hippocampus revealed a significantly lower number of fluorogold positive cells in aged brain. Despite a lower density of phospho-TrkA immunoreactivity in the cortex and hippocampus of aged rats, there was no difference in NGF expression. In young animals, P-Tau404, P-Tau231 and GSK3β immunoreactivity was observed mainly in neuronal fibers with minimal staining in somata in the main subdivisions of basal forebrain. By contrast, Tau and GSK3β labeling was confined to the cell bodies in aged rats. This is confirmation that aging leads to a redistribution of cytoskeletal proteins in BFCNs. Since a somatic localization of phospho-Tau is indicative of cytoskeletal breakdown, we suggest that failure of axonal trafficking may be responsible for the lack of trophic support in aged cholinergic neurons of the basal forebrain. Aging BFCNs lose their ability to sustain retrograde transport of target-derived NGF and then become vulnerable to degenerative changes.

Modulation of muscarinic cholinergic receptors in the central nervous system of galanin-treated rats

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The neuropeptide galanin (gal) is involved in diverse neurological processes and is altered in neurodegenerative diseases such as Alzheimer’s disease. Thus, a gal hyperinnervation of the basal forebrain cholinergic cells has been described in Alzheimer’s disease patients. In addition, some studies have observed cognitive deficits in rodents after treatment with gal. Furthermore, gal provokes an inhibition of acetylcholine release in the hippocampus. Therefore, the aim of this study was to evaluate the effect of intracerebroven-
Failure of mitochondrial respiration affects dopamine metabolism and its transporter function

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The mitochondrial toxin, 3-nitropipionic acid (3-NPA), is a specific inhibitor of succinate dehydrogenase, complex II in the mitochondrial respiratory chain. This toxin is widely used for generation of animal models of Huntington’s disease (HD). The striatum is the primary site of neuronal loss in HD. It has been suggested that dopaminergic (DA) system perturbation is responsible for vulnerability of this brain region to damage.

The aim of our study was to determine the relationship between inhibition of mitochondrial complex II and DA metabolism and transport in the rat striatum after exposure to 3-NP (ip, cumulative dose of 100 mg/kg in 4 days). The study was carried out using spectrophotometric, radiochemical and HPLC methods. Our data showed an increase in DA metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), indicating that DA turnover was exacerbated.

Moreover, synaptosomes exposed to 1 mM 3-NP showed substantial decrease in [(3)H]DA uptake by specific dopamine transporter (DAT). The activity of DAT could be affected by energy disturbances or free radical generation as a result of respiratory complex II inhibition. We also observed in in vitro experiments that DA itself decreased lipid peroxidation, while its metabolite, HVA, activated oxidative damages suggesting that accumulated DA metabolites might be involved in the alteration of DAT function.

Our results indicate that mitochondrial complex II inhibition, through the modification of DAT function, is responsible for disturbances of dopaminergic neurotransmission.

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Regulation of nitric oxide-stimulated cytosolic phospholipase A₂ activity and its role in PC12 cells death

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Phospholipase A₂, particularly its cytosolic isoform (cPLA₂), is activated by various signals such as growth factors and produces very potent lipid mediators, free fatty acids including arachidonic (AA), docosahexaenoic acids (DHA) and lyso-phospholipids. The level of AA plays a critical role in regulation of eicosanoids synthesis among them prostaglandins, leukotrienes and in generation of free radicals. The alterations of these processes have important implication in pathomechanism of Alzheimer’s disease and other neurodegenerative diseases.

The aim of this study was to investigate mechanism of NO-evoked AA release and cell death in PC12 cells line. Our data indicated that incubation of [³H]AA prelabelled PC12 cells with NO donors (1 mM SNP or 10⁻⁶ M SNAP) for 30 min stimulated [³H]AA release and free radical generation (assayed by using DCF method). Inhibition of cPLA₂ by ACOOF₃ (10⁻⁶ M) significantly by 50% decreased these processes. Moreover, it was found that DHA inhibited NO-induced cPLA₂ activity. Our data indicated that also PKG, PKC and ERK 1 and 2 were involved in basal and NO-induced AA release by cPLA2 activation. Elevated concentration (0.05–1 mM) of NO donor, SNP decreased neuronal viability determined by 3-[4,5-dimethylthiazol-2]-2,5-diphenyltetrazolium bromide (MTT) assay. SNP (1 mM) decreased cell viability by about 80% after 24-h treatment compared to untreated control cells. These results indicated that NO/cGMP/PKG pathway was involved in modulation of cPLA2 activity and in enhancement of AA release. Both cPLA2 and PKG inhibitors have no ameliorating effect on PC12 cells death evoked by excessive NO release.

