Special section

Abstracts

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Topics:

LECTURES OF INVITED SPEAKERS:

Mitochondria in neurodegenerative diseases
Adam-Vizi, V.
Department of Medical Biochemistry, Semmelweis University of Medicine, Budapest, Hungary

Estrogens as neuroprotective hormones: modes of action
Behl, C.
Insitut für Physiologische Chemie und Pathobiochemie, Johannes-Gutenberg Universität, Mainz, Germany

P2Y receptors and control of neuronal Ca^{2+} and K^+ channels
Böhm, S.
Institut für Pharmakologie, Universität Wien, Austria

Calcium, mitochondria and neurotoxicity
Duchen, M.
University College, London, UK

The pain receptor VR1
Hucho, F.
Institut für Biochemie, Freie Universität Berlin, Berlin, Germany

Interaction between purinergic, glutamatergic and dopaminergic systems of the brain
Illes, P.
Rudolf-Boehm-Institut für Pharmakologie und Toxikologie, Leipzig, Germany

Activity dependent synaptic plasticity and neurotrophins
Korte, M.
Max Planck Institute of Neurobiology, Department of Cellular and Systems Neurobiology, Martiensried, Germany

Lipid mediators and microglial activation in neurodegenerative diseases
Minghetti, L.
Instituto Superiore Di Sanita, Rome, Italy

Neuroimmunology and Inflammatory processes
Neumann, H.
European Neuroscience Institute Goettingen Goettingen, Germany

Proinflammatory cytokines and deletion of TNF receptors
Raivich, G.
Department of Anatomy and Developmental Biology, University College, London, UK

Mitochondria - a source and target for oxidative stress in neurodegeneration Toescu, Reynolds, I. J.
University of Pittsburgh School of Medicine, Pittsburgh, USA

Endocytosis and regulation of G protein-coupled receptors
Rosenthal, W.
Forschungsinstitut für Molekulare Pharmakologie, Berlin, Germany

Spatially resolved detection of neurotransmitter secretion
Schuhmann, W.
Ruhr-Universität, Bochum, Germany

Role of acetyl-CoA metabolism and NGF in cholinergic neurotoxicity
Szutowicz, A.
Department of Laboratory Medicine, Medical University, Gdansk, Poland

Nucleotide receptors in the nervous system: Molecular determinants of $P2Y^2$ receptor function

Weisman, G.
University of Missouri-Columbia, Columbia, U.S.A.

Docosahexaenoic acid accumulation in the prenatal brain: A purported antioxidant role

Yavin, E.
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ABSTRACTS OF SHORT TALKS AND POSTER PRESENTATIONS:

Identification of syndapin protein interactions and their biochemical and functional characterizations

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Neurotransmitter release requires that synaptic vesicles fuse with the plasma membrane when intraterminal calcium rises, after which the synaptic vesicle membrane is rapidly recycled and refilled with neurotransmitter. Recovery of plasma membrane after stimulated exocytosis is commonly referred to as compensatory endocytosis. Syndapins are a family of proteins that can interact with the cellular machinery for vesicle budding as well as with that for actin polymerization via its single C-terminal Src homology 3 (SH3) domain. This family of dynamin-associated proteins includes the highly brain-enriched syndapin I (synaptic, dynamin-associated protein I) and the more ubiquitously expressed syndapin II, which exists in several splice variants If it were somehow possible that syndapins form larger complexes by undergoing interactions other than those of its SH3 domain, they could not only play a dual role in both of these cellular processes but instead physically and functionally link them. In the further work we want to identify new syndapin binding partners using the yeast two hybrid system. In this work we used constructs encoding the NPF motif-containing regiond of syndapin II and the full length syndapin Iconstruct, respectively, to find the interaction partners After these interactions have been identified using yeast two hybrid system, they will be further verified in vitro as well as in vivo. These new syndapin interacting partners will be further characterized functionally and biochemically to understand their relevance in biological functions.

Phosphatidylserine Triggers specific signalling pathway in microglial cells

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The clearance of apoptotic neurons by microglia requires the interaction of phosphatidylserine (PS), exposed on the external plasma membrane in the early phase of apoptosis, with its specific receptor PtdSerR. We recently demonstrated that this interaction is crucial to prevent the acquisition of pro-inflammatory functions by microglial cells, similarly to that reported for peripheral macrophages. Indeed, PS-liposomes, used to mimic apoptotic cells, or PS-exposing apoptotic PC12 cells, strongly reduced the LPS-induced release of inflammatory mediators. In order to elucidate the signal transduction elicited by the interaction of PtdSerR with its natural ligand PS in non-stimulated or LPS-stimulated neonatal rat microglial cells, we analysed the effect of PS-liposomes on the activation of the nuclear transcription factor (NF)-kB and the cyclic AMP responding element binding protein (CREB), two transcription factors playing crucial roles in the processes of microglia activation and de-activation, respectively. We found that PS did not affect the activation of NF-kB, consistently with the observation that phagocytosis of

apoptotic cells does not trigger NF-kB activation in peripheral macrophages. On the contrary, PS-liposomes induced CREB phosphorylation in resting microglia, but delayed the phosphorylation induced by LPS. Moreover PS-liposomes inhibited the LPS-induced activation of the p38 mitogen activated protein kinase (p38). In resting microglia, PS-liposomes did not activate p38, in line with the non-inflammatory consequences of the recognition and removal of apoptotic cells by macrophages. To our knowledge, this is the first biochemical evidence of the molecular signalling evoked by PS/PtdSerR interaction possibly related to repression of pro-inflammatory activities in microglial cells.

Developmentally induced oxidative stress coincides with beta-amyloid plaque deposition in transgenic Tg2576 mice with Alzheimer-like pathology

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The molecular mechanisms of beta-amyloidogenesis in Alzheimer´s disease are still poorly understood. Anti-inflammatory and antioxidant agents have been observed to delay onset or to slow down the progression of Alzheimer´s disease. To reveal whether oxidative stress and local inflammatory events may trigger or contribute to beta-amyloid deposition, a transgenic mouse (Tg2576) that express the Swedish double mutation of human amyloid precursor protein and develop Alzheimer-like beta-amyloid deposits at late ages may represent an appropriate approach. In Tg2576 mouse brain, cortical levels of beta-amyloid(1-40) and (1-42) steadily increase with age, but significant deposition of fibrillary beta-amyloid into cortical areas does not occur before postnatal age of 10 months.The aim of the present study was to address the hypothesis whether the age-related occurance of oxidative stress and proinflammatory cytokines coincide with the developmental pattern of beta-amyloid plaque deposition in Tg2576 mouse brain. The activities of superoxide dismutase assayed in cerebral cortical tissue from Tg2576 mice steadily increased between postnatal ages of 9 and 12 months at which age the highest activity during the whole period of life was detected. A similar developmental profile was observed for the activity of glutathione peroxidase. The levels of cortical nitric oxide, reactive nitrogen species (NOx), and interleukin-1beta demonstrated peak values around 9 months of age. The developmental temporal coincidence of increased levels of reactive nitrogen species and antioxidative enzymes with the onset of beta-amyloid plaque deposition strongly suggests the involvement of oxidative stress in triggering beta-amyloidogenesis.
Supported by the Interdisciplinary Centre for Clinical Research (IZKF), University of Leipig, project TP C18.

Expression profile of the SK-N-MC neuroblastoma cell line

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Modern microarray technology enables to record the expression profile of thousands of genes in a single experiment. The large volume of data generated from these experiments provides solid evidence of transcription profiles and allows to gain insight into functional gene networks. As an introduction to functional studies we used the Affymetrix GeneChip® technology to collect and analyse the gene expression profile of SK-N-MC-cells. The used Human Genome U133 A chip is part of a set comprising two microarrays containing over 1,000,000 unique oligonucleotide features. This set covers more than 39,000 transcript variants

which in turn represent more than 33,000 well-substantiated human genes. SK-N-MC-cells, one of two neuroblastoma cell lines isolated in September of 1971 from metastatic neuroblastoma, are frequently used in cell culture for a growing number of studies. Several receptor systems and signalling cascades are already described in the literature. We attempted to gain a cell type-specific characterization of active genes in our culture condition. The cells used for the experiment were cultured according to standard protocols. Five independent samples were pooled and the extracted RNA was used for two replicate measurements. The detection algorithm used for the analysis compared probe pair intensities to generate a detection p-value and assign a Present, Marginal, or Absent call. Significant expression (p-Value <= 0.01) in both replicates for example was found in 9,507 of 22,283 analysed genes (43%). The number of 39 G-protein coupled receptors was found in 337 annotated GPCR-sequences (12%). In 1,120 sequences with annotated relation to signalling pathways and signal transduction processes there were 761 (68%) transcripts found to be present. These first results show that microarray technology is also a valuable tool to further characterize the used model system.

Effectors of the Innate Immunity in Neurodegeneration lead to signalling through the extracellular matrix

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It is a general concept that in CNS diseases, effector molecules of the innate immunity contribute to the progression of the pathology. Proinflammatory cytokines such as Interleukin-1ß (IL-1ß) and Tumor Necrosis Factor alpha (TNF-α) regulate e.g. reactive gliosis, microglia activation and permeability of the blood brain barrier. In order to understand the enigmatic nature of cytokine activation it is important to define target genes of these effector molecules in the CNS. Extracellular proteases such as serine proteases (tPA) or metzincins, matrix metalloproteinases (MMPs) and metalloproteinase-disintegrines (ADAMs) are regulated by proinflammatory cytokines and since they are able to remodel the ECM in the CNS, it is crucial to understand their functions. In several mouse models for neurodegeneration we detected elevated expression levels of membrane-typed Matrixmetalloproteases, either MT-MMPs or ADAMs. They are particularly important for signalling in the ECM in response to injury. Among the substrates of these enzymes are receptors for immunomodulatory molecules such as the low affinity receptor for IgE, CD23. Moreover, also neural cell adhesion molecules are cleaved by ADAMs, demonstrated by the proteolytic release of L1 by ADAM10 or of CHL1 by ADAM8 (1). ADAM8, originally cloned from monocytes, was significantly upregulated in CNS regions excerting neurodegeneration and was localized to reactive astrocytes, microglia, oligodendrocytes and degenerating neurons. In the same cell types, we detected elevated levels of tumor necrosis factor alpha (TNF-α, a cytokine with broad-range effects in the CNS. Recombinant TNF-α was able to stimulate ADAM8 expression in a dose-dependent manner (2) demonstrating that ADAM8 is a TNF-α target gene in CNS diseases. ADAM8 is located to the cell surface, has autocatalytic activity leading to protease activation and proteolytic activity against myelin basic protein (MBP). As the cleaved extracellular part of CHL1 is able to stimulate neurite outgrowth, there is a link between TNF-α and nerve regeneration (1). In addition, ADAM8 has a direct effect on cell adhesion, as the disintegrin domain of ADAM8 is able to mediate cell adhesion in both, a homophilic manner and by binding to integrins (3). Both functions, proteolysis and cell adhesion by ADAM8 are functionally relevant for neurodegenerative diseases. Work was supported by the German Research Council (DFG), SFB 549, A4.

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S., Yamamoto, S., Jockusch, H., Bartsch, J.W. (2000). J. Neurosci. 20, 7964-7971.
Schlomann, U., Wildeboer, D., Webster, A., Antropova, O., Zeuschner, D., Knight, C.G., Docherty, A.J.P., Lambert, M., Skelton, L., Jockusch, H., Bartsch, J.W. (2002) J. Biol. Chem. 277, 48210-48219.

Heat-shock protein 70, heme oxygenase-1, and selenite protect immortalized hippocampal neurons against glutamate-induced cell death

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HT22 immortalized hippocampal neurons serve as a cellular model system to study oxidative stress, an imbalance of the cellular redox homeostasis. Glutamate induces HT22 cell death by inhibiting the uptake of cystine into the cells via the cystine/glutamate transport system x_c^-, thus leading to reduced levels of glutathione. Here, we show that HT22 cells overexpressing heat-shock protein 70 (Hsp70) or heme oxygenase-1 are protected against glutamate-induced cell death. Likewise, sodium selenite supplementation of the culture medium displayed a strong neuroprotective activity. In contrast, brain-derived neurotophic factor (BDNF) stimulation of HT22 cells expressing the TrkB neurotrophin receptor did not provide protection against oxidative glutamate toxicity, but efficiently counteracted withdrawal of trophic support. These data indicate that glutamate-induced cell death differs substantially from that induced by growth factor deprivation. Inhibition of macromolecular synthesis protected HT22 cells, in part, against oxidative glutamate toxicity, indicating that the glutamate-induced cell death pathway further requires the synthesis of pro-apoptotic proteins.

Molecular Mechanisms of Aggregate Formation in OLN t40 Cells

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The accumulation of proteins is tightly linked to cellular degeneration in a variety of neurodegenerative diseases. Protein aggregates are defined by poor solubility, aberrant subcellular distribution and non-native secondary structure. Under normal conditions, protein accumulation is prevented by highly specialized quality control mechanisms, like the chaperone response and selective degradation via the ubiquitin-proteasome pathway. Altered proteasomal function and proteasome impairment have therefore been implicated in a number of diseases. Furthermore, the presence of different small heat shock proteins (HSPs), including ubiquitin and αB-crystallin, has been reported in tau-positive inclusions in oligodendrocytes, which represent a common neuropathological hallmark in corticobasal degeneration (CBD), progressive supranuclear palsy (PSP) and frontotemporal dementias with Parkinsonism linked to chromosome 17 (FTDP-17). To test the hypothesis, that proteasomal inhibition contributes to inclusion body formation, we have studied the effects of the proteasomal inhibitor MG-132 on an oligodendroglial cell line stably transfected to express the longest human isoform of the microtubule (MT) associated protein tau, namely OLN-t40 cells. Treatment with MG-132 caused the induction of ubiquitin, αB-crystallin, HSP 32, HSP 70, and HSP 90 in a time- and concentration-dependent manner, as shown by Western blot analysis. Using indirect immunofluorescence, we demonstrated that αB-crystallin was translocated to the perinuclear region and ubiquitin-positive aggregates formed in the vicinity of the MT organizing center. Concomitantly, staining with MitoTracker and anti-HSP 60 antibodies revealed the assembly of mitochondria around the nucleus. MG-132-

induced aggregates did not contain tau accumulations and did not stain with Thioflavine-S. In contrast thereto, fibrillary aggregates, containing tau, αB-crystallin and ubiquitin, were observed when cells were treated with protein phosphatase inhibitor okadaic acid (OA), which induces tau hyperphosphorylation. The transient nature of these OA-induced aggregates indicates that ubiquitination marks them for proteasomal degradation. On the other hand, proteasomal inhibition by MG-132 caused their stabilization and deubiquitination. Proteasomal inhibition led to the recruitment of αB-crystallin to the inclusion bodies and its association with tau, as indicated by immunoprecipitation with antibodies against tau. In immunoprecipitates of MG-132 treated cells, αB-crystallin coprecipitated with tau in a time-dependent manner. Hence, tau-positive inclusion bodies, similar to those observed in a variety of neurodegenerative diseases, possibly form due to hyperphosphorylation and proteasomal impairment. They might initially protect the cells but eventually contribute to in the induction of cell death.

Ethanolaminephosphoglyceride species as molecular targets for oxidative stress and as early markers for apoptotic cell death.

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Following oxidative stress (OS) a complex network of kinases carrying signals from the cell surface to the nucleus have been shown to be activated. The involvement of plasma membrane phospholipids (PL) has been inferred from several studies, yet, little is known about the molecular mechanisms involved in such processes. Activation of members of the MAP kinase family in response to the oxidative stressor H_2O_2 has been shown by us to involve a rapid translocation of ethanolaminephosphoglyceride (EPG) particularly when either arachidonic acid (AA, 20:4 n-6) or docosahexaenoic acid (DHA, 22:6 n-3) were added to cells for several days before stress. Under these conditions exposure of DHA-enriched cells to OS caused a rapid translocation (max. within 30 min) of EPG from the inner to the outer plasma membrane. Within 2-3 h, EPG returned to normal levels indicating a possible regulatory role in cellular signaling. Translocation of EPG was followed by apoptotic cell death (ACD). Under these conditions, activation and nuclear translocation of ERK, notable at very early times after stress, persisted up to 24 h at the time ACD became apparent. Upstream inhibition of ERK prevented EPG outward flip, impaired ERK activation and translocation and prevented cell death indicating a linkage between these events. ACD could be prevented by substituting the ethanolamine moiety of EPG with monomethyl- (mEa) or dimethylethanolamine (dEa). dEa/DHA-treated cells showed neither EPG outward flip, nor significant ERK nuclear translocation, and were rescued from death indicating that prevention of EPG translocation by either scrambling membrane asymmetry or via inhibition of ERK signaling protects cells from ACD. Thus, EPG translocation is a major consequence of OS and seems to be a necessary signal to trigger apoptosis, which acts most likely via ERK activation. A detailed analysis of all cellular PLs was performed using thin layer and gas chromatography. Introduction of dEa, being a non naturally abundant free N-methyl base, resulted in the formation of a new dEPG lipid analog accounting for about 50 % of the total PL content, and a great reduction in EPG. Additionally, high-performance liquid chromatography/mass spectrometry (LC/MS) was used to quantify the phospholipid molecular species following polar head group (PHG) and fatty acid (FA) modifications. A number of distinct molecular species disappeared in the course of PHG modification whereas new ones appeared as a result of FA supplements. In particular, diacyl EPG and plasmalogen EPG (p-EPG) were highly enriched in DHA. Following dEa supplements EPG, p-EPG and oxidized p-

EPG showed highly unusual fatty acyl compositions. These data strongly indicate that oxidation of these FAs may elicit a cellular response which causes EPG species' translocation and activates signaling cascades leading to ACD. *Supported by a grant from the Gulton Foundation, New York.*

Regulation of ligand-gated ion channel function – local perturbations of protein structure determine glycine receptor activation and desensitisation

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The strychnine-sensitive glycine receptor is the principal mediator of rapid synaptic inhibition in mammalian spinal cord and brain stem. Deficient glycinergic signaling underlies the hypertonic motor disorder, hyperekplexia. Functional channels are homo- or heteropentameric transmembrane complexes composed of ligand-binding α- and structural β-subunits. Each subunit is characterised by a large extracellular N-terminal domain comprising ca. 50 % of the total protein, followed by four membrane-spanning domains and an extracellular C-terminus. Transmembrane domain 2 (TM 2) forms the inner lining of the ion pore, while the large intracellular loop linking TM 3 and TM 4 mediates receptor anchoring and posttranslational modification. The intracellular loop preceding TM 2 has previously been identified as a key regulatory element. Here, chemical parameters, such as hydrophobicity and polarity of this region were varied using site-directed mutagenesis. Ion channel function of receptor variants was studied by a combination of electrophysiological recording methods and rapid kinetic techniques. Increased hydrophobicity within the TM 1-2 loop favoured a non-active, rapidly deactivating channel by stabilising desensitised receptor states. Single-channel conductance was reduced, but gating, the open-closed transition of the receptor was not affected. Structural consequences of the introduced mutations were examined by molecular dynamics simulation of the TM 1 – TM 2 region. The localised increase in hydrophobic volume of an individual amino acid side chain increased the motility of the TM 1-2 loop region. Thus, structural alterations and changes in protein flexibility resulting from mutagenesis could be directly correlated to receptor ion channel function. Supported by Deutsche Forschungsgemeinschaft (SFB 353) and Sofie-Wallner Foundation.

Antidepressant drugs more potently inhibit activity of human corticotropin-releasing-hormone gene promoter in differentiated than in intactneuro-2a cells

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Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis is frequently observed in patients suffering from major depression. Clinically effective therapy with antidepressant drugs normalizes the disturbed HPA axis activity, in part, by decreasing corticotropin-releasing hormone (CRH) synthesis. However, molecular basis of interaction between antidepressants and CRH is poorly recognized. The aim of the present study was to compare the effect of various antidepressant drugs on human CRH gene transcription in undifferentiated and differentiated, neuron-like cells. The experiments were carried out on Neuro-2A cells stably transfected with a human CRH promoter

fragment (-663 to +124 bp) linked to the chloramphenicol acetylotransferase (CAT) reporter gene. The cells were cultured in medium supplemented with 10% fetal calf serum or in order to promote *in vitro* differentiation, the cells were grown for 3 days in medium containing 1 % serum. The observation that forskolin was equally efficient in stimulating CRH transcription in differentiated and intact Neuro-2A cells indicates that cAMP/PKA-dependent pathway plays an important role in stimulation of CRH gene in both investigated cell cultures. Imipramine, amitryptyline, desipramine, fluoxetine and mianserin, present in culture medium for 5 days, inhibited basal hCRH gene promoter activity in undifferentiated Neuro-2A cells, while other drugs under study (citalopram, tianeptine, moclobemide, venlafaxine, reboxetine, mirtazapine and milnacipram) were inactive. In the differentiated cells, all examined antidepressants, except moclobemide and tianeptine, inhibited hCRH gene transcription. Moreover, in differentiated cells, the drugs acted stronger and were effective at lower concentrations. These results indicate that differentiated, i.e. neuron-like cells, are a better model, then intact neuroblastoma cells, to investigate mechanism of psychotropic drug action. The ability of various antidepressant drugs to inhibit CRH transcription may be a molecular mechanism by which these drugs inhibit CRH synthesis and thus HPA axis activity.

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Activation of neurotrophin-like signaling by protein tyrosine phosphatase inhibitors in hippocampal neurons

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In neurons, nerve growth factor (NGF) exerts protective effects through enhanced tyrosine-phosphorylation of the high affinity NGF-receptor TrkA and subsequent activation of downstream signaling pathways such as the phosphoinositide-3-kinase/protein kinase-B (PI_3K /Akt) pathway and activation of the mitogen-activated protein kinases (MAPK) extracellular-regulated kinase-1/2 (Erk1/2). Activation of TrkA requires phosphorylation at multiple intracellular tyrosine residues. Here, we investigated whether inhibition of protein tyrosine phosphatases (PTP) was sufficient to enhance TrkA-phosphorylation and subsequent neuroprotective signaling in embryonic hippocampal neurons. Primary hippocampal neurons were exposed to the protein tyrosine phosphatase inhibitors orthovanadate and Et-3,4-dephostatin (DPN). Changes of TrkA tyrosine phosphorylation as well as (phospho-)Akt and (phospho-)Erk1/2 were investigated by immunoblot analysis and immunocytochemistry at 10, 30, 60 and 180 min after exposure of the neuronal cultures to the PTP inhibitors. In addition, hippocampal neurons were exposed to staurosporine (STS, 200nM) in the presence or absence of the PTP inhibitors. Staurosporine-induced apoptosis was quantified 24 h later after staining of the nuclei with the DNA-binding fluorochrome Hoechst 33258. Both PTP inhibitors orthovanadate (1-10μM) and DPN (0.1-10μM) reduced the percentage of apoptotic nuclei in STS-treated neurons. Six to twelve hours preatreatment with the PTP inhibitors were necessary to achieve this neuroprotective effect. Similar to NGF, the PTP inhibitors enhanced TrkA tyrosine phosphorylation in the neurons within 10 min of exposure. Western blot analysis revealed that PTP inhibitor-induced TrkA phosphorylation was evident up to 180 min after onset of the treatment, while NGF only

induced transient TrkA-phosphorylation. Most interestingly, PTP-inhibitors also induced TrkA-phosphorylation and exerted neuroprotective effects in the presence of neutralizing NGF-antibodies. In addition, the PTP inhibitors accelerated levels of phosphorylated Akt and Erk-1/2. Co-treatment with wortmannin, an inhibitor of the PI_3K/Akt pathway, or UO126, an inhibitor of MAPK-kinases, blocked neuroprotection by PTP inhibitors against STS-induced apoptosis. Our results suggest that PTP inhibitors induce prolonged TrkA tyrosine phosphorylation and subsequent activation of Akt and MAPK pathways. In conclusion, PTP inhibitors are capable of inducing NGF-like neuroprotective signaling in neurons without binding of the neurotrophin to its high affinity receptor.

Cloning and expression of orexin-1-receptor mutants

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Orexin A and orexin B, two recently discovered neuropeptides play an important role in the sleep-wake-cycle and are involved in food intake. Both peptides are ligands of the orexin-1-receptor and the orexin-2-receptor, two G-protein-coupled receptors [1]. The focus of this study was the comparison of the orexin B binding properties to the orexin-1-receptor and to an orexin-1-receptor mutant. It has been known that the SK-N-MC cell line endogenously expresses an other orexin binding site than the recombinant orexin-1-receptor. These receptors show a different affinity to orexin analogues compared to the wildtype receptor [2]. To further investigate the diverse binding properties, the orexin-1-receptor cDNA and the mutant were cloned into the pEGFP-N1 vector. As the receptor and its mutant were expressed by the corresponding EGFP fusion proteins, the expression efficiency and the receptor localisation of transiently receptor-EGFP-N1 transfected BHK and CHO cells was investigated. Subsequent to the synthesis of human orexin B and its tritium labeling, binding experiments on SK-N-MC, CHO-OxR1 cells and transiently transfected OxR1-pEGFP-N1 or Mu1-pEGFP-N1 CHO cells were performed. The potency of orexin B to activate the receptors was examined in a functional assay. In addition the increase of intracellular calcium concentration in CHO-OxR1 cells after binding of human orexin B was measured by using the video supported calcium imaging method. Preliminary results show that this approach will allow to indirectly define the binding activity of the peptide after optimizing the test system.

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Expression of α-Synuclein in Oligodendroglial OLN-93 Cells is Cytotoxic and Leads to Cell Death

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α-Synuclein is a neuronal protein, predominantly located at presynaptic terminals where it may play a role in synaptic function. Under pathological conditions, however, α-synuclein is found in various intracellular aggregates in neurons as well as in glial cells. Multiple system atrophy (MSA), a sporadic adult onset neurodegenerative disease with Parkinsonism, ataxia and autonomic failure, is characterized by the presence of glial cytoplasmic inclusions (GCIs) with α-synuclein as a major component originating in oligodendrocytes. The function of α-synuclein in glial cells and its contribution to the pathology of neurodegenerative diseases remains unclear. Here we present a cellular model for

investigating the effects of α-synuclein expression in glial cells. Cells of the oligodendroglial cell line OLN-93 were transiently transfected with a plasmid encoding the human wildtype α-synuclein. Transfection was carried out with Metafectene, a polycationic transfection reagent based on RMA-Technology (Repulsive Membrane Acidolysis) (Biontex). As determined by comparatively transfecting OLN-93 cells with a plasmid encoding the enhanced green fluorescent protein (egfp), 60-85% of the cells were successfully transfected and expressed the gene of interest. Expression level of α-synuclein was surveyed by indirect immunofluorescence and immunoblot analysis using antibodies against α-synuclein. Morphological evaluation indicates that α-synuclein causes cytotoxic effects, specifically after a post transfection period exceeding 24h. Processes are retracted, cells appear flattened and the nucleus protrudes. Indirect immunofluorescence shows that α-synuclein in the individual cells is expressed at a high level, and is distributed throughout the cytoplasma and processes. Using antibodies against α-tubulin, immunofluorescent staining further reveals the disorganisation of the microtubule network. With DAPI staining deformed and pycnotic nuclei are observed. Although cytotoxic effects were detected indicating stress responses, Western blot analysis demonstrates that expression of α-synuclein in OLN-93 cells does not induce heat shock proteins (HSPs), including Grp 94, HSP90, HSP70, HSP25, and αB-crystallin. In contrast thereto, a slight activation of extracellular signal-regulated kinases 1 and 2 (ERK1,2) was observed, which have been implicated in the regulation of cell death and survival. In summary, expression of α-synuclein in oligodendroglial cells has cytotoxic effects, leads to changes in morphology and eventually to cell death.

Crosscoupling of NF-kB and TGFß signaling in cerebellar granule cells

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During cerebellar development NF-kB is activated during an early phase form birth until day 7 in developing cerebellar granule cells. Previously, we used an unbiased cDNA array approach to screen for genes which were differentially expressed in the two developmental stages. TGFß-2 was identified as a more than 4-fold up-regulated gene in the developmental stage with low NF-kB activity. This was confirmed with in situ hybridisation and immunolocalisation. The function of TGFß2 in the cerebellum was analyzed in organotypic cerebellar slices, which were treated with different amounts of recombinant TGFß2. Using this ex vivo approach we could show that TGFß2 is essential to induce proliferation in cerebellar granule cells.

Determination of levels of „Advanced Glycation Endproducts“ in cerebrospinal fluid in Alzheimer‘s Disease and Frontotemporal Dementia by LC-MS/MS

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The diagnosis of Alzheimer‘s disease (AD) is predominantly based on neuropsychological tests and imaging techniques. More recently, there is increasing interest to find biomarkers in the cerebrospinal fluid (CSF), which could assist in the diagnosis of AD. One interesting candidate are „Advanced Glycation Endproducts (AGEs)“, oxidized sugar fragmentation products, which accumulate in amyloid plaques and neurofibrillary tangles and at the border of brain tissue and CSF. The aim of this study was to determine whether the level of AGE-modified proteins in CSF of AD reflects the increased tissue levels in the AD brain. Levels of defined AGEs in AD patients were compared to those in non-demented controls and to patients with Pick‘s disease. 35 CSF samples were assayed by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) using internal standards of isotope-substituted AGEs. Protein-bound and free AGEs were measured separately. Among the protein-bound AGEs, concentrations of MG-H1, CML, CEL were significantly higher in AD and significantly lower in frontotemporal dementia (FTD) compared to controls. Very interestingly, AGE levels and the severity of dementia correlated significantly in AD patients. Among the free AGEs, MG-H1, G-H1, 3-DG-H1, CEL and CML were significantly higher in AD and FTD compared to controls. However, the high correlation of the concentration of free AGEs with CSF protein in the patients indicate that these AGEs are not produced in the brain but rather diffuse from the blood into the CSF. In conclusion, protein-bound MG-H1, CML, CEL could be used as a laboratory parameter for the differential diagnosis of dementias.

Carbonyl stress by methylglyoxal causes ATP depletion, mitochondrial dysfunction and dopamine release in human neuroblastoma cells by a NMDA receptor-mediated mechanism

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Methylglyoxal (MG) a physiological precursor of advanced glycation end products (AGEs) accumulates during decreased metabolic utilisation of triosephosphates, or glutathione depletion, both of which have been described in neurodegenerative disease. The aim of this study was to investigate the metabolic and functional effects of the reactive dicarbonyl MG in the human neuroblastoma (SH-SY5Y) cell line. Furthermore, we tested the potential protective effects of carbonyl scavengers (aminoguanidine, and tenilsetam), antioxidants (lipoid acid and ß-estradiol), the nitric oxide synthase (NOS) inhibitor, L-NAME and NMDA receptor antagonists (MK-801, memantine, and D-AP7). MG showed a dose-dependent toxicity on neuroblastoma cells in the µM concentration range. This toxicity was associated with increased production of intracellular reactive oxygen species (ROS), decreased intracellular ATP, and significantly decreased retention of rhodamine123 (Rh123). The use of carbonyl scavengers, antioxidants, the NOS inhibitor L-NAME and NMDA receptor antagonists prevented ATP depletion in the presence of MG. Interestingly, only ß-estradiol antagonized ATP depletion by a concomitant increase in lactate production. More importantly, mitochondrial function impaired in the presence of MG, was prevented by the use of carbonyl scavengers, L-NAME and NMDA receptor antagonists and not by antioxidants. In addition, MG induced increased release of the neurotransmitter dopamine in a time- and dose-dependent manner. The latter effect was partially prevented by carbonyl scavengers and NMDA receptors antagonists whereas antioxidants were ineffective. Therefore, antioxidants provide only

a partial protection against disorders related to carbonyl stress. Scavenging of reactive carbonyl groups, or interference with their downstream pathways should offer a promising therapeutic strategy to reduce the pathophysiological changes associated with dicarbonyl mediated energy depletion and neurodegeneration.

Signal transduction pathways in mouse microglia N-11 cells activated by "Advanced Glycation Endproducts" (AGEs)

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Deposition of crosslinked insoluble protein aggregates such as amyloid plaques is characteristic for Alzheimer's disease. Microglial activation by these extracellular deposits has been proposed to play a crucial role in functional degeneration as well as cell death of neurons. A sugar-derived posttranslational modification of long-lived proteins which accumulates on amyloid plaques, advanced glycation endproducts (AGEs) activate specific signal transduction pathways, resulting in the up-regulation of various pro-inflammatory signals such as cytokines (IL-6, TNF-α) and inducible nitric oxide synthase (iNOS). Our goal was to study AGE-activated signal transduction pathways involved in the induction of pro-inflammatory effectors in the murine microglial cell line N-11. Chicken egg albumin-AGE (CEA-AGE), used as model AGE, induces both NO, TNF-α and IL-6 production. The AGE receptor, RAGE, and the transcription factor NF-κB appear to be involved in all pathways, since a neutralising RAGE antibody and a peptide inhibiting NF-κB translocation down-regulated NO, TNF-α and IL-
6 production. NO and TNF-α, but not IL-6 production appear to be regulated independently, since NOS inhibitors did not decrease TNF-α secretion and a neutralizing TNF-α antibody did not reduce NO production, while employment of NOS inhibitors reduced significantly the secretion of IL-6. Inhibition of the MEK and PI_3K pathway, but not that of MAPK-p38, reduced NO, TNF-α and IL-6 significantly, suggesting that simultaneous activation of the first two pathways is necessary for the AGE-induced induction of these pro-inflammatory stimuli.

Interaction of interleukin-1β with muscarinic acetylcholine receptor-stimulated signaling cascade in cell culture

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β-amyloid plaque-mediated glial upregulation of the pro-inflammatory cytokine interleukin-1β (IL-1β) has been assumed to contribute to the impairments of cortical cholinergic neurotransmission observed in Alzheimer´s disease. To test for this hypothesis, a murine cholinergic septal cell line SN56 was exposed to IL-1β followed by agonist stimulation of muscarinic acetylcholine receptors (mAChR) and detecting key molecules of both signaling cascades. The activities of acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) of SN56 cells were dose-dependently increased following stimulation with carbachol, while stimulation of cells with the M1-mAChR-specific agonist talsaclidine did not affect cholinergic enzyme activities, suggesting that the carbachol-induced increases in cholinergic enzyme activities are mainly mediated through M2-mAChR signaling. Pre-exposure of SN56 cells to IL-1β (1ng/ml) for one hour did not affect the carbachol-stimulated formation of inositol phosphates, but significantly induced the

expression of ChAT mRNA and AChE activity while ChAT activity was not affected by IL-1β Stimulation of IL-1β-pre-exposed cells with carbachol resulted also into upregulation of AChE activity and ChAT mRNA but to a lower extent as compared to incubations in the absence of carbachol, indicating interactive mechanisms between IL-1β and mAChR signaling cascades which may contribute to the cholinergic deficits in Alzheimer´s disease.

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Modulation of the expression of transferrin receptor and ferritin in astrocytes by application or deprivation of iron

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Cellular iron uptake and storage have to be tightly regulated in order to provide essential iron while preventing iron-catalysed generation of radicals. In contrast to the periphery, little is known about the regulation of iron metabolism in brain cells. To investigate the regulation of iron metabolism in astrocytes we have used astroglia-rich primary cultures from the brains of newborn rats. After application of iron (ferric ammonium citrate) astrocytes strongly accumulated iron in a time- (0 to 48 h) and concentration-dependent (0.01 to 1 mM) manner. This iron accumulation was prevented if iron was applied in combination with an excess of the iron-chelator deferoxamine. Application of iron to astroglial cultures caused a strong increase in the cellular content of ferritin and decreased the amount of transferrin receptor, which is involved in transferrin-mediated iron uptake into cells. In contrast, application of deferoxamine strongly increased the expression of transferrin receptor. Both, the upregulation of ferritin expression by iron application and of transferrin receptor expression by deferoxamine was prevented in the presence of the protein synthesis inhibitor cycloheximide. These results indicate that brain astrocytes respond strongly to the presence or the absence of iron by up- or down-regulation of proteins involved in iron uptake and iron storage.

Effects of NMDA receptor antagonists and agonist on spontaneous alternation in mice exposed to cerebral oligemic hypoxia

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It has been shown that a transiently reduced oxygen supply as produced by bilateral clamping of the common carotid arteries (BCCA) in rodents, causes changes in metabolic and transmitter systems of certain brain areas. Cerebral oligemic hypoxia leads to increased extracellular dopamine, aspartate and glutamate, hippocampal cholinergic dysfunction or a longlasting increase in GABA content in structures associated with learning and memory processes. Further, increased inositol phosphate metabolism and the production of hydroxyl radicals are also observed. Cerebral oligemic hypoxia induced by BCCA is not followed by any neuronal damage as assessed by light-microscopical studies. However, it was possible to demonstrate deficits in spatial learning in BCCA rats. The purpose of the present study was to examine the amnesic effects of NMDA receptor antagonists, competitive, CGP 39551 (D,L-(E)-2-amino-4-methyl-5-phosphono-3-pentanoic acid ethyl ester) and non-competitive, dextromethorphan, or agonist, N-methyl-D-aspartate (NMDA) on spontaneous alternation in BCCA mice. It was reported that NMDA-dependent processes are involved in the mechanisms of working memory. On the other hand, glutamatergic system is one of the affected neurotransmitter systems by cerebral oligemic hypoxia. It was therefore of interest to examine

NMDA receptor antagonist or agonist effects in mice following cerebral oligaemia and compare the cognitive capacities of these mice with animals affected by BCCA or NMDA modulating agents alone. Transient cerebral oligemic hypoxia was induced by bilateral clamping of carotid arteries (BCCA) for 30 min under pentobarbital anaesthesia. To examine spontaneous alternation, mice were tested in the Y-maze for 8 min, 48 h or 7 days after surgery. According to previous reports, alternation behaviour can be regarded as a measure involving spatial working memory. NMDA receptor antagonists were given i.p. and NMDA was injected s.c. In BCCA mice, dextromethorphan at a dose of 20 mg/kg impaired spontaneous alternation when given on 7 postsurgical day. CGP 39551 at a dose of 3 mg/kg or 5 mg/kg failed to affect mice performance in the Y-maze. NMDA (50 mg/kg) ameliorated spatial working memory in mice exposed to cerebral oligaemia when evaluated 48 h after surgery. These results suggest that cerebral oligemic hypoxia induced by BCCA leads to functional disturbances in the brain, such as working memory impairment and increased susceptibility to NMDA receptor related drugs. NMDA receptors seem to play some role in these processes.

Ca^{2+} homeostasis and ROS generation is differently regulated in hippocampal neurons and astrocytes in defined mixed cultures

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Different vulnerabilities of astrocytes and neurons to energy limitations were frequently reported. Because of the close functional relation between astrocytes and neurons it is necessary to investigate the sensitivities of both cell types in a culture system allowing those interactions. We have established a mixed cell culture system of hippocampal origin which allows the correlation between immunhistochemical cell identification and functional measurements. The influx of increased glutamate concentrations (100 µM) to Ca^{2+} homeostasis and ROS generation in neurons and astrocytes was monitored. In contrast to hippocampal neurons, astrocytes are able to regulate the cytosolic Ca^{2+} concentration after glutamate challenge. The cytosolic Ca^{2+} in neurons remains elevated until extracellular glutamate is removed. The ability to remove increased cytosolic Ca^{2+} loads is maintained in astrocytes even under inhibition of glycolysis or oxidative phosphorylation. The glutamate mediated-Ca^{2+} load in respiration inhibited neurons is increased above the glutamate-evoked Ca^{2+} level in energetic intact neurons. The inhibition of glycolysis has no additional effect. The glutamate-mediated plateau is absent in Ca^{2+}- free buffers, indicating that the influx of extracellular Ca^{2+} is nearly exclusively responsible for the Ca^{2+} deregulation in neurons. In astrocytes the glutamate-evoked transient Ca^{2+} peak was only slightly reduced in Ca^{2+}-free buffers. The generation of ROS was measured by the oxidation of DHE in neurons and astrocytes. In control conditions a higher ROS generation is found in neurons in comparison to astrocytes. Application of glutamate further increased the neuronal ROS generation, whereas the withdrawal of extracellular Ca^{2+} reduced the neuronal ROS generation. The ROS generation in astrocytes , however, was independent of cytosolic Ca^{2+} loads. In summary, we have shown that identical insults have very different physiological effects on different cell types. Glutamate mediated excitotoxicity can not be.

Impairments of cholinergic and non-cholinergic neurotransmission in transgenic Tg2576 mouse brain with Alzheimer-like pathology

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The cortical cholinergic deficits observed in brains from Alzheimer patients are highly correlated with the extent of neuropathological changes. To address the question whether beta-amyloid may contribute to these deficits, brain tissue from transgenic Tg2576 mice with Alzheimer plaque pathology at ages of 5 (still no significant plaque load) and 17 months (high cortical plaque burden) were examined for a number of cholinergic and non-cholinergic markers. Transgenic mice with no significant plaque load demonstrated reduced hemicholinium (HC)-3 binding to choline uptake sites in anterior brain regions as compared to nontransgenic littermates, while in aged transgenic mice decreased HC-3 binding levels regionally correlated with high plaque load. The beta-amyloid-associated decrease in high-affinity choline uptake sites in some cortical brain regions was accompanied by an increase in vesicular acetylcholine transporter binding of vesamicol. Alpha1-, alpha2-and beta-adrenoceptor binding levels were hardly affected in aged transgenic mice. The data provide evidence of a modulatory role of beta-amyloid on cortical cholinergic neurotransmission but with differential actions on distinct cholinergic synaptic markers. The development of changes in cholinergic synaptic markers in transgenic Tg2576 mouse brain already before the onset of progressive plaque deposition provides *in vivo* evidence of a modulatory role of soluble beta-amyloid on cholinergic neurotransmission and may be referred to the deficits in learning and memory observed in these mice also before significant plaque load.

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The role of methylglyoxal and glyoxalase I on tau phosphorylation and aggregation in human neuroblastoma SH-SY5Y-cells

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Methylglyoxal (MG), a product of triose phosphate, hydroxy/ amino aceton decomposition and non-enzymatic degradation of sugars is converted by glyoxalase I and II to D-lactate. In order to study the role of MG on tau phosphorylation and formation of tau aggregates in SH-SY5Y human neuroblastoma cells, we specifically inhibited glyoxalase-I with *p*-bromobenzyl glutathione cyclopentyl diester ($Cp_2GSpBrBz$). Application of the glyoxalase-I inhibitor leads to a 3-fold increase of intracellular MG concentration, measured by GC/MS, after 24h. When the MG concentration was increased by extracellular application in µM concentrations or by inhibition of glyoxalase I, it could be demonstrated by Western Blot that both extracellular and intracellular MG dramatically changed AT8-tau phosphorylation pattern in a time dependent manner (epitope of AT8 antibody: Ser199, Ser202 and Thr205). In a first attempt to identify the responsible kinases for MG-induced tau phosphorylation, we investigated a possible activation of Cdk5 and p38-MAPK. Decreased p35/p25 levels indicate that Cdk5 might be responsible for the increase in tau phosphorylation. In contrast, p38 MAPK is not activated by MG in SH-SY5Y cells. The involvement of critical phosphatases remains to investigate. Since tau is 'hyper'phosphorylated in neurofibrillary tangles, these results indicate that dysfunction of glyoxalase I may contribute to the pathology in Alzheimer´s disease and tauopatic brains.

Structural investigations of the cytoplasmic loop of the nicotinic acetylcholine receptor

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The nicotinic acetylcholine receptor (nAChR) is a ligand-gated ion channel that mediates signal transduction at the postsynaptic membrane of cholinergic synapses. *Torpedo* as well as muscle nAChR consists of five subunits ($\alpha_2\beta\gamma\delta$) arranged around a central axis. Each subunit contains a large N-terminal extracellular fragment, four transmembrane fragments (M1-M4) and a large cytoplasmic loop connecting the M3 and M4 transmembrane sequences (100-150 amino acid residues in different receptor subunits). The structure and the function of this cytosolic domain are poorly defined so far. In order to produce this cytoplasmic loop in sufficient quantities for NMR spectroscopic structural investigations, we are trying to express it heterologously. We found that nAChR α, β, γ and δ subunit cytoplasmic loops from *Torpedo* as well as the δ-subunit cytoplasmic loop from *Rattus norvegius* can be expressed in *E.coli* with relative ease. All these loop proteins, however, were expressed in insoluble form and could be purified only from bacterial inclusion bodies. Secondary structure investigations have been performed on the δ-subunit intracellular domain after refolding. The study of the intracellular domain shortened at its C-terminus, based on computational predictions, showed that the removal of 10 amino-acid residues increased the solubility of the heterologously expressed protein. A concentration of 0.3-0.4 mg/ml was repeatedly obtained. According to CD data, the intracellular domain contains about 60% of ordered secondary structure. Fluorescence spectra revealed the exposed position of tryptophan residues. *In vitro* phosphorylation experiments with PKA and Ab1 tyrosine kinases demonstrated that the isolated intracellular domain is phosphorylated at the same sites as the δ subunit in native nAChR. CD spectra show that the phosphorylation does not grossly change the secondary structure of the cytoplasmic loop. We have created a vector system to express α, β, γ, δ subunit intracellular domains simultaneously in one bacterial cell. When applying this polycistronic expression, the chromatographic profile was different from monocistronically expressed δ subunit cytoplasmic loop. Preliminary gel filtration and cross-linking experiments suggested that the polycistronically expressed protein might form a pentamer.

Diadenosine polyphosphates mediated arachidonic acid release via P2Y receptors

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P2 receptors are classified into two families, inotropic P2X receptors and G protein-coupled P2Y receptors. Both of them are activated by extracellular nucleotides. Seven P2Y subtypes, namely $P2Y_{(1,2,4,6,11,12,13)}$ have been cloned and functionally characterized in humans. These receptors on stimulation by nucleotide triphosphates exhibit a variety of physiological actions via different signaling pathways. Recently it has been reported that P2 receptors can be stimulated by diadenosine polyphosphates (Ap_nA). We decided to investigate the effects of Ap_nA on P2Y receptors. We used HEK 293 cells stably expressing the rat brain $P2Y_1$ ($P2Y_1$-R), the rat lung $P2Y_2$ receptor ($P2Y_2$-R), both tagged to green fluorescent protein, and A549 cells to investigate the release of archidonic acid, a major player in inflammatory processes. ATP and ADP stimulate the P2Y1-R, whereas the $P2Y_2$-R responds equipotently to ATP and UTP. The functional expression of the transfected receptors in HEK cells was confirmed by agonist evoked intracellular Ca^{2+} rise. Endogenously the A549 cells do not express $P2Y_1$, as ascertained by RT-PCR. The arachidonic acid release from the cells was quantified by using ^{3}H-labelled arachidonic acid. A significant difference in the arachidonic acid release was observed between HEK 293 and A549 cells on stimulation of the cells with identical concentrations of nucleotide

triphosphates. A similar difference was observed in the case of the diadenosine polyphosphates. These results demonstrate that the degree of activation of the P2Y receptors depends on the agonist and the cell type. This could be important for P2Y receptor-mediated regulation of inflammatory responses.

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High frequency stimulation is mediated by activated GABAergic neurons in rat striatum in vitro

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Deep brain stimulation (DBS) is used as therapy in Parkinson's Disease. The basic mechanisms of high frequency DBS in the subthalamic nucleus or globus pallidus internus (GPi) remain unclear. Since both nuclei predominantly receive GABA inputs, it is hypothesized that the GABA system is involved. Most of the striatal cell bodies and efferent neurons are GABAergic. Therefore, in this study, slices of rat caudate nucleus were used with a thickness of 250 µm that were incubated in perfusion chambers. The effects of high frequency stimulation (HFS) of 130 Hz on both basal and veratridine (VER) -induced GABA and glutamate outflow of striatal slices were investigated in an in vitro model. GABA and glutamate outflow were measured by OPA-sulphite derivatization and HPLC with electrochemical detection. HFS did not modulate basal GABA outflow of striatal slices. Coincubation with VER led to a concentration-dependent increase of GABA outflow. In the presence of 1 or 3 µM VER, GABA outflow significantly enhanced after simultaneous application of HFS. With 10 or 50 µM VER, a marked increase of GABA outflow was observed. However, addition of HFS did not further increase GABA outflow, probably due to a ceiling effect. No significant change of glutamate outflow was detected in all experiments. The above results suggest that the effect of HFS is mediated by activated GABAergic neurons.

Microarray analysis of tumor necrosis factor α induced gene expression of U373 human glioblastoma cells identifies MCP-1 as disease relevant target

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Tumor necrosis factor α (TNF) is able to induce a variety of biological responses in the nervous system including inflammation and neuroprotection. Human astrocytoma cells U373 have been characterized as a model for inflammatory cytokine actions in the nervous system. We used cDNA microarrays to analyze the time course of the transcriptional response from 1h for up to 12h post TNF treatment in comparison to untreated cells. TNF activates many different transcriptional pathways. One major mediator of TNF-induced gene expression is the transcription factor NF-κB. NF-κB comprises a family of transcription factors that are involved in the inducible expression of genes regulating neuronal survival, inflammatory response, cancer and innate immunity. To separate potential NF-κB regulated genes from those regulated by other TNF-induced pathways, gene expression patterns induced by TNF alone were compared to those obtained with TNF and pharmacological inhibition of NF-κB. In this study we show that many genes responded to TNF (> 880 from 7500 tested) with a more than two-fold induction rate. In U373 cells known TNF-responsive genes including IκB, ICAM, VCAM-1 and B94 and additional genes such as

TRAIL, TRAILR2, IRF-1, MCP-1 and ribosomal protein genes were detected. Surprisingly a strict cell specificity of TNF induced gene expression is evident. There is a striking difference of the here described gene expression profile in comparison to published data analyzing TNF induced gene expression in 3T3 or Hela cells. This suggests that the majority of the here described genes are novel TNF targets. Expression of the chemokine MCP-1 was top in height and duration in our microarray analyzisis. Therefore MCP-1 expression was monitored in addition to microarrays with immunofluorescence and ELISA. Based on these data and on evidence from literature a model for the potential neurodegenerative effect of NF-κB is provided. Conclusion: In addition to previously unreported TNF responsible genes kinetics and height of induction were analyzed in detail, showing striking co-regulation for several functional groups.

Attenuated hippocampal cell proliferation and reduced caspase 3 in synras mice expressing constitutive active ras in neurons

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Adult neurogenesis in the dentate gyrus of the hippocampus is thought to be correlated with the modulation of cognitive functions. In order to study the mechanism of neurogenesis in the hilus and dentate gyrus, we have used a transgenic synRas mouse model expressing constitutively activated V12-Ha-Ras selectively in mature neurons. We have demonstrated previously that lesion-induced neuronal degeneration is greatly prevented in synRas mice (Heumann et al,.2000 J Cell Biol 151, 1537-1548). Here we show that in synRas mice endogenous caspase 3 immunoreactivity is decreased by 90% in the subgranular region of the dentate gyrus. Proliferation or survival rate of newborn cells was measured in animals that were perfused at day 1 or day 28 after treatment with BrdU. There was a significant 69% reduction in number of BrdU-labeled cells within the dentate gyrus in synRas mice as compared to wt siblings at day 1 after BrdU injections. However, while in wt animals only 35% of the newborn cells were present after 28 days, in synRas mice the survival of new born cells was strongly increased to 62%. Double labeling experiments using glial fibrillary acidic protein (GFAP) and calbindin as markers show that the reduction in total cell BrdU labeled number after 28 days from 26 cells/ 10 mm^2 to 17 cells/ 10 mm^2 was greately at the expense of neuronal cells while the number of labeled GFAP positive cells remained constant. The results indicate that neuronal Ras activation attenuates the generation of cells and modulates numbers in calbindin positive neurons in the dentate gyrus.
Analysis of the spatial memory abilities in a radial arm maze task revealed that in contrast to wt siblings synRas mice were unable to reach the learning criteria indicating impairments in working memory performance. We propose the hypothesis that Ras-activation mediated stabilization of mature neurons in the hippocampal granule layer of synRas mice reduces generation of cells and hippocampus-dependent learning abilities.

c-Cbl binds to tyrosine phosphorylated neurotrophin receptor p75 and induces its Ubiquitination

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Neurotrophins are necessary for survival, differentiation and apoptosis of diverse neuronal and non-neuronal cell types. They interact with two classes of receptor proteins, the Trk receptor tyrosine kinases, with TrkA preferentially binding to NGF, TrkB to BDNF and NT4/5 and TrkC to NT3 and the p75 neurotrophin receptor

($p75^{NTR}$), a member of the tumor necrosis factor receptor family, binding to all neurotrophins. The p75 neurotrophin receptor ($p75^{NTR}$) has dual functions in cell survival and cell death but its intracellular signalling pathways are not understood. Here we show that $p75^{NTR}$ tyrosine phosphorylation can occur in exogenously expressed $p75^{NTR}$ in PCNA and HEK293 cells as well as endogenous $p75^{NTR}$ in rat brain. $p75^{NTR}$ is phosphorylated at tyrosine 308 within the cytosolic C-terminus. To characterize proteins which bind to $p75^{NTR}$ dependent on its tyrosine phosphorylation we performed two-hybrid and co-precipitation studies with phosphorylated $p75^{NTR}$. We identified the E3-ubiquitin ligase c-Cbl as a binding protein of $p75^{NTR}$ which interacts only when $p75^{NTR}$ is phosphorylated at Y308. This $p75^{NTR}$ tyrosine phosphorylation is a prerequisite for a c-Cbl dependent ubiquitination of $p75^{NTR}$ in PCNA and HEK293 cells. Our data suggest a c-Cbl dependent ubiquitination of $p75^{NTR}$ involved in the regulation of $p75^{NTR}$ signalling.

Ribozyme-mediated knock-down of peripheral tryptophan hydroxylase

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Serotonin is not only a neurotransmitter in the central nervous system, but also a ubiquitous hormone in the periphery involved in vasoconstriction and platelet function. Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in serotonin biosynthesis.[1] By gene targeting we have shown that serotonin is synthesized independently by two different TPH isoenzymes in peripheral tissues and neurons and identified a neuronal tryptophan hydroxylase isoform.[2] Mice selectively deficient in peripheral TPH (TPH1) and serotonin exhibit a reduced risk of thrombosis and thromboembolism (see elsewhere this postersession). Therefore, *Tph1*-specific ribozyme may provide a novel and effective form of gene therapy that may be applicable to a variety of thrombotic diseases. We designed several *antitph* hammerhead ribozymes and tested their cleavage activity against short synthetic Tph1 RNA substrates. In vitro cleavage studies demonstrated site-specific leavage of Tph1 mRNA that was dependent on substrate/ribozyme ratio and duration of exposure to ribozyme. This experiments for which the ribozymes and Tph1-fragments were transcribed in vitro via the T7 promotor, show an efficiency of 0, 16 and 68 %, respectively. For experiments in cell culture we cloned the ribozymes into tRNA expression constructs to promote constitutive expression and to direct the chimeric tRNA-ribozymes into the cytosolic compartment. Interestingly, we detected completely different *in vitro* cleavage rates of the chimeric tRNA-ribozymes in preliminary experiments, but obtained one with a high cleavage rate (approx. 66 %). This active chimeric tRNA-ribozyme is capable of selectively cleaving native *Tph1* mRNA in P815 mouse mastocytoma cells, as detected using RNase protection assays, with concomitant downregulation of the serotonin biosynthesis, as detected in TPH activity assays. Thus, this ribozyme may provide a novel and effective gene therapeutic strategy to ameliorate thrombotic diseases.

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Mechanisms of VEGF mediated survival and proliferation in endothelial cells

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Vascular endothelial growth factor (VEGF) mediates survival and mitogenicity of endothelial cells. VEGF binds to the tyrosine kinase receptors VEGFR1 and VEGR2. We focused on intracellular mechanisms of VEGF signalling for survival, stimulation of DNA synthesis and change in cell number (generation and survival of cells) in transformed human umbilical vein endothelial cells (t-HUVEC). In order to be able to dissect the specific signalling capacities of VEGFR1 and VEGFR2, respectively a chimeric receptor approach was used as well as the receptor specific ligands PlGF-1 and VEGF-E. Activations of VEGFR1 and VEGR2 stimulated DNA synthesis as measured by BrdU incorporation. However, the mitogenic effect but not the survival promoting activity of VEGF could be abolished by inhibition of the proto-oncogene protein Ras using pharmacological and immunological methods. Furthermore, only VEGFR2 but not VEGFR1 mediated survival of t-HUVEC cells. Thirdly, increase in cell number

was strongly enhanced by selective activation of VEGFR2 as compared to VEGFR1. Towards identification of the possible survival promoting effect by VEGFR2 we measured the time-resolved release of NO by T-HUVEC using electrochemical microsensor techniques [1]. Interestingly, release of NO was only found after stimulation of VEGFR2 but not of VEGFR1. Inhibition of NO synthesis by analogs of L-arginine (L-NAME) abolished the VEGF and VEGF-E mediated survival effect. Addition of NO-donors could mimic and even enhance the effect of VEGF. We conclude that VEGFR2 but not VEGFR1 develops autocrine survival activity that is mediated by NO. Comparing the two different signalling profiles in survival and stimulation of cell division between VEGFR1 and VEGFR2, we conclude that the increase in cell number by VEGFR2 results from a combined effect of mitogenic activity and survival while VEGFR1 stimulates DNA synthesis alone. These findings were confirmed by measuring cell survival, BrdU incorporation and increase in cell number in primary HUVEC. The knowledge of these two distinct VEGFR signalling pathways could open new therapeutical potentials for specific inhibition of blood vessel maintenance and new blood vessel formation.

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TNFα increases the activity of γ-glutamyl transpeptidase in cultured rat astroglial cells

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The enzyme γ-glutamyl transpeptidase (γGT) is involved in the metablic cooperation between astrocytes and neurons in the glutathione (GSH) metabolism of the brain. Using the GSH exported from astrocytes as substrate, γGT catalyses the generation of the neuronal GSH precursor cysteinylglycine. To investigate the presence of γGT in brain cells, cultures enriched for astroglial cells, neurons, oligodendroglial cells and microglial cells derived from rat brain were studied. Astroglial cultures contained a specific γGT activity of 2.2 ± 0.7 nmol x min^{-1} x mg $protein^{-1}$. Significantly lower specific activities of γGT were detectable in the other brain cell cultures investigated. The specific activity of γGT in astroglial cultures was strongly elevated by the presence of tumor necrosis factor-α (TNFα) in a time- and concentration-dependent manner. Maximal activity of γGT was observed after 3 d incubation of astroglial cultures with 30 ng/mL TNFα. Under these conditions the specific γGT activity was threefold increased compared to controls. Presence of the γGT-inhibitor acivicin completely inhibited γGT activity both in TNFα-treated and in control cells. In addition, the increase in astroglial γGT activity after

application of TNFα was completely prevented in the presence of the protein synthesis inhibitor cycloheximide. To test for consequences of an elevated γGT activity, the extracellular accumulation of GSH in astroglial cultures was investigated. After TNFα-treatment the concentration of GSH in the medium of astroglial cells was significantly reduced compared to control cells. The data presented demonstrate that TNFα stimulates the synthesis of γGT in astroglial cells and, thereby, improves the capacity to process the GSH exported by these cells.

The Alzheimer's disease β -secretase (BACE1) is not a neuron-specific enzyme

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The beta-site amyloid precursor protein (APP)-cleaving enzyme (BACE1) is a prerequisite for the generation of β-amyloid peptides, which give rise to β-amyloid deposits in the brains of Alzheimer's disease patients. It is believed that BACE1 is exclusively expressed by neurons. However, in the brains of transgenic Tg2576 mice that overexpress human APPsw, the BACE1 protein is also expressed by reactive astrocytes in proximity to β-amyloid plaques. Employing six different experimental strategies to activate brain glial cells we demonstrate the expression of BACE1 by reactive astrocytes but not by activated microglial cells in models of chronic rather than acute gliosis. To identify regulatory elements which drive tissue- or cell type-specific BACE1 expression we cloned and sequenced a 1,5 kb fragment of the rat BACE1 promoter and generated BACE1 promoter-luciferase reporter constructs. The basal activity of this promoter construct was highest in neuronal cell lines and in the pancreatic cell line AR42J, somewhat lower in rat primary neurons, astrocytic and microglial cultures, very low in hepatocytes and almost absent in fibroblasts and in the monocyte-macrophage cell line RAW264.7. Similar results were obtained by using immunocytochemical analysis and by a fluorimetric assay to quantify BACE1 enzymatic activity. The analysis of promoter activities of deletion mutants suggests the presence of activators of BACE1 transcription between bases -514 to -753 and the existence of inhibitors of BACE1 transcription between bases -754 and -1541. Together, our data indicate that brain glial cells, in particular astrocytes, may contribute to increased generation of β-amyloid peptides and to accelerated formation of β-amyloid plaques in the course of Alzheimer's disease. This hypothesis is supported by our observation of BACE1-immunoreactive astrocytes in the brains of Alzheimer's disease patients.
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Multiple biochemical, developmental, and neurological defects in mice lacking selenoprotein P (SePP)

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The human genome contains 25 genes encoding selenoproteins with selenocysteine in their primary sequence. In those with identified enzymatic function, selenium (Se) is involved in catalysis. We have genetically deleted the gene for selenoprotein P (SePP) in mice and could show that it functions in Se transport and distribution within the body. SePP-deficient mice display reduced activities of selenoenzymes like glutathione peroxidase and thioredoxin reductase in kidney, testis, and brain. The growth spurt that normally starts around P14 in mice does not take place in SePP-deficient mice and thus these mice remain considerably smaller. Rotarod and footprint analyses revealed a movement disorder. Moreover, SePP-deficient mice display occasional seizures. (108)

Long-term functional, morphological and molecular consequences of neurotrauma

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The molecular mechanisms leading to late consequences of neurotrauma and posttraumatic stress disorder are largely unclear. Retrospective clinical studies have established a causal relationship between traumatic brain injury (TBI) and neurodegenerative diseases such as Alzheimer disease. Also, a causal relationship between TBI and psychosis has been recognized in clinical studies. These pathologies are all associated with decreased cognitive function, significant disability and disturbed quality of life. Prospective human studies are missing, however, and our knowledge of the functional, morphological and molecular consequences of experimental neurotrauma is limited to observations with a short follow-up of a few weeks. Thus, relevant animal models to be used in long-term follow-up setting are of pivotal importance for advancement of knowledge in this field. We have established a rodent neurotrauma model that allows long-term functional and morphological follow-up. We have shown that a small, standardized unilateral parietal cortical trauma in rats and mice results in long-lasting bilateral hippocampal alterations in gene and protein expression, in abnormal behavior, and in bilateral hippocampal cell loss. Our data on the hematopoietic growth factor, erythropoietin (EPO) has revealed that EPO can act neuroprotective both in animals and man via multiple mechanisms of action during neurodevelopment and metabolic stress. EPO treatment in the mouse model of neurotrauma improved functional and morphological recovery after neurotrauma. Based on these findings we have formulated the following working hypothesis: Neurotrauma, in particular in the presence of a genetic predisposition, can act as an inducer / enhancer of neurodegeneration via activation of cellular signaling cascades that conjure to a final common deleterious pathway. Our goal is to characterize the genes of this "final common pathway", to understand the interplay between neurotrauma and genetic predisposition in activation of this cascade and to test the characterized novel strategies for therapeutic neuroprotective interventions.

Activation of phopholipase A_2 and availability of endogenous arachidonic acid is crucial for acute synthesis of prostaglandins in astrocytes.

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Arachidonic acid (AA) is an important polyunsaturated fatty acid which serves as a precursor of physiologically active substances, such as prostaglandins (PGs). AA and PGs participate actively in development of different pathological states in brain, which are mainly related to inflammation. We have studied mechanism of PGs synthesis in rat brain astrocytes. These cells conduct most of the inflammatory reaction in the brain and are important suppliers of PUFAs in neuronal tissue. Using EIA and HPLC procedures we have studied kinetics of PGs synthesis by astrocytes stimulated by Ca-ionophore A23187 and by exogenous AA. A23187, which is a putative activator of PLA_2 and of endogenous AA release, stimulated acute release of PGs into culture medium already after 5 min of stimulation, whereas stimulation by endogenous AA produced detectable amounts of PGs only after 30 min with slow increase up to 2 h. We have also found radioactivity release from astrocytes labeled with [^{3}H]AA which were stimulated by exogenous AA. This release had kinetics similar to the AA-stimulated PG synthesis. It was detectable at 30 min with

increase to 2 h. Inhibition of PLA_2 by methyl arachidonyl fluorophosphonate lead, in all cases, to significant decrease in radioactivity release and PG synthesis even in the presence of exceeding amounts of exogenous AA. These results suggest that in astrocytes prostaglandins are preferentially synthesized from endogenous AA and activation of PLA_2 is crucial for this process.

Role of basic region leucine zipper transcription factors in cAMP response element-mediated transcription.

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The cAMP-response element (CRE) is a point of convergence for many extracellular and intracellular signals, including cAMP, calcium, or neurotrophins. The CRE binding protein CREB, a member of the basic region leucine zipper (bZIP) family of transcription factors, requires phosphorylation to connect signaling pathways with transcriptional changes of CRE containing genes. We have constructed a constitutively active CREB mutant that allowed us to uncouple the investigation of transcriptional activation via CREB from the variety of signaling pathways that lead to an activation of CREB. This constitutively active CREB mutant stimulated the tyrosine hydroxylase promoter and a model promoter containing four copies of the CRE derived from the c-*Fos* gene. The transcriptional activity of the CREB mutant could be impaired by a dominant-negative form of CREB, but not by dominant-negative mutants of the bZIP proteins CREB2, ATF2, c-Jun or C/EBP, indicating that these proteins are not able to heterodimerize with CREB. Thus CREB mediated activation of transcription is very specific. The data also indicate that CREB2, described as a negative regulator of CREB and CRE-mediated transcription, does not interact with CREB and does not negatively interfere with CREB-mediated transcription.

The effect of systemic N-methyl-norsalsolinol administration on rat behavioural activity, serotonin metabolism and opioid receptor mRNA expression

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The TIQ derivative N-methyl-norsalsolinol (NMNorsal) was identified as endogenously synthesized neurotoxin in parkinsonian lumbar cerebrospinal fluid. We could demonstrate that NMNorsal intraperitoneally injected is able to pass through the blood-brain barrier. However the pathophysiological role of NMNorsal remains still unclear. After systemic NMNorsal (20 mg/kg resp. 40 mg/kg) administration to conscious, freely-moving rats their behavioural activity was examined in the following 48 hours. The activity was classified in arbitrary units for each animal. In parallel, we used in vivo microdialysis technique to measure striatal serotonin (5-HT) levels. Finally, the striatal serotonin and opioid receptor mRNA expression was determined in ex vivo RT-PCR experiments. All analyses were done 1 h, 24 h, and 48 h after NMNorsal injection. During the experiments, low-dose-treated rats were almost always sleeping, but high-dose-treated (40 mg/kg) rats were active with a significant increase of mean behavioural score 24 hours (9 points) and 48 hours (15.6 points) when compared to controls (1.3 points). After high-dose NMNorsal application, 5-HT levels increased to approximately 2-fold during the 48 hours while HIAA decreased to approximately 50 % in the dialysate. The level of 5-HT2A as well as δ-opioid receptor mRNA increased obviously with a maximum after 48 h, while the mRNA level of the μ-opioid-receptor significantly decreased. Our results demonstrate delayed effect of NMNorsal on the serotonergic and opioid receptor systems in the rat caudate-putamen. This led us to suggest that this compound may

be involved in the occurrence of hallucinosis or psychosis following L-Dopa,.

Acetyl-CoA metabolism in noncholinergic terminals after selective basal forebrain cholinergic immunolesion by 192IgG-saporin

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Indirect data indicate that acetyl-CoA content and metabolism in cholinergic and noncholinergic brain compartments may be similar. However, cholinergic encephalopathies are accompanied by decline in metabolism of glucose and other acetyl-CoA precursors in the brain. The aim of the present study was to reveal whether reduced cortical cholinergic input affects the acetyl-CoA metabolism in cholinoceptive cortical target regions which may play a causative role for the deficits in cerebral glucose metabolism observed in Alzheimer´s disease. Cortical cholinergic denervation produced by a single intracerebroventricular application of the cholinergic immunotoxin 192IgG-saporin and its effect on activities of pyruvate dehydrogenase and ATP-citrate lyase as well as on the level of synaptoplasmic and mitochondrial acetyl-CoA and acetylcholine release in cortical target regions was studied. Cholinergic immuno lesion produced 83%, 72% and 32% decreases in the activities of choline acetyltransferase, acetylcholinesterase and ATP-citrate lyase in nerve terminals isolated from rat brain cortex, respectively, but no change in pyruvate dehydrogenase activity. Spontaneous and Ca^{2+}-evoked acetylcholine release from synaptosomes was inhibited by 76% and 73%, respectively, following immunolesion. However, ratio of quantal to spontaneous acetylcholine release remained about four in both groups. The lesion induced 39% decrease of acetyl-CoA level in synaptosomal mitochondria and 74%-increase in synaptoplasmic fraction. On the other hand, levels of acetyl-CoA and CoASH in whole brain mitochondria from lesioned cortex were 61 and 48%, respectively higher as compared to controls. These findings indicate a preferential localization of ATP-citrate lyase in cholinergic nerve terminals, where it may contribute to the transport of acetyl-CoA from the mitochondrial to the cytoplasmic compartment. They also suggest a differential distribution of acetyl-CoA in subcellular compartments of cholinergic and noncholinergic nerve terminals. In addition loss of cholinergic innervation or/and glia activation could evoke marked shift of acetyl-CoA from mitochondrial to cytoplasmic compartment of noncholinergic tereminals. Thus we postulate that cholinergic activity plays a role in maintaining proper intracellular distribution of acetyl-CoA in glial and other noncholinergic cortical cells. Significance of these changes for viability of brain noncholinergic neurons in course of cholinergic encephalopathies remains to be tested. Supported by KBN project 6P05A 01020.

Endocytosis of $P2Y_2$ receptor

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The role of extracellular nucleotides as signaling molecules is widely accepted. The effect of nucleotide triphosphates is mediated by the P2Y receptors, which belong to the G protein coupled receptor family. Desensitization and endocytosis are the two well-known modes of termination of response of a receptor to agonist-mediated stimulation. Endocytosis and recycling of the receptors are complex processes involving a number of interacting proteins and their phosporylation. We investigated the effect of different players on the endocytotic pathway of $P2Y_2$ receptor ($P2Y_2$-R). We used HEK 293 cells stably expressing the rat lung $P2Y_2$-R tagged to green fluorescent protein (GFP) to

investigate the endocytosis of the receptor. The $P2Y_2$-R responds equipotently to ATP and UTP. Complete endocytosis of the receptor was observed on stimulation of the cells with 100 μM UTP for 30 min at 37^0C. Endocytosis of the receptor involves a re-arrangement of the actin cytoskeleton. The inhibition of receptor phosphorylation by a CaM Kinase-II inhibitor delays endocytosis of the receptor. The inhibition of the dephosphorylation of the internalized receptor by okadaic acid results in delayed processing of the receptor. Inhibition of clathrin-mediated internalization, too, results in delayed endocytosis of the receptor. Internalization of the receptor could be completely inhibited only in the presence of 0.45 M sucrose. Thus, the above findings indicate the involvement of the actin cytoskeleton in endocytosis. The phosporylation and dephosphorylation of the receptor affect the rate of turnover and final fate of the receptor. Endocytosis of the $P2Y_2$-R seems to mainly take place via the clathrin mediated pathway.

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Cannabinoid CB1 and endothelin B receptor interactions in the hippocampus

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The endogenous cannabinoid and endothelin systems in the brain are involved in vasoaction and have been suggested to be neuroprotective. However, the physiological role of the brain cannabinoid system and its possible interactions with the endothelin system are not fully understood. We used the endothelin B (ETB)-deficient spotting lethal (sl/sl) rats to study the cannabinoid 1 (CB1) receptor and the ETB receptor interplay in the hippocampus. Sl/sl-rats have a natural mutation in the ETB-gene leading to a complete lack of functional ETB receptors that is accompanied by an increased cell death of cultured hippocampal neurons and a higher rate of neuronal apoptosis in the dentate gyrus especially at the third postnatal week. The interactions of the two endogenous neuromodulator systems were investigated using immunohistochemistry and functional bioassays in astrocyte and neuronal cultures. Immunohistochemistry showed an age-dependent increase in CB1 receptor expression from postnatal day 4 through 21 in the hippocampus of wildtype, heterozygous and mutant rats. The increase in CB1 receptor expression in the granule cells of the dentate gyrus was significantly higher ($p<0.05$) in the 14-day-old mutant rats (n=7) than in wildtype animals (n=6). No significant differences in CB1 receptor expression between the three genotypes were found in 21-day-old rats. To test cannabinoid effects on cell survival in mutant and wildtype cultures, postnatal hippocampal neurons were exposed to hypoxia (15h, <1% oxygen). Preliminary data from these experiments demonstrated a protective effect on hypoxia-induced cell death by noladin, an endogenous cannabinoid agonist. This effect was more pronounced in mutant sl/sl neurons. We further developed a ”mild in-vitro ischemia” model for primary astrocytes to simulate stroke conditions (glucose deprivation + hypoxia for 15h, <1% oxygen). Administration of noladin to astrocyte cultures had no protective effect against ischemia. In summary, the CB1 expression increases age-dependently as the rate of apoptosis in the dentate gyrus decreases in healthy wildtype animals. ETB deficiency aggravates neuronal cell death in the hippocampus. Interestingly, this effect is most intense at postnatal day 21 at a time when the CB1 receptor expression in mutant rats is no more significantly different from wildtype animals. Since endogenous cannabinoids show neuroprotective actions in culture, neuronal CB1 overexpression in sl/sl rats may reflect a natural substitution effect for ETB-deficiency in hippocampal neurons.

Peroxynitrite- Mediated Nitration of Microtubule Associated Protein Tau and Cytotoxic Effects in Differentiated PC12 Cells

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Oxidative and nitrative injury is implicated in the pathogenesis of neuro-degenerative disorders, including Alzheimer`s disease (AD), Parkinson`s disease, frontotemporal dementias, multiple system atrophy and multiple sclerosis. Reactive oxygen and nitrogen species are produced *in vivo* and may act synergistically to form nitrating agents. The reaction of superoxide radicals and nitric oxide generates peroxynitrite (PN), which can convert native tyrosine residues to 3-nitrotyrosine (3-NT). Tau is a microtubule associated protein (MAP) which is abundantly expressed in the CNS. It is the major building block of paired helical filaments in AD and also found in neuronal and glial cell inclusions in brain lesions of other diseases. Tau hyperphosphorylation contributes to the formation of cytoplasmic inclusions. The present study was undertaken to investigate if tau is modified by nitrative stress, and whether tau nitration impairs its microtubule binding properties similar to the effects of tau phosphorylation. PC12 rat pheochromocytoma cells were differentiated in the presence of NGF (50ng/ml; 7 days) and treated with PN (0.5mM or 1mM) for up to 48h. This treatment caused morphological changes, i.e. retraction of cellular processes and membrane blebbing, indicating the involvement of apoptotic cell death. This was further confirmed by the appearance of a DNA-ladder. Also, the induction of the small heat shock protein HSP32/HO-1 was observable, which is an indication of oxidative stress. Tau phosphorylation was not affected under these conditions. To investigate the effects of PN on nitration of proteins, two antibodies were used: polyclonal anti 3-nitrotyrosine antibodies (3-NT) and a monoclonal antibody specifically recognizing nitrated tau, namely MAb n847. Immunoblot analysis revealed that PN leads to the nitration of a number of proteins including tau. Tau was nitrated already 30 min after the treatment and tau nitration was maximal after 24h. Microtubule-binding assay revealed that nitrated tau did not bind to microtubules. Furthermore, incubation with PN resulted in an increase in abnormal tau, which could not be solubilized in high salt or Triton X-100 buffers, but only in formic acid. Taken together, tau is nitrated by PN and this nitrated tau was not associated with microtubules. Nitrative damage impairs its solubility and might be causally related to pathological events leading to the formation of neuronal and/or glial tau inclusions.

Biochemical properties of the neuronal tryptophan hydroxylase

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Serotonin is a neurotransmitter synthesized in the raphe nuclei of the brain stem and involved in the central control of food intake, sleep, and mood.[1] Accordingly, dysfunction of the serotonin system has been implicated in the pathogenesis of psychiatric diseases. At the same time, serotonin is a peripheral hormone produced mainly by enterochromaffin cells in the intestine and stored in platelets, where it is involved in vasoconstriction, haemostasis, and the control of immune responses.[2] Moreover, serotonin is a precursor for melatonin and is therefore synthesized in high amounts in the pineal gland. Tryptophan hydroxylase (TPH) catalyzes the rate-limiting step in serotonin synthesis. Until recently, only one gene encoding TPH was described for vertebrates. By gene targeting, we functionally ablated this gene in mice. To our surprise, the resulting animals, although being deficient for serotonin in the periphery and in the pineal gland, exhibited close to normal levels of serotonin in the brain stem. This led us to the detection of a second *Tph* gene in the genome of

humans, mice, and rats, called *Tph2*.[3] This gene is predominantly expressed in the brain stem, while the classical *Tph* gene, now called *Tph1*, is expressed in the gut, pineal gland, spleen, and thymus. These findings clarify puzzling data, which have been collected over the last decades about partially purified TPH proteins with different characteristics and justify a new concept of the serotonin system. In fact, there are two serotonin systems in vertebrates, independently regulated and with distinct functions. Here we describe some physical properties and kinetic constants of recombinant *Tph2*.

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Serotonylation of small GTPases: A receptor-independent signaling pathway for granule secretion

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Recently, we have shown that serotonin is synthesized independently by two different tryptophan hydroxylase isoforms in peripheral tissues and neurons, and identified a neuronal tryptophan hydroxylase isoform.[1, 2] Mice selectively deficient in peripheral tryptophan hydroxylase and serotonin exhibit an impaired hemostasis, resulting in a reduced risk of thrombosis and thromboembolism, while the ultrastucture of the platelets is not affected as demonstrated by electron microscopy.[3] Interestingly, the experimental aggregation of serotonin-deficient platelets is unaffected *in vitro* but their adhesion *in vivo* is reduced due to a blunted secretion of von Willebrand factor and other α-granular contents. Here we present that serotonin is covalently attached to small GTPases in a transglutaminase-dependent reaction during activation and aggregation of platelets, rendering these GTPases constitutively active. Our data provide evidence for a novel, receptor-independent signaling pathway for serotonin that we named "serotonylation", which leads to α-granular exocytosis from platelets. Furthermore, several lines of evidence lead to the conclusion that serotonylation could be also involved in neuroendocrine and immunological exocytotic processes. Since transglutaminases also accept other monoamines, such as histamine and catecholamines as substrates, it can be expected that histaminylation and catecholaminylation could be involved in analogous signaling mechanisms.

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Jacob and Caldendrin: A protein/protein interaction in control of dendritic spine number and formation?

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The neuronal Ca2+-sensor protein Caldendrin is a component of the postsynaptic density (PSD) with high similarity to Calmodulin (CaM). In search for Caldendrin binding partners we identified a novel brain-specific protein only expressed in mammalia termed Jacob. Jacob is highly enriched in the PSD and in neuronal nuclei, exhibits a strikingly restricted expression in limbic brain and cortex with a dendritic mRNA and is like Caldendrin localized to only a subset of synapses. Caldendrin but not CaM binds to Jacob in a Ca2+-dependent manner in a α-helical region harboring an IQ-like-motif and a bipartite nuclear localization signal. Jacob is recruited to the nucleus in response to NMDA-receptor activation and this recruitment results in a drastically altered morphology of the dendritic cytoarchitecture, which is preceded by a rapid stripping of synaptic contacts. Overexpression of a Jacob Wild-type constructs in hippocampal primary neurons caused the formation of an increased number of neurites, while transfection with constructs that are targeted extranuclear also led to an increased number and size of dendritic spines. These results suggest that Jacob might be involved in the coupling of morphogenetic signals from excitatory synapses to nuclear gene transcription that control the cytoarchitecture of the dendritic tree.

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Expression of beta-secretase BACE is differentially controlled through muscarinic acetylcholine receptor signalling

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β-amyloid peptides derived by proteolytic cleavage from a much larger amyloid precursor protein (APP), play a major role in the pathogenesis of Alzheimer´s disease by forming aggregated, fibrillary complexes that have been shown to be neurotoxic. Therefore, preventing b-amyloid formation by inhibition of APP-processing secretases is considered as potential strategy to treat Alzheimer´s disease. Recently, the transmembrane aspartyl protease β-site APP-cleaving enzyme (BACE) has been identified as β-secretase. As cholinergic mechanisms have been shown to control APP processing, the present study intends to reveal whether the expression of BACE is particularly driven by muscarinic acetylcholine receptor (mAChR) using neuroblastoma cell line SH-SY5Y as a model. Stimulation of cells with the M1- and M3- selective mAChR agonist talsaclidine for one hour resulted in a dose-dependent increase in BACE expression up to 2.5-fold over basal level, detectable already 24h following stimulation. Similar effects of BACE upregulation were observed, when protein kinase C was directly activated by phorbol esters. However, in the presence of PD98059, an inhibitor of the MAP kinases MEK/ERK, BACE expression is no longer upregulated by activation of M1-mAChR through talsaclidine. In contrast, BACE expression is suppressed below the basal level by stimulating M2-mediated pathways by both selective M2-agonist binding or by direct activating of adenylate cyclase with forskolin. When protein kinase A is inhibited by H89, forskolin does no longer affect BACE expression. The data indicate that BACE expression is differentially controlled by mAChR signaling. Agonist binding to M1/M3-mAChR upregulates BACE expression, through activation of both protein kinase C and MAP kinase signaling cascade. In contrast, BACE expression is downregulated through activation of M2/M4-mAChR and protein kinase A-mediated pathways. The data suggest that selective inhibition of brain M1/M3-mAChR signaling in combination with stimulation of M2/M4-mAChR should be potential to suppress excessive β-amyloid formation in Alzheimer´s disease.

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PREVENTIVE LIPOPROTEINS 1HNMR SPECTROSCOPIC BLOOD SERUM ANALYSIS OF ALZHEIMER'S DISEASE AND STROKE.

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Abstract

The etiopathology of Alzheimer's disease (AD) is still unknown after innumerable studies and publications.

However,beyond the genetic factors,which are not considered causative,there is evidence,more and more, that cerebrovascular atherosclerosis with lipoproteins alterations is one of risk factors in stroke and AD.Our preventive and comparative study is the first attempt reported in the literature to quantify the 1HNMR spectra of lipoprotein fractions VLDL,LDL and HDL of blood serum of atherosclerosis,stroke and AD, compared to normal healthy subjects.We applied the line shape fitting analysis with mathematical separation of the overlapping resonances from the VLDL,LDL and HDL. HDL was decreased in stroke and more dramatically decreased in AD. LDL was increased in stroke and in AD while VLDL was increased in stroke and poorly detected in AD.Our Investigation might suggest that the AD & stroke have some etiopathological factors of arteriosclerosis in common and further studies are worthwhile to continue.Our study confirms the potential role of 1HNMRS quantification analysis of lipoproteins with more rapidity and accuracy than the routine lipid testing .

Late abstracts

Effects of A_3-receptor activation during hypoxia and possible signalling pathways in rat cortical neurons

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Adenosine is involved in various regulatory mechanisms within the CNS referring to neuroprotection. Whereas four G-protein-coupled receptors exist in the brain (A_1, A_{2A}, A_{2B}, A_3), the neuroprotective effects are mainly mediated by A_1-receptors. Previously we described pharmacologically the existence of A_3-receptors on rat cingulate cortical neurons. Their activation leads to an inhibition of postsynaptic potentials (PSPs) evoked by electrical stimulation. The present study was aimed to investigate whether the activation of A_3-receptors is additionally involved in neuroprotective effects of endogenous adenosine during hypoxia and by which signalling pathways A_3-receptors mediate the inhibition of the PSPs. The experiments were done with intracellularly placed microelectrodes in rat brain slices. Hypoxia (95%N_2+ 5%CO_2, 5 min) induced an inhibition of the PSP-amplitude which was more pronounced in the presence of the adenosine re-uptake inhibitor NBTG (30 µM) and the deaminase inhibitor EHNA (5 µM) and completely reversed by adenosine deaminase (1 U/ml). A hypoxia-induced inhibition of the PSPs in the presence of NBTG was also found after blockade of the A_1-receptors with DPCPX (0.1 µM). This inhibition was completely reversed by the A_3-antagonist MRS 1220 (0.2 µM). For further studies the selective A_3-agonist IB-MECA was used. IB-MECA (10 µM) induced an inhibition of the PSP-amplitude while the A_1-receptors were blocked. Application of GDP-β-S (100 µM) which inhibits the coupling of G-proteins completely abolished the IB-MECA-induced effect. The inhibition was also abolished in the presence of N-ethylmaleimide (150 µM) pointing to a G_i-coupling. The phospholipase C inhibitor U73122 (10 µM) but not its negative control U73343 (10 µM) prevented the IB-MECA-induced inhibition of the PSPs. The effect of IB-MECA was partially abolished by the IP_3-antagonist heparin (2 U/ml) and completely blocked by BAPTA-AM (10 µM), a chelator of intracellular Ca^{2+}. The calmodulin-antagonist W7 (30 µM) completely abolished the effect of IB-MECA on the PSPs. KN-93 (3 µM), an inhibitor of the Ca^{2+}/calmodulin-dependent protein kinase II but not its negative control KN-92 (3 µM) prevented the IB-MECA-induced inhibition. We assume that in situations of increased adenosine concentrations, as seen in hypoxia, the activation of A_3-receptors could be an additional mechanism to protect against neurotoxic effects of hypoxia. Phospholipase C and Ca^{2+}-dependent processes are involved in the signal transduction of the A_3 receptor on rat cingulate cortical neurons.

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Selective inhibition of ST8SiaII by unnatural sialic acids

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Polysialic acid (polySia) is a unique and highly regulated posttranslational modification of the neural cell adhesion molecule (NCAM). The presence of polySia affects NCAM-dependent cell adhesion and plays an important role during brain development, neural regeneration and plastic processes including learning and memory. Polysialylated NCAM is expressed on several neuroendocrine tumors of high malignancy and correlates with poor prognosis. The biosynthesis of polySia is catalyzed by two closely related enzymes, the polysialyltransferases ST8SiaII and ST8SiaIV. However, the impact of each enzyme in NCAM polysialylation is not understood. Here we describe the selective *in vivo* inhibition of ST8SiaII using synthetic sialic acid precursors. For the first time, we provide evidence for different substrate affinities of ST8SiaII and ST8SiaIV. These data open the possibility to study the individual role of the two enzymes during various aspects of brain development and function and in tumorigenesis.