MOLECULAR BASIS OF PATHOLOGY AND THERAPY IN NEUROLOGICAL DISORDERS

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Guest Editor:
Barbara Lukomska

MOLECULAR BASIS OF PATHOLOGY AND THERAPY IN NEUROLOGICAL DISORDERS

The 9th International Symposium

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PROGRAMME

Thursday, November 27th, 2008

09:15 Opening of the Conference

Session I

NEW THERAPEUTIC STRATEGIES IN TREATMENT OF CNS DISEASES

Chairs: **Jerzy Lazarewicz (Warsaw, Poland)**
Wojciech Danysz (Frankfurt/M, Germany)
Barbara Zablocka (Warsaw, Poland)

09:30–10:00 **Wojciech Danysz, (Frankfurt/M)**
Glutamate receptors as therapeutic targets: present status and future perspectives

10.00–10.30 **Andrzej Lipkowski (Warsaw)**
Neuropeptide analogues as prospective new medicines for cancer pain treatment

10.30–11.00 **Stanislaw J. Czuczwar (Lublin)**
Management of drug-resistant seizures based
on experimental models of epilepsy

11.30–12.00 **Adam Plaznik (Warsaw)**
New therapeutic targets in treatment of affective disorders, anxiety and depression

12:00–12:15 **Anna Kazmierczak (Warsaw)**
Extracellular alpha-synuclein and its neurotoxic fragment NAC peptide induce cell death by different molecular pathways

12:15–12:30 **Krzysztof Wieczerzak (Krakow)**
Imipramine-induced changes in expression of G proteins and ERKs in cerebral cortex of rat

Session II

EXPERIMENTAL APPROACHES IN ACUTE AND PROGRESSIVE CNS INJURY

Chairs: **Andrzej Szutowicz (Gdansk, Poland)**
Klaus Reymann (Magdeburg, Germany)
Teresa Zalewska (Warsaw, Poland)

13.30–14.00 **Klaus G. Reymann (Magdeburg)**
Endangered neurons after ischemia are getting help from microglia

14.00–14.30 **Bozena Kaminska-Kaczmarek (Warsaw)**
Signal transduction underlying stroke-induced gliosis and inflammation as therapeutic target

14.30–15.00	**Jacek Kuznicki (Warsaw)** Proteins of GSK3β pathways as targets for Alzheimer's disease drugs
15.00–15.30	**Grzegorz Wilczynski (Warsaw)** Matrix metalloproteinase-9 – a culprit in epilepsy?
15:30–15:45	**Magdalena Karetko (Warsaw)** Comparison of developmental composition pattern of perineuronal nets in the visual and the barrel cortex
15:45–16:00	**Joanna Klimaszewska-Lata (Gdansk)** Relationships between acetyl-CoA level and function of fenotypically modified N9 murine microglial cells
16:30	*Poster sessions*

Friday, November 28th, 2008

Session III

STEM CELLS FOR THE TREATMENT OF CNS DISEASES

Chairs:	**Krystyna Domanska-Janik (Warsaw, Poland)** **Nico Forraz (Lyon, France)** **Barbara Lukomska (Warsaw, Poland)**
9.30–10.00	**Colin P. McGuckin (Lyon)** Umbilical Cord Blood – models for neurodegeneration and drug testing
10.00–10.30	**Leonora Buzanska (Warsaw/ISPRA)** Human cord blood stem cells in neurotoxicology: advantage of emerging technologies
10.30–11.00	**Jukka Jolkkonen (Kuopio)** Cell-based therapies and functional recovery in experimental stroke rats
11.30–12.00	**Alexander Storch (Dresden)** Stem cell-based therapy in Parkinson disease
12:00–12:15	**Joanna Sypecka (Warsaw)** The crucial role of the local microenvironment in fate-decision of neonatal rat NG2 progenitors
12:15–12:30	**Piotr Rieske (Lodz)** Inducing efficient catecholaminergic differentiation of GFAP, SOX2-positive neural progenitors (NHA) by means of kinetic factors
13:30–15:30	*Poster session*

Session I

NEW THERAPEUTIC STRATEGIES IN TREATMENT OF CNS DISEASES

SI-L1
Glutamate receptors as therapeutic targets: present status and future perspectives

Danysz W.
Merz Pharmaceuticals, Frankfurt am Main, Germany

In spite of intensive research for over 2 decades, number of drugs affecting glutamatergic system that are clinically used is very limited. This presentation will be devoted to overview of selected glutamate targets, current drug development, their potential and pitfalls. In particular, NMDA receptors (channel blockers and NR2B antagonists) and mGluR5 positive and negative modulators will be discussed more extensively. In case of NMDA receptors, some channel blockers (e.g. memantine) or NR2B antagonists are in clinical use for dementia or are in late stages of development for neuropathic pain respectively. In contrast, mGluR5 modulators are less advanced. Recently, several such substances have been introduced such as MTEP, MPEP, CDPPB or ADX47273. mGluR5 negative allosteric modulators (NAMs) e.g. MTEP are active in some models such as formalin, and Freund adjuvant model of inflammatory pain. Additionally they have potential in the treatment of L-DOPA induced dyskinesia. mGluR5 positive modulators (PAMs, e.g. CDPPB) may have potential as antipsychotics and improve learning. As it will be presented, in our hands these expectations can be only partially fulfilled as no clear convincing picture arises from testing in various animal models of schizophrenia (amphetamine-induced hyperactivity or apomorphine-induced prepulse inhibition). In learning models positive effect was seen in object recognition task (spontaneous forgetting).

SI-L2
Neuropeptide analogues as prospective new medicines for cancer pain treatment: Opioid-tachykinin chimera

Lipkowski A.W., Kosson D., Klinowiecka A., Misicka A.
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Multidrug therapies became routine approach in modern medical treatment protocols. However, simple combinations of drugs have disadvantages, including differences in pharmacological profiles of single drug component. Therefore, over twenty years ago we have proposed development of multitarget medicines as a new avenue of drug discovery. Identification of numerous endogenous components that participate in the formation, transmission, modulation and perception of pain signals offers numerous strategies for the development of new analgesics. One of them is hybridization of opioid pharmacophores with tachykinin receptor ligands. Tachykinins, like substance P (SP) produces both hyperalgesia and, at low doses, a naloxone-sensitive analgesia. Very likely, these opposite effects of SP in the spinal cord, are mediated through activation of various self-regulatory mechanisms. Modulation of tachykinin receptor system is probably significant component of tolerance and dependence development. Therefore, various new opioid agonist-tachykinin antagonist and opioid agonist-tachykinin agonist have been synthesized and tested to develop new medicines for chronic pain treatment. The presentation will focus on new group of opioid agonist-tachykinin agonist that expresses strong analgesic activity even after peripheral application. These new compounds are interesting candidates for treatment of chronic pain because they express very low tolerance development properties.
Presented studies have been supported in part with EU grant Normolife (LSHC-CT-2006-037733)

SI-L3
Management of drug-resistant seizures based on experimental models of epilepsy

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Approximately 30% of epileptic patients are resistant to monotherapy and may require combinations of antiepileptic drugs (AEDs). The combinations listed below have been evaluated in the maximal electroshock test in mice which is a model of human tonic-clonic and complex partial seizures. Gabapentin when combined with carbamazepine (CBZ), oxcarbazepine (OXC), lamotrigine (LTG), phenytoin (PHT), phenobarbital (PB), topiramate (TPM), or tiagabine (TGB) resulted in synergy with no neurotoxicity. However, LTG and PB produced elevations of the plasma concentrations of gabapentin. A synergy was also observed when LTG was combined with valproate (VPA) or TPM and these combinations were accompanied by antagonism concerning adverse effects. In contrast, LTG co-administered with CBZ produced a clear-cut anticonvulsant antagonism and neurotoxic additivity. Even worse results were found for OXC+LTG where anticonvulsant antagonism and neurotoxic synergy were evident. TGB combined with PHT, CBZ, PB, LTG or TPM led to additivity in the convulsive test and an apparent synergy of TGB+VPA was associated with a pharmacokinetic interaction. Additive effects were also observed for OXC+PB, CBZ, or VPA as regards anticonvulsant activity or neurotoxicity. In contrast, a combination of OXC+PHT was antagonistic in the seizure test and additive in neurotoxic tests, although OXC combined with TPM was clearly synergistic in terms of anticonvulsant activity and additive in terms of neurotoxicity. One of the newer AEDs, levetiracetam, produced anticonvulsant synergy when combined with CBZ, OXC and TPM. In conclusion, one can hardly predict an outcome of a drug combination basing upon the mechanisms of action of AEDs. Also, only beneficial combinations of AEDs need to be clinically evaluated.

SI-L4

New therapeutic targets in the treatment of affective disorders, anxiety and depression

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I have selected problems that focus on innovative approaches rather than reviewing existing mechanisms and their well known limitations. Small molecules capable of blocking neuropeptide receptors have the potential to become novel antidepressants. Likely candidates have been corticotropin releasing factor CRF antagonists and neurokinin-1 receptor antagonists. However, the efficacy of this treatment was not confirmed in clinical phase III trials. Preclinical data from α2,3-subunit selective GABA-A receptor agonists suggest that these targets may yield anxioselective agents. However, the α1 subtype mediates a substantial proportion of the anxiolytic actions of benzodiazepines. Considering that GABA-A1 receptors are expressed in higher densities than GABA-A2 or -A3 receptors and that there is substantial overlap of their localization, it may remain necessary to have a residual degree of their activation to achieve clinically significant anxiolysis. The discovery of novel molecules designed to regulate the specific aspects of neurogenesis may lead to improved CNS therapeutics, including antidepressant drugs. The majority of antidepressant drugs currently prescribed for the treatment of depression inhibits the reuptake of serotonin and/or norepinephrine. The strategy for developing triple reuptake inhibitors (TRIs) is based on preclinical and clinical studies indicating that adding a dopaminergic component will improve these therapies. Metabotropic glutamate receptors mGlu2,3 and mGlu5, and the cannabinoid receptors CB1 and CB2 are also attractive therapeutic targets. It is concluded, that a deeper understanding of the breadth of neuromodulators structural diversity as well as the components required for their stimulation/inhibition will guide a rational drug development.

SI-01

Extracellular alpha-synuclein and its neurotoxic fragment NAC peptide induce cell death by different molecular pathways

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Extracellular alpha-synuclein (ASN, NACP – NAC Precursor Protein) was suggested to play a crucial role in the pathogenesis of various neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease. The fragment corresponding to the region 61-95 of the protein, originally termed NAC (non-amyloid-beta component), has been found in amyloid plaques associated with Alzheimer's disease, and several reports suggest that this region is responsible for the toxicity of alpha-synuclein. However, the precise mechanism of ASN and NAC action remains unclear. The aim of the present study was to investigate the signaling events in ASN and NAC mediated apoptosis of neuronal PC12 cells. Immunochemical, spectrophotometrical and spectrofluorometrical methods were used in this study. Our data evaluated by MTT assay and Hoechst 33342 showed that soluble ASN and NAC peptide, in concentration dependent manner, induce enhancement of free radicals level and apoptotic death of PC12 cells by 50% and 70%, respectively. ASN induced caspase-3 activation by 40% with concomitant decrease in poly(ADP-ribose) polymerase (PARP) immunoreactivity and had no effect on AIF release from mitochondria. On the contrary, NAC peptide had no effect on caspase-3 activity and PARP protein level, but enhanced PARP activity and induce AIF release. Inhibitor of caspase-3 (Z-DEVD-FMK, 100 microM) prevented large population of cells against ASN-evoked cell death, but had no significant protective effect on cells treated with NAC peptide. These findings indicate that ASN and its liberated neurotoxic fragment induce different signaling pathways of programmed cell death.

Supported by the MS&HE Grant No 2PO5A4129 and MS&HE Scientific Network No 28/E-32/SN-0053/2007

SI-02

Imipramine-induced changes in expression of G proteins and ERKs in cerebral cortex of rat

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Imipramine belongs to a class of tricyclic antidepressants which augment monoaminergic transmission in a brain and are prevailingly used in treatment of depressive disorders. Increased availability of neurotransmitters (e.g., noradrenaline and serotonin) results among others, in modulation of the activity of G protein-coupled receptors and other proteins involved in intracellular signaling. The study was aimed to assess the effects of single and repetitive treatment with imipramine (10 mg/kg, twice daily, for 21 days) on the expression of G11, GQ and G12 proteins and protein kinases, ERK1/ERK2 and pERK1/pERK2 in the rat prefrontal cortex. Animals were sacrificed 4 and 24 h after the last drug injection. Single and chronic treatments with imipramine decreased similarly the Gq protein level (by 29, 36 and 48%), while G11 and G12 were unchanged. In contrast, the acute imipramine dose increased the level of ERK1 (by 32% above saline control) that was further enhanced by the chronic treatment (by 48% and 64%, respectively). The increase in ERK2 level was similarly marked (at both time-points) after chronic (by 39% and 31%) and after single dose of drug (by 28% vs. saline). Interestingly, the ERK1/ERK2 ratio was changed only

at 24 h after completion of chronic treatment (127% vs. saline control). The same direction of changes, independently on amount of imipramine doses, was observed in the case of pERK1 and pERK2 (increase by approx. 25% of saline group). Our data demonstrate that treatment with imipramine causes downregulation of Gq protein level and upregulates the ERK1/ERK2 pathway(s). The results suggest that imipramine-induced changes in ERKs can result from other than Gq-linked intracellular signaling.
Supported by Polish MNSW Scientific Network fund

SI-P1

Pretreatment is not mandatory for neuroprotection with 1α,25-dihydroxyvitamin D3 in perinatal hypoxic-ischemic rat brain damage *in vivo* and in excitotoxic injury of primary neuronal cell cultures

Makarewicz D.[1], Kajta M.[2], Zieminska E.[1], Jantas D.[2], Domin H.[2], Lason W.[2], Kutner A.[3], Lazarewicz J.W.[1]
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Previous *in vivo* and *in vitro* studies demonstrated neuroprotective potential of pretreatment with 1α, 25-dihydroxyvitamin D3 (calcitriol). The aim of present study was to determine effectiveness of calcitriol administered *in vivo* after brain ischemic episode in the rat model of perinatal asphyxia, or co-applied with some delay during 24 h exposure to glutamate of the mice hippocampal, cortical and cerebellar neuronal cultures at 7th and 12th day *in vitro*. In some experiments calcitriol was given after acute exposure to glutamate of the rat cerebellar neurons. Our results demonstrated, that in the 7 day old rat pups submitted to hypoxia – ischemia acute application of calcitriol in one dose of 2 μg/kg 30 min after termination of the insult or sub-chronic, 7-day post-treatment with calcitriol effectively diminished brain damage. The rate of such accomplished neuroprotection exceeded that achieved by hypoxic preconditioning, used as the reference neuroprotective method. Moreover the results of our *in vitro* experiments revealed the ability of calcitriol to reduce excitotoxicity in a way dependent on origin of neuronal cells, stage of their development and duration of excitotoxic insult. Calcitriol was neuroprotective when it was applied together with glutamate or even with up to 6 h delay during 24-h excitotoxic challenge to the hippocampal and neocortical, but not cerebellar neuronal cultures. In addition calcitriol inhibited glutamate-induced caspase-3 activity in hippocampal cultures. We ascribe these protective effects of calcitriol to a rapid, possibly non-genomic modulation by this compound of the mechanisms that are instrumental in its direct neuroprotective action.
The study was supported by Polish MNSW Scientific Network Fund no 26/E-40/SN-0023/2007

SI-P2

The protective effect of TRH and its analogues on staurosporine – but not doxorubicin-induced apoptosis in primary cortical neurons

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In the present study we investigated the influence of thyrotropin-releasing hormone (TRH, pGlu-His-Pro-NH2) and its more stable analogues: CG-3703 (Montirelin), RGH-2202 (L-6-keto-piperidine-2carbonyl-L-leucyl-L-prolinamide) and Z-TRH (Z-pGlutamyl-Histydyl-Proline) on neuronal apoptosis evoked by staurosporine – or doxorubicin, agents activating mitochondrial or extracellular (FAS) apoptotic cell death, respectively. We showed that TRH (0.001–10 μM) in U-shape concentration dependent way (effective concentrations: 0.01 and 0.1 μM) partially attenuated the staurosporine (0.5 μM) – but not doxorubicin (0.5 μM)-evoked cell damage in mouse 7 DIV cortical neurons only when added 24 h before toxin administration. The TRH analogues (MON, RGH, Z-TRH) were also effective in lower concentration (0.001 μM) than TRH in attenuation of the staurosporine-induced LDH release. Moreover, that beneficial effect of TRH and its analogues was not accompanied with its influence on caspase-3 activity, though the attenuation of number of apoptotic cells was observed in Hoechst's staining. Furthermore, we found that neither PI3-K (wortmannin 10 μM, LY294002 1 μM) nor MAPK/ERK1/2 (PD098059 1 uM and U0126 1 μM) inhibitors were able to abolish protection served by TRH and MON. There was no protection observed when peptides were added concomitantly with staurosporine and doxorubicin. The obtained data showed ameliorating effect of pretreatment with low concentrations of TRH and its analogues on neuronal cell death mediated by agent activating mitochondrial pathway of apoptosis. That effect seems to be caspase-3-independent and does not engage the PI3-K/Akt and MAPK/ERK1/2 cellular prosurvival pathways.
Supported by grant No. 2PO5A15530 from the Ministry of Education and Science (Warsaw, Poland)

SI-P3

Assessment of antiproliferative potency of opioid peptide analogue biphalin in human glioblastoma cell line T98G

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Biphalin is a new type of opioid peptide analogue with high analgesic potency that is over 1000-fold greater than morphine, a

well-known opiate compound widely utilized in pain management. Because of its less addictive nature than morphine, this substance has been suggested as an useful analgesic drug. Biphalin's high analgesic activity may be related with interaction with all three types of opioid receptors (mu, delta and kappa), belonging to G protein-coupled receptors (GPCR) family. These members of GPCR are expressed by astrocytes, including neoplastic glioma cells. It has been evidenced, that opioid receptors, particularly MOR (mu opioid receptor) and KOR (kappa opioid receptor) are involved in growth regulation of glioma cells. The alteration of tumor cell proliferation might be associated with adenylate cyclase inhibition, that results in decrease of intracellular cAMP level and prevention of PKA activation. The present study was performed on human glioblastoma cell line TG98 to establish the effect of biphalin on neoplastic cell growth and proliferation abilities. The glioma cell line exposed to biphalin at increasing concentrations exhibited the decrease of growth rate, reduction of cell ability to form colonies and alteration of Ki-67 proliferation index. These results suggest that this opioid peptide analogue is promising medicine in simultaneous analgesic and anti-cancer therapy.

The work was supported by the Ministry of Science and Education, Grant No. NN401228334 and European Grant Normolife No. LSHC-CT-2006-037733

SI-P4

Involvement of non-receptor protein kinases in the mechanism of imipramine action

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Chronic treatment with antidepressant imipramine increases synaptic plasticity and connectivity in the rat brain. Signals that orchestrate changes associated with neuronal plasticity derive in part from extracellular matrix (ECM). Two homologous tyrosine kinases – FAK and PYK2 are thought to play a major role in transducing signals from extracellular matrix to the cell interior. This prompted us to examine the effect of acute and chronic imipramine treatment on the activity of FAK and PYK2-dependent signaling pathway in the rat brain cortex. To approach this problem we aimed to quantify the level of FAK and PYK2 phosphorylation of their tyrosine residues as well as interaction of these kinases with downstream signaling substrates such as the Src kinase, adaptor protein p130Cas, and cytoskeletal protein-paxilin. Our results demonstrate different responses of the two kinases to the imipramine administration. Imipramine leads to the suppression of FAK-dependent pathway with simultaneous stimulation of the pathway coupled with PYK2 kinase. The reduction in FAK Tyr 397 phosphorylation, in particular after chronic administration of the drug, was translated into a decreased association of FAK with downstream molecular partners, Src kinase and p130Cas. In contrast, the acute and chronic treatment with imipramine leads to activation of 402 tyrosine phosphorylation of PYK2 kinase and in consequence increased interaction with kinase Src and adaptor protein p130Cas. Because both kinases appear to be well suited to play a role in synaptic plasticity, it seems probably that PYK2 may function in a compensatory manner for the FAK inhibition and may be responsible for neuronal plasticity-connected events after imipramine treatment.

Supported by Polish MNSW Scientific Network Fund

SI-P5

Neuroprotective effects of Y2 and Y5 neuropeptide Y receptor agonists

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Neuropeptide Y (NPY), a 36 amino acid peptide widely distributed in the nervous system, inhibits glutamatergic transmission and decreases hippocampal epileptiform activity which may lead to neuroprotection. Such effects were observed in some earlier studies, but results are divergent and the role of particular Y receptors remains unclear. In the present study we investigated a possibility of neuroprotective action of neuropeptide Y1, Y2 and Y5 receptor specific ligands in rats in two *in vivo* models of brain damage. In the first model, kainic acid (KA)(2.5 nmol/1 µl) was microinjected into the CA1 region of the rat dorsal hippocampus and the peptide compounds (470 pmol/1 µl) were injected in the same region 30 min, 1 h or 3 h after the kainate. Seven days later the brains were taken for histology and number of neurons in CA pyramidal layer was evaluated by stereological counting. It was found that, Y2 agonist (NPY13-36) and Y5R agonist ([CPP1-17,NPY19-23,Ala31,Aib32Gln34] hPP), injected 30 min or 1 h but not 3 h after the KA, significantly diminished KA-induced hippocampal lesion. Contrary Y1 agonist ([Leu31,Pro34]-NPY) did not induced any protection but had a tendency towards an increase of the degeneration. The most promising Y2 agonist was tested also in the second model, focal cerebral ischemia after transient middle cerebral artery occlusion (MCAO). The peptide was injected icv (10 µg/6 µl,), 30 min after MCA occlusion. It significantly diminished MCAO-induced brain damage evaluated by TTC staining. Our results indicated neuroprotective effects of Y2 and Y5 activation. Moreover we found that the peptides may be effective after delayed (30–60 min) application.

SI-P6

Dose-dependent neuroprotection by 17β-estradiol following MPTP intoxication in male mice: Role of the neuroinflammatory reaction in estrogen-mediated neuroprotection

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17β-estradiol (E2) have been shown to reduce damage of the nigrostriatal system following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The immunosuppressive properties of this hormone may be involved in estrogen-mediated nigro-striatal neuroprotection. We studied the chronic effects of two doses of E2: 0.25 and 2.5 mg per pellet (21-days release) administered 7 days prior MPTP treatment in C57BL male mice (12 months old) on MPTP-induced dopaminergic neurons degeneration and inflammatory reaction in nigrostriatal pathway. We estimated striatal: tyrosine hydroxylase (TH) and dopamine (DA) level; glial fibrillary acidic protein (GFAP), cytokines (IL-1β, IL-6, TNFα, TGFβ1, IFNg), trophic factor (GDNF) and adhesion molecules (ICAM-1 and VCAM-1) gene expression at 1,7 and 21 days post MPTP intoxication. We showed that only the lower E2 dose (0.25 mg) exerted a neuroprotective effect upon nigrostriatal system. E2 0.25 pre-treatment attenuated the MPTP-induced loss of striatal DA at 1 day time-point and TH at 7- and 21-day time-points. E2 0.25 also diminished the early MPTP-induced increase of the striatal IFNg, IL-1β, TGFβ, ICAM-1, VCAM-1 and GFAP levels but failed to suppress the MPTP-induced increase of striatal TNFα. E2 0.25 also induced an increase of the striatal GDNF and IL-6 gene expression. In contrast, higher E2 doses did not affect the expression of the investigated inflammatory mediators, expect the GFAP gene expression (increase of the GFAP expression after E2 2.5 administration). We conclude that E2 has a critical dosing effect on dopaminergic neurons survival, only physiologic levels of E2 are neuroprotective in male mice. The neuroprotective effects of E2 might mediate through a modulation of molecular factors of neuroinflammatory reaction.

SI-P7

Inhibition of 12-lipoxygenase protects against amyloid beta peptides-evoked toxicity, memory impairment and alteration of locomotor activity

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The pro-inflammatory enzyme 12/15-lipoxygenase (12/15-LOX) is upregulated in Alzheimer's disease (AD), but the role of the enzyme in a amyloid beta (AB)-evoked toxicity is not fully understood. Its pro-oxidative activity may contribute to the pathophysiology of AD. The aim of this study was to analyze the expression and activity of 12-LOX in animal model of AD. The role of 12-LOX in AB42-evoked memory impairment and locomotory activity and the effect of systemic inflammation on AB-dependent alterations were also studied. Then the relationship between AB concentration and 12-LOX was examined using PC12 cells transfected with human wild-type and mutant AB precursor protein (APP) gene. Twelve-month-old C57Bl6 mice were injected with AB42 (1 nmol, icv) alone or simultaneously with lipopolysaccharide (LPS; 1 mg/kg, ip). Some mice received 12-LOX inhibitor, beicalein (10 mg/kg, ip). Our results indicated that AB significantly increased 12-LOX expression and activity in hippocampus. Beicalein effectively prevented AB-induced 12-LOX activation and protected mice against memory deficit and locomotory disturbances. *In vitro* studies demonstrated the significant relationship between AB level and 12-LOX expression, oxidative stress and NF-κB activation. Beicalein protected PC12 cells against NF-κB nuclear translocation. Our data indicated that 12-LOX is involved in AB toxicity. Beicalein protected mice against memory deficit and locomotory disturbances, suggesting that 12/15-LOX inhibitors may provide new therapeutic opportunities in treatment of AD.
Supported by MS&HE scientific network 28/E-32/SN-0053/2007

SI-P8

Antinociceptive interaction of substance P and dermorphin applied intrathecally

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In the clinic, multidrug therapies become standard of modern successful therapies. However, the different pharmacodynamic profile of the drugs creates limits of using of a mixture. The discovery of numerous endogenous components which participate in the formation, transmission, modulation and perception of pain signals offers numerous strategies for the development of new analgesic. One of them is Substance P (SP). This undecapeptide Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH2 is widely distributed throughout the central nervous system and is highly expressed on areas that are critical for the regulation of pain influx, affective behavior and stress. For activation of the receptor neurokinin-1 (NK-1) the C-terminal sequence of SP is essential but not all the effects of SP are mediated through the C-terminal fragment. N-terminal fragment, such as SP(1-7) induces antinociception and desensitization of several SP-induced behaviors. SP has been suggested to modulate the expression of opiate tolerance and withdrawal behaviours in rodents. This communication will present the results of creating the mixture SP and dermorphin administered together at the same time, because it is very important to describe pharmacological mechanism of these two compounds. This is the first step to design new analgesics hybrids where in one molecule we have two different pharmacophores: C-terminal fragment of substance P and N- terminal fragment replaced with dermorphin.
Presented studies have been supported with EU grant Normolife (LSHC-CT-2006-037733)

SI-P9

Blood-brain barrier permeability and analgesic activity of morphine and endomorphine-1 in mice bred for swim-stress induced analgesia

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In 1983 prof. Boguslaw Sadowski from Institute of Genetic and Animal Breeding in Jastrzebiec developed genetic mouse lines selectively bred for high (HA) and low (LA) swim-stress induced analgesia. Selected animals differ in sensitivity to both, pain or stress responses. Many experiments were conducted but the mechanism which is responsible for different sensitivity to both, pain and stress in these animals has been unknown till today. We hypothesized that difference in blood-brain barrier (BBB) permeability is the reason of different response of HA and LA mice. According to our hypothesis the β-endorphins which are released on the periphery after stressful stimuli, can cross the BBB and reach the brain, and analgesia is observed. The aim of the studies is to present the analgesic activity of opioid compounds in selected mice to examine their BBB permeability. This communication will also present influence of stress on analgesic activity of the given compounds. Different analgesic potency of alkaloid and opioid peptide in selected mice was a premise to conduct an ultramicroscopic studies which displayed pathological changes in morphology of BBB in HA and LA mice. These findings may explain different analgesic potency of the given compounds. These animals may well simulate human high and low sensitivity to both, pain or stress response.

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SI-P10

Selected biochemical characteristics of the rat mhSOD1(G93A) transgenic model of familial amyotrophic lateral sclerosis (fALS) and the effects of cytidine 5'-diphosphocholine (sodium salt, CDPch) treatment

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Background: Massive expression in rats of the mutated human superoxide dismutase-1 gene (mhSOD1G93A) causes an incurable, fast-progressing fatal illness that is an established model of fALS. We showed earlier that CDPch can slightly but significantly defer the onset of neurologic symptoms and extend life of the carriers. Here we report effects of the drug on some biochemical indices. Methods: Transgenic mhSOD1G93A(+) (Tg+) rats were randomized by gender and litter between study groups. The treatments began on postnatal day (PD) 61, consisted of a daily ip dose of CDPch (0.5 g/kg) or isotonic NaCl, and continued for a preset time or until an arbitrary (the rats were euthanized when unable to feed voluntarily) death point. Untreated Tg+ rats (PD 50–60, 94 and 108–129) and their Tg-siblings were used for additional controls. After decapitation, blood serum and CNS were harvested and stored at −80°C till analyzed. Results: ANOVA showed significant (P<0.001) age-related elevation of serum immunoreactive mhSOD1 (s-ir-mhSOD1) in NaCl-treated Tg+ rats, significantly (P=0.011) higher s-ir-mhSOD1 level in NaCl-treated terminal stage Tg+ rats than in their CDPch-treated counterparts, and a significant interaction (P=0.02) between these factors' effects on s-ir-mhSOD1 level; no such effect was found in serum VEGF or spinal cord ir-mhSOD1 level. There was significant (P<0.01) lowering effect of CDPch treatment, a tendency (P=0.09) for age-related lowering and a tendency (P=0.10) for interaction between these factors' effects on serum total thiol (sTT) level; *post-hoc* analysis showed significantly lower sTT level in CDPch-treated terminal stage rats than in their NaCl-treated counterparts. Western blots showed the existence of multiple oligomeric forms of s-ir-mhSOD1 in Tg+ rats.

SI-P11

Spinal cord hydrolysate peptides ameliorate immunological response in Experimental Autoimmune Encephalomyelitis (EAE)

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Background: Recently has been proposed to apply a method of oral tolerance to ameliorate auto-immune reactions. The aim of this study was to use the hydrolysate of pig spinal cord proteins (mixture of neuroantigens) to induce oral tolerance in the animal model of sclerosis multiplex (SM) – experimental allergic encephalomyelitis (EAE). Methods: The female Lewis rats were fed with pig spinal cord hydrolysate in two doses for one week before immunization, which was induced by injection of guinea pig spinal cord homogenate. The clinical course was observed and evaluated in a five grade scale. At the peak of clinical symptoms (the 13th day post immunization) the rats were sacrificed and the spleen removed. Splenocytes were suspended in a culture medium and placed in microculture plates. The cells were stimulated with homogenate alone, hydrolysate alone, mixture of homogenate +

hydrolysate, and medium alone. The cells were cultured for seven days. Subsequently, proliferation of splenocytes was estimated by means of [3H]thymidine incorporation and expressed in cpm (average of triplicate samples). In supernatants of cultures of splenocytes the level of cytokines interferon gamma (IFN-γ), interleukin (IL)-10, IL-4, and tumor growth factor (TGF)-α was measured. Results: It was demonstrated that homogenate-induced splenocytes of hydrolysate-fed rats gave rise to low proliferation as compared to the controls used. The IFN-γ was inhibited in hydrolysate-fed animals as well as in hydrolysate-stimulated samples. Conclusion: The results show that the hydrolysate of pig spinal cord proteins has a modulatory effect on the immune reaction, particularly on the orally-induced antigen-specific modulation of autoimmune response. It might have a clinical implication in SM treatment.

SI-P12

Effects of FK506 or cyclosporine A on the developing hippocampal formation

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Tacrolimus (FK506) and cyclosporin A (CsA) are immunosupressants widely used in transplantology. They can also protect neurons in several models of brain damage. Prolonged administration of the drugs has many negative neurological side-effects. The effects might depend on the developmental stage but appropriate investigations have been particularly rare. The present study focuses on long-term changes evoked by the drugs in the developing hippocampal formation. Six- and 30-day-old rats (P6s and P30s, respectively) were injected with FK506 or CsA in their pharmaceutical formulas containing a mixture of ethyl alcohol and Cremophor as a vehicle. Controls received the vehicle alone. When the rats were 60-day-old, sizes of their hippocampal formation, densities of calretinin-(CR+), parvalbumin-immunopositive (PV+) neurons and S100β protein-positive (S100β+) astrocytes were assessed. In P6s and P30s treated with CsA in its farmaceutical formula, the size of hippocampal formation was reduced. However, injections of the vehicle alone led to similar effects. In P30s, FK506 decreased the density of CR+ neurons but the vehicle had again the same negative effect. The only significant change in relation to vehicle-treated animals was a decrease in density of PV+ neurons in CsA-treated P30s. In P6s, FK506 dissolved in the vehicle increased the density of S100β+ astrocytes only in relation to naive but not vehicle-treated controls. Long-term effects of FK506 or CsA in their pharmaceutical formulas were mostly negative. Interestingly, they could also be obtained by application of the vehicle alone. Therefore, clinical and experimental effects of FK506 or CsA cannot exclusively be attributed to the drugs themselves abut also to the vehicle which appears to be not biologically neutral.

SI-P13

CXCL11 and Interleukin-18 in relapsing-remitting multiple sclerosis patients treated with methylprednisolone

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Chemokines may play a role in the pathogenesis of multiple sclerosis (MS), facilitating the trafficking of immune cells across the blood-brain barrier. Interferon-inducible T cell alpha chemoattractant (CXCL11) recruits activated Th1 cells to sites of inflammation. We have estimated the levels of CXCL11 chemokine and IL-18 also known as IFN-gamma inducing factor in sera of 30 relapsing-remitting MS patients during relapse, both before and after methylprednisolone (MP) treatment and compared the results with those in control group. The serum CXCL11 and IL-18 concentrations were measured by the ELISA method. The serum levels of CXCL11 were detectable in 23 relapsing-remitting MS patients. The levels were significantly higher in sera of studied patients before (55.4 ± 63.1 pg/ml) and after steroid therapy (40.7 ± 43.2 pg/ml) in comparison with control group (17 ± 18.3 pg/ml, P=0.002). The serum levels of CXCL11 before and after MP treatment did not differ significantly. The levels of IL-18 were detectable in the sera of all studied MS patients. The serum concentration of IL-18 in relapsing-remitting MS patients before MP therapy was 246.6 ± 143.5 pg/ml and was significantly higher than the level of IL-18 in healthy controls (171.06 ± 56.8 pg/ml, P=0.008). IL-18 levels in the sera of MS patients after steroid therapy was 258.76 ± 292.5 pg/ml and was higher than that in the control group (P=0.006) but did not differ significantly from the serum concentration of IL-18 in the same patients before the onset of MP treatment. The results suggest involvement of CXCL11 and IL-18 in immunopathogenesis of MS.

SI-P14

Cytogenetic analysis of MSRV pol, gag, env sequences and genome instability in multiple sclerosis

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Among of the potential agents causing multiple sclerosis MSRV virus (multiple sclerosis-associated retrovirus) is often taken into consideration. Aims of the study were (1) an assessment of

MSRV potential role in MS, and (2) test of genome instability in MS patients. The material was peripheral blood lymphocytes from 92 patients with MS, 12 patients with myasthenia and 20 healthy persons. The FISH studies with labeled PCR products of pol gag and env MSRV genes in nuclei, chromosomes and chromatin fibers were done. Classical cytogenetic techniques were introduced into karyotypes and micronuclei analyses. MSRV pol, gag and env sequences were found in both MS patients and controls. The copy number of MSRV pol sequence was significantly greater in MS patients (6–24 copies on nucleus) than in myasthenia (4–5 copies) and normal individuals (3–6 copies). MSRV gag sequence was found in a range of 5–20, 4–5, and 2–4 copies in MS patients, patients with myasthenia and healthy donors, respectively. MSRV env was found in a range of 6–22, 4–5, and 2–4 copies in MS patients, patients with myasthenia and healthy donors, respectively. Moreover, the number of spontaneous micronuclei was significantly greater in MS patients compared to control. In patients with MS diversity of chromosome aberrations was observed. In conclusion, evident difference in MSRV pol, gag and env copy number between MS patients and control suggests that MSRV may play some role in the etiology of multiple sclerosis (latent viral infection). The presence of chromosome aberrations and high amount of micronuclei in MS patients shows that the instability in MS genome often occurs.
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SI-P15
Extent of the oxidative damage to DNA (8-oxo-2dG) in multiple sclerosis

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There are some hypotheses that oxidative damage to DNA secondary to inflammation may contribute to irreversible alterations in MS plaques. To test this assumption, we have estimated the level of a DNA oxidative marker, the level of a purine oxidation product, the 8-oxo-2-deoxyguanosine (8-oxo-2dG) in lymphocytes of MS patients. Material and methods: Peripheral blood was collected from 28 patients with clinically definite MS aged from 19 to 46 years. The duration of MS was 4.2 ± 3.1 years. The mean of EDSS was 2.0 ± 0.8. In MRI study 14 cases were gadolinium positive and 14 negative. Thirty healthy volunteers make up the control group. DNA was isolated from peripheral blood lymphocytes and then hydrolyzed to nucleosides using P1 nuclease. In order to determine 8-oxo-2dG level, the nucleoside mixture was applied to the HPLC/UV system, coupled to an electrochemical detector. Results and discussion: The mean level of 8-oxo 2dG in MS patients was 19.6 ± 35.1 and, compared to that established for control subjects (12.3 ± 7.2), showed no statistically significant differences. The comparison of 8-oxo-2dG in subgroups of patients divided according to duration of the disease showed the higher number of cases with DNA damage in patients of the subgroup with shorter duration of the disease. The mean level of 8-oxo-2dG in gadolinium positive MS cases was 17.3 ± 13.1 and in gadolinium negative ones it was 9.6 ± 4.0. The difference was significant. Conclusions: (1) Oxidative damage to DNA is not a general feature in MS patients but it may frequently appear in the early period of the disease; (2) The higher level of oxidative marker of DNA damage in MS, noted in active period of the disease, testifies to the relationship between the studied variable and MS process.

SI-P16
Recombinant forms of myelin antigens expressed on CHO cells as a tool for identification of autoantibodies in serum of MS patients

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A contribution of B cells and autoantibodies has been demonstrated in MS leading to interest in the use of such autoantibodies as diagnostic or prognostic markers and as a basis for immunomodulatory therapy. ELISA and Western blotting fail to detect reactivity against epitopes displayed by native antigens expressed on myelin sheats. We describe a cell-based assay that specifically identifies serum antibodies directed against myelin autoantigens: MBP, PLP and MOG. The method detects antibody binding to recombinant antigens in their native conformation on MBP, PLP or MOG transfected mammalian (hamster ovary) cells. 36 patients with relapsing-remitting MS diagnosed according to criteria of Mc Donald were recruited. Age 38.2 and duration of the disease 7.1. Serum anti-MBP, anti-PLP and anti MOG IgG autoantibodies were detected in MS patients and 35 healthy donors by FACS analysis. Compared with healthy controls the titers of IgG autoantibodies directed against membrane-bound recombinant myelin antigens were most significantly increased for PLP, no quite significant for MBP and not significant for MOG. The titers of anti-MBP antibodies were low in contrast to high titers of anti-MOG antibodies in both groups suggesting a nonspecific binding. The cell-based assay detection of autoantibodies directed against recombinant myelin antigens could be a useful tool providing the serological markers in diagnosis and progression of MS. Indeed, it could allow to obtain molecular characteristics of disease in each patient in term of an antibody response against certain myelin and non-myelin antigens. We have shown

that in RRMS patients elevated level of serum antibodies against PLP is significant, what should be considered in search for specific immunomodulatory therapy in MS.

SI-P17

Antibodies against oxidized low density lipoproteins (LDL) and apolipoprotein E polymorphism in demented patients

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In serum of 114 patients with dementia and of 102 controls the titer of G class immunoglobulins directed against oxidized low density lipoproteins (LDL) was determined by ELISA method using OLAB kits. In isolated DNA apolipoprotein E (APOE) gene polymorphism was identified. In the group with dementia a tendency to lower antibodies levels in e4 allele carriers and to higher ones in the e2 group was observed. In some individuals very high levels of the antibodies were stated exceeding the 90 percentile of the investigated groups. The prevalence of very high anti-ox LDL antibodies level was significantly more frequent in the carriers of e2 allele and less frequent in the carriers of e4 allele. These results could suggest a role of apolipoprotein E polymorphism in the immune response against oxidized low density lipoproteins. This could play an additional role in the pathogenesis of dementia.

SI-P18

Polymorphism of glycogen synthase kinase 3 Beta and cyclin-dependent kinase 5 genes in early and late onset Alzheimer's disease

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Alzheimer's disease (AD) is the main cause of dementia in the elderly. Over-activation of Glycogen Synthase Kinase 3 beta and Cyclin-Dependent Kinase 5 has been implicated in the aberrant phosphorylation of tau – the major component of the neurofibrillary tangles, which besides deposits of amyloid β are pathological hallmarks of AD. In this study we assessed the association between single nucleotide polymorphism (SNP) in those kinases genes and the risk of early (EOAD) and late onset (LOAD) Alzheimer's disease. TaqMan SNP genotyping assay or polymerase chain reaction-restriction fragments length polymorphism (PCR-RFLP) assay were used to genotype 4 SNP sites in 198 Polish LOAD cases, 71 EOAD cases and 104 controls. The distribution of genotypes in rs334558 SNP in GSK3β gene significantly differed between patients with late onset AD and aged related, healthy control group. No significant association between rs9278, rs2069454 and rs2069442 SNPs in CDK5 gene and AD was found and none of the examined alleles can be considered now as a genetic risk factor in AD in Polish population. The analysis of environmental factors showed higher serum level of total cholesterol and lower LDL (low density lipoprotein) level in EOAD and LOAD groups compared to control group. Moreover lower level of vitamin B12 and higher homocysteine level were observed only in LOAD group compared to controls.

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SI-P19

The activity of serum paraoxonase and level of conjugated dienes in stroke patients

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Paraoxonase (PON) protects low-density lipoproteins from oxidation. Gln192Arg genotype of PON was identified as a risk factor for stroke. The aim of our study was to evaluate the activity of the esterase and concentration of conjugated dienes (CD) in the early phase of stroke and its clinical significance. Material and methods: The PON activity and conjugated dienes (CD) concentrations were analyzed within first 24 hours after stroke onset by means of spectrophotometric methods. Results: We included into the study 424 persons [403 ischemic stroke (IS) patients, aged 67 ± 12 and 21 patients with IS secondary hemorrhagic transformation (SHT), aged 69 ± 11 and 12 age-matched healthy controls]. The PON activity was lower in SHT patients (13.2; interquartile range 2.3–67.1 U/L) comparing to IS patients (63.7; 40.2–145.9 U/L) and controls (42.34; 38.98–57.44 U/L) ($P<0.05$). There was no significant difference in CD concentration between IS and SHT patients ($P>0.05$). Multiple regression analysis revealed correlations between CD concentration and Barthel index in IS and SHT, and between PON activity and Barthel index in HT patients. Conclusion The serum PON activity in early phase of stroke was significantly lower in SHT than in IS patients. PON activity showed correlation with clinical outcome in SHT patients whereas CD concentrations correlated with the outcome in both IS and SHT groups.

SI-P20

An association of the MTHFD1 c.1958G>A polymorphism in Polish women with epilepsy

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The genetic basis of epilepsy has been substantiated by numerous examples of familial forms of epileptic syndromes. Among these, autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) and idiopathic generalized epilepsy (IGE) can be mentioned. Most previous studies of epilepsy genetics have implicated ion channel genes. Other studies have noted an increased frequency of the c.677C>T MTHFR gene polymorphism in women with IGE. This study was designed to explore an association of three polymorphisms of the key genes encoding enzymes involved in folate and methionine metabolism with the epileptic disorders in Polish women. The study includes 15 female patients with ADNFLE, 75 female patients with IGE and 110 unrelated healthy women used as a control. In each group the allele and genotype frequencies of MTHFR c.677C>T, MTR c.2756A>G and MTHFD1 c.1958G>A polymorphisms were determined using PCR-RFLP analyzes. Our results supported the association between MTHFR c.677C>T polymorphism and IGE in women, since frequencies of TT homozygotes in IGE female patients were different from the controls as compared with CC homozygotes (P=0.033). However, no statistical differences in the allele frequency and in the proportion of TT and CT *versus* CC genotypes in these patients and the controls were observed. Otherwise, inclusion of ADNFLE female patients significantly altered genotype frequencies of MTR c.2756A>G polymorphism (GG vs. AA: OR=5.818; P=0.0157). However, statistical differences in allele frequencies of MTR c.2756A>G polymorphism were observed when both idiopathic epilepsies were analyzed with the control group (P=0.0153) as well as when IGE patients were compared separately with the healthy women (0.0388). Similar results were obtained for MTHFD1 c.1958G>A polymorphic transition, although, the differences in allele and genotype frequencies remained statistically significant for each group of the patients as compared to the controls. We found that the 1958A allele appeared with a significantly higher frequency in the IGE subgroup and in both idiopathic epilepsies than in the controls (P=0.0176 and P=0.011, respectively). Moreover, as compared with the 1958GG genotype, the AA and combined GA+AA genotypes were associated with a significantly increased risk of IGE (AA vs. GG: OR=2.647; P=0.0245 and GA+AA vs. GG: OR=2.218; P=0.0136) as well as IGE and ADNFLE considered together (AA vs. GG: OR=2.625; P=0.0197 and GA+AA vs. GG: OR=2.354; P=0.0068). An association between the c.1958G>A polymorphism of the MTHFD1 and IGE was evidenced suggesting a significant role of the methyl cycle in the women with idiopathic epilepsies.

SI-P21

Serum VEGF levels in migraine patients

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Introduction: Migraine is a known risk factor for ischemic stroke. Recent studies have shown endothelial dysfunction in migraine patients. The aim of this study was to examine the levels of circulating Vascular Endothelial Growth Factor (VEGF) during interictal period. Material and methods: We included in the study 52 migraineurs