Changes in apoptotic DNA fragmentation in mouse brain caused by aging or genetic deletion of the neuronal-nitric-oxide-synthase (NOS-1) gene


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Nitric oxide (NO) is produced by three isoforms of NO synthases (NOS), the neuronal form of NOS (nNOS or NOS-1), the inducible form of NO synthase (iNOS or NOS-2), and the endothelial form of NO synthase (eNOS or NOS-3). NO is critical for normal physiological regulation of the nervous system and plays an important role in learning and memory [Zhang, Eur J Neurosci, 1998]. Also, NO is believed to play a critical role in regulating apoptosis of neurons [Fiscus, Neurosignals, 2002]. NO has either pro-apoptotic effects or anti-apoptotic effects on neurons, depending on the concentration of NO and on the concentration of other chemicals (e.g. superoxide anion that can be combined with NO to form the very toxic peroxynitrite). In the present study, we determined if apoptosis in the brain is affected by aging and by the genetic deletion of nNOS, in order to gain further insight concerning the neurotoxic or neuroprotective effects of nNOS. Whole brain from young and aged nNOS-knockout mice and appropriate wild-type controls were used. Levels of apoptosis were determined by a new ultra-sensitive quantitative technique pioneered in our laboratory for accurately quantifying apoptotic DNA fragmentation using capillary electro-
phoresis with laser-induced fluorescence detector (CE-LIF). Furthermore, the levels of expression of different isoforms of NOS were also determined by immunohistochemistry. The results of the present study show that there is a significantly higher level of apoptosis in the brain of aged (18-month) mice compared to the young (2–3-month) mice (11-fold increase; p < 0.0001). On the other hand, in the nNOS knockout (nNOS-KO) mice, the result is just the opposite, the young knockout mice have higher levels (38-fold increase; p < 0.05) of apoptosis compared to the aged knockout mice. When compared to young wild-type control, nNOS-KO mice have 72-fold (p < 0.01) higher level of apoptosis. When comparing the aged knockout and the aged wild-type, the wild type shows a higher number of apoptosis (8 fold; p < 0.01). The results suggest that in young mice nNOS has antia apoptotic effects, whereas in the aging model nNOS has proapoptotic effects.

The effect of lipopolysaccharide on endothelial cells in the brain

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Lipopolysaccharide (LPS)-evoked oxidative stress is responsible for endothelial cell damage, circulatory dysfunction, and in consequence organs ischemia and death. It is believed that activated cells of inflammatory system are the main source of free radicals in endotoxemia.

The aim of this study was to analyze the effect of LPS on ultrastructure of brain capillaries after ip injection of LPS (1 mg/kg) into C57BL6 mice. Moreover, direct effect of LPS on cultured endothelial cells (HUVEC), and the role of endogenous NO in free radical damage were determined.

Our results indicated that LPS induced pathological alterations in brain endothelial ultrastructure. We observed endothelial cells (EC) with shrunken and dark cytoplasm and swollen EC completely closing vessel lumen without any changes in basement membrane. However, vessels with basement membrane proliferation, but without alterations in EC were also present. Many vessels had reduced lumen due to swelling of neuropile elements: glial and neuronal processes and synapses. In vitro studies showed that LPS reduced viability of HUVEC and enhanced free radical formation determined by fluorogenic probe H2DCF. NO synthase inhibitors, 1400 W and NNLA had no effect on LPS-evoked alterations in HUVEC.

Our results indicated that systemic LPS induced considerable alterations in endothelial cells of brain microcapillaries, which potentially might affect the function of blood-brain barrier. In vitro studies demonstrated that direct effect of LPS on endothelium might play an important role in inflammation-related endothelial dysfunction.

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Changes in cGMP level and nitric oxide synthase activity during maturation of the rat brain

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Nitric oxide (NO), an important messenger molecule in the brain is synthesized mainly by the neuronal isoform of the nitric oxide synthase (nNOS) that is present at different amounts in all brain regions. The main target for NO is the soluble isoform of guanylyl cyclase (sGC). Using immunohistocolocalization of cGMP and cholinergic marker, we have indicated that NO-stimulated cGMP synthesis occurs in all cholinergic fibers in the cortex and caudate putamen. It is more widespread after birth (between day 1 and 10) and significantly decreases during maturation (21 days–4 months) and aging (24 months). Therefore, we decided to study NOS activity and correlate it with cGMP level. The activity of NOS was determined in different brain parts: caudate putamen, hippocampus and cerebral cortex during development. Our data have indicated that NOS activity is decreasing with brain maturation in the following order: hippocampus < caudate putamen < brain cortex. Comparing three investigated brain areas in 10 days old (p10) animals, the highest activity of NOS was observed in the caudate putamen, slightly lower in the cerebral cortex and the lowest in the hippocampus. In p21 and in 4-months-old (adult) animals, the NOS activity decreased in the hippocampus and caudate putamen, compared to p10 but in the brain cortex it remained at the similar level. Therefore, we have concluded that NO/cGMP signaling pathway is involved in the development of the cholinergic system of the brain.

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Differential expression of nestin in glial cells in murine and rat hippocampus during trimethyltin-induced neurodegeneration

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Several data suggest that in different brain pathological conditions, adult glial cells are induced to revert to more primitive glial forms and transiently re-express phenotypic features characterizing early developmental stages. In the present studies, we used immunocytochemistry to investigate whether a neurodegeneration induced by a potent neurotoxicant, trimethyltin (TMT) will be accompanied by a re-expression of nestin, an embryonic intermediate filament component. TMT evokes different pattern of neurodegeneration in murine (dentate gyrus granular cell apoptosis) and rat (pyramidal cells of CA4/CA3 and CA1 region, mainly of non-apoptotic character). In both models, a strong re-expression of nestin in activated glial cells was proved. However, while glial cells in the zone of degenerated CA4/CA3 pyramidal neurons in rat hippocampus were a subpopulation of astrocytes, those in the region of apoptotic murine dentate granular cells represented mainly a novel type of ameboid cells bearing both oligodendroglia progenitor (expression of NG2 and O4) and macrophage-microglia (expression of OX42 and ED1) features. It may be supposed
that in both cases the re-expression of nestin in glia may promote the neuroprotective/recovery processes of injured neurons, however, the mechanism of such effects may be different depending on the type of cells within which this developmental protein is re-expressed.

Presenilin 1 promoter polymorphism is not a risk factor for Alzheimer’s disease

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The genetic determinants of Alzheimer’s disease (AD) are heterogeneous. *PSEN1* is one of a few susceptibility genes of AD. There are several epidemiological data showing that *PSEN1*-22c/t promoter common variant increases a risk of AD by altering *PSEN1* expression and thereby influencing Aβ production. According to in vitro studies –22c allele is associated with a two-fold decrease in promoter activity. We performed a systematic screening of a *PSEN1* promoter region encompassing –22c/t polymorphism to test the frequency of this substitution in a Polish cohort of 115 LOAD, 51 EOAD, 98 mild cognitive impairment (MCI), and 275 age-matched control subjects. Our results suggest that there is no statistically significant correlation between –22c/t polymorphism and a risk for AD.

The mechanism of age-related alteration of endothelial nitric oxide synthase in striatum and other parts of the brain

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Endothelial nitric oxide synthase (eNOS) is present in endothelial cells of blood vessels and in neurons of the central nervous system. Recent data associate this isoform with neuronal plasticity and learning phenomena including long term potentiation in the hippocampus. Our last results indicated that NO was involved in the development and maturation of cholinergic system in the brain. However, little is known about the role of eNOS in brain aging and available results are inconsistent. The aim of this study was to investigate age-related changes in eNOS activity in the striatum and in other parts of the brain and to elucidate mechanisms underlying these alterations. The levels of eNOS mRNA, protein and activity were measured using RT-PCR, immuno- and radiochemical methods, respectively. Our studies indicated that eNOS activity is lower in the striatum and other parts of aged brain comparing to adults although it decreases significantly only in the cerebellum. The expression of eNOS determined at mRNA level was enhanced to about 140% of adult value in the striatum, cortex and hippocampus while it was increased to 190% in the cerebellum. The higher level of mRNA may be an adaptive response to lower NOS activity. However, the Western-blot signal of eNOS protein was unchanged in aged brain parts compared to adults.
suggesting age-related disturbances of its translation. To elucidate causes of decreased activity of this isoform despite unchanged protein level, we investigated the synthesis of NO in the presence of 7-nitroindazole and inhibitors of protein kinases and phosphatases. A change in eNOS phosphorylation may occur in the aged rat brain lowering specific eNOS activity similarly to the change reported in blood vessels. The lower eNOS activity in aged brain may significantly affect the signal transduction processes and neuronal plasticity.

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Interleukin (IL)1-α and IL1-β gene polymorphisms and risk for sporadic late-onset Alzheimer’s disease

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Inflammatory processes play a key role in the pathogenesis of Alzheimer’s disease (AD). In the brains of AD patients, microglial cells are activated and produce a large number of inflammatory mediators including cytokines such as interleukin (IL)1-α, IL1-β, IL-6 and tumor necrosis factor α (TNF-α). Overexpression of IL1 by activated microglia across brain regions correlates with the pattern and distribution of amyloid plaques in AD suggesting that IL1 may be a principal factor in the initiation and spread of Aβ plaque pathology in AD. Recently several papers have reported that genetic variation within genes encoding IL1-α and IL1-β is associated with an increased risk of AD, perhaps through upregulation of the cytokine cascade.

A case-control association study was performed for IL1-α, IL1-β and IL1-receptor antagonist (IL1-RA) genes. The following DNA polymorphisms may influence the protein production: 1) IL1-α-889 (promoter region), 2) IL1-β-511 (promoter region), 3) IL1-β+3953 (exon 5), and 4) a variable number tandem repeats (86 bp VNTR) polymorphism in the IL1-RA gene were analyzed in a cohort of 95 patients with sporadic probable AD (41 cases of early-onset AD, EOAD and 54 cases of late-onset AD, LOAD) and 127 controls. We found a significant increase in the IL1-β-511*2 allele frequency (p < 0.02, OR = 2.42) and homozygotes of IL1-β-511 2/2 genotype (p < 0.047) among LOAD patients than in controls. Moreover, we observed a slight but not statistically significant difference in a distribution of IL1-β+3953 heterozygotes in patients with LOAD and controls (p < 0.08). We compared the obtained results with earlier reports on the association between AD and DNA heterogeneity within the IL1 gene family and we also discussed possible reasons of conflicting data.
Consequences of spinal cord lesion for changes in NOS pools

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Spinal cord injury causes a disruption of motor function. It produces changes in affected cells and in parts of the spinal cord, occurring below the site of the injury. In the present study, the hemisection at lower thoracic level was done and used as a model of traumatic injury of the spinal cord in order to characterize 1) both constitutive NOS and inducible NOS activities at the site of injury and 2) the changes in constitutive NOS activity, neuronal NOS protein and neuronal NOS immunoreactivity in lumbosacral spinal cord 7 days after spinal hemisection. Our data show the increase in inducible NOS activity and a decrease in constitutive NOS activity at injured site analyzed 7 days after spinal cord hemisection. We propose that a rapid formation and release of NO at the site of injury accelerates neurodestructive events leading to a secondary inactivation of constitutive NOS, a process possibly caused by NO binding to the heme iron of NOS and/or by the phosphorylation of NOS by protein kinases. An almost equal decrease in constitutive NOS activity was detected in contra- and ipsilateral dorsal part of the lumbosacral segments on the 7th day of hemisection. This finding was accompanied by a statistically significant changes in the number of nNOS-IR neurons. While a quantitative assessment of nNOS immunostaining disclosed a strong decrease in the number of small neurons in superficial laminae (laminae I-III), the number of large nNOS-IR neurons located in the deep dorsal horn layers (laminae IV-VI) was not significantly affected. Completely different results were found in ventral horn of lumbosacral segments where constitutive NOS activity was considerably increased on ipsilateral side of spinal hemisection. Such increase may account for a high number of retrogradely-responding NOS-IR neurons, found in ventral horn of lower lumbar segments [Maršala et al., J Chem Neuroanat, 2004]. The level of neuronal NOS protein in this part of the spinal cord tended to increase, but the changes were non-significant. Our results show that NO represents a neuromodulator influencing NOS pools not only at the site of the injury but also in remote parts of the spinal cord. These findings suggest the participation of NO in the processing of the information arising from the initial insult, i.e. from thoracic level to affected neurons located far beyond the original lesion.

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Presence of serine base exchange enzyme in triton-insoluble floating fraction from rat cerebral cortex and stimulation of the plasma membrane enzyme by hypoxia

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The impairment of signal transduction machinery has dangerous consequences for cell function and the uncontrolled activity of enzymes of this complex network of events contributes to development of brain damage. This statement can be valid not only for glycerophospholipids that represent a source of lipid
mediators, which play a pivotal role in signal transduction and in neurodegeneration, but also for phosphatidylinerine (PS). We have recently revised metabolic peculiarities of PS metabolism in the brain, with a particular attention to the enzyme responsible for its synthesis by serine base exchange (SBEE), to the involvement of PS in signal transduction and to the possibility that an increase in PS synthesis in brain cortex can be one of the early events in the development of brain damage [Mozzi, Neurochem Res, 2003].

Results of this study done on cerebral cortex of 60-day-old rats demonstrate that: a) SBEE activity is present in Triton X-100 insoluble floating fractions (TIFF) whose enrichment in signaling molecules, including PKC, is well assessed; b) TIFF almost exclusively possess the isoform of SBEE capable of utilizing also ethanolamine as free exchanging base, that is the isoform responsible for stimulation of PS synthesis in hypoxia (unpublished results); c) hypoxia stimulates the activity of SBEE present in plasma membranes (PM). The following experimental procedures were used:

a) TIFF were prepared according to Olive et al. [J Neurochem, 1995] and identified by alkaline phosphatase assay and immunoblotting for flotillin. The region of the gradient corresponding to TIFF contained two visible bands. 1 ml fractions from TIFF containing area were diluted with buffer and centrifuged at 100000 × g, and then membranes corresponding to each band were combined and assayed for SBEE activity (pH 7.4 or pH 8.2 with 2 mM Ca²⁺). Enzyme activity, expressed as pmol PS synthesized/µmol lipid P/30 min, was 14 at pH 8.2 and 8.0 at pH 7.4 (upper band); 11 at pH 8.2 and 4.5 at pH 7.4 (lower band).

b) SBEE assay medium contained, in addition to 8 µM [14C] serine, unlabeled ethanolamine at the concentration (1 mM) which, in PM, maximally lowered by 80% serine incorporation into PS. No radioactivity was found in TIFF PS.

c) Slices were incubated for 10 min before PM preparation. SBEE activity (pH 8.2, 2 mM Ca²⁺) of PM from N₂-treated slices was by 30% greater than that of PM from oxygenated slices.

Taken together these results support our hypothesis that SBEE is a component of signal transduction and that stimulation of its activity is involved in the development of brain damage due to this peculiar role.

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**Effect of carvedilol on neuronal survival and poly(ADP-ribose) polymerase activity in hippocampus after transient forebrain ischemia**

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Carvedilol, a β-adrenoreceptor antagonist with potent antioxidant properties may offer high therapy expectations. In this study the effect of carvedilol on neuronal survival after transient forebrain ischemia in gerbils was investigated. The role of poly(ADP-ribose) polymerase (PARP-1) in this process was evaluated. Our data indicated that carvedilol administered subcutaneously at a dose of 7 or 70 mg/kg directly after 5 min of transient forebrain ischemia, protected significant population of neurons in CA1 hippocampal layer but had no effect after induction of prolonged 10-min ischemia. Carvedilol significantly decreased PARP activity in the hippocampus which was potently biphasically enhanced during 15 min and 4 days of reperfusion after 5 min ischemia. Moreover, carvedilol prevented NAD⁺ depletion after ischemic-reperfusion insult. These results indicated that carvedilol protected neurons against death and suggested that suppression of PARP activity during reperfusion might be involved in this process.

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Regional differences in the expression of glutamate transporters in lead toxicity

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Excitatory amino acid transporters are membrane-bound proteins localized mainly in astroglial but also in neuronal cells. They regulate the postsynaptic response to the released glutamate, terminating its action. Inactivation of glutamate and limiting of glutamatergic signaling restricts overstimulation of glutamatergic receptors and protects neurons from excitotoxic damage. Dysfunction of the transporters may be the initiating event or part of the cascade leading to the cell death. It was shown that glutamatergic component underlies Pb-induced neurotoxicity. Thus, we investigated the response of main glutamate transporters – GLAST and GLT-1 (astrocytic) and EAAC1 (neuronal) to short-term lead exposure in the adult rat brain. Highly decreased expression of GLT-1 mRNA and protein was observed in forebrain and hippocampus of lead-exposed rats. In contrast, GLAST was overexpressed in forebrain and in cerebellum. The enhanced expression of EAAC1 was observed only in forebrain. The results revealed that of the two astroglial transporters GLT-1 was more susceptible than GLAST to the neurotoxic Pb effects. It seems that in the forebrain, unlike in the hippocampus, downregulation of GLT-1 is compensated by overexpression of GLAST, suggesting the protective role of astroglia against elevated extracellular glutamate in this part of the brain. Undoubtedly, the hippocampus is the most vulnerable to excitotoxic cell damage under Pb toxicity conditions due to the impaired clearance of glutamate.

Changes in the expression of glutamate transporters and metabotropic glutamate receptors (group I) in experimental autoimmune encephalomyelitis (EAE)

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Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS), which primarily affects young adults and in most cases leads to chronic disability. Little is known yet about the pathogenesis of the disease. Several hypotheses have been proposed, including viral infection and autoimmunity. Recent studies have suggested a possible role for glutamate excitotoxicity in the pathogenesis of this disease. Patients with MS were found to have increased levels of excitatory amino acids. To investigate the pathogenesis of MS in vivo, a number of experimental models have been developed that reproduce various aspects of the disease. Our present studies have been performed with the rodent model of experimental autoimmune encephalomyelitis (EAE), which is the main animal model used for the investigation of MS. We have focused specifically on the expression of group I metabotropic glutamate receptors (mGluRG I) and excitatory amino acid transporters (EAATs) which are membrane bound proteins localized in glial cells and presynaptic glutamatergic nerve endings. The expression of protein of group I receptors: mGluR1alpha and mGluR 5, and EAATs (EAAT 1–3) was studied. The patterns of mGluR1 and
mGluR 5 expression in EAE tissue revealed significant increase compared to the control pattern. Changes in the expression of the two glutamate transporters – EAAT2 and EAAT3 – were also observed. The results suggest that glutamate is involved in the progressive brain damage in multiple sclerosis. Glutamate transporters, like mGluRG1, may play a role in disturbed glutamate homeostasis. It may be suggested that the future therapeutic strategies for MS should be complemented by mGluRG1 antagonist to reduce neurological disability and protect neurons from neurotoxicity.

Differences in apolipoprotein E and interleukin 1β gene polymorphisms between dementia with neurodegenerative traits and vascular dementia

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In 229 patients with dementia and in 144 control subjects polymorphisms of apolipoprotein E, LDL receptor related protein, α2-macroglobulin, interleukin 1β, angiotensin converting enzyme and of methylene-tetrahydrofolate reductase genes were investigated by DNA analysis. Plasma lipids were also determined. Dementia was classified as probable Alzheimer’s disease, probably dementia of vascular origin and mixed dementia where both neurodegenerative and vascular symptoms were present. An association of the disease with apolipoprotein E and interleukin 1β polymorphism and with increased level of LDL cholesterol was observed in Alzheimer’s disease and in mixed dementia but not in dementia of vascular origin.

The effect of nicotine on the kynurenic acid production in rat brain – in vitro and in vivo study

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Kynurenic acid (KYNA), the endogenous neuroprotectant, is a recognized broad-spectrum antagonist of excitatory amino acid receptors with a particularly high affinity for the glycine co-agonist site of the NMDA receptor complex. Recent studies show that kynurenic acid strongly blocks alpha 7 nicotinic receptor. The present study was performed to assess the effect of nicotine on KYNA synthesis in rat brain slices in vitro and in vivo. In brain slices, only 10 mM nicotine significantly increased KYNA production in normal Krebs-Ringer buffer. Nicotine in modified Ca++-free Krebs-Ringer buffer did not change its ef-
fect on KYNA synthesis. The lack of Na\textsuperscript{+} reversed the effect of nicotine on KYNA production in brain slices – nicotine at 10 mM concentration lowered KYNA production about 25\% vs. control. In vivo assays evaluated the effect of acute exposure of rats to nicotine (1 mg/kg ip) and chronic po administration of nicotine (100 μg/ml) during 10 and 30 days on KYNA concentration in rat brain. Acute exposure of rats to nicotine (1 mg/kg mc ip) did not affect KYNA level in rat brain, 10 days of exposure of rats to nicotine in drinking water increased KYNA level to 143\% vs. control group, while 30 days of exposure of rats to nicotine in drinking water decreased KYNA level in brain to about 53\% vs. control group.

It seems that nicotine effect on KYNA production is not a simple direct receptor effect but it is possible that more sophisticated metabolic changes are involved in its effect on KYNA synthesis in the brain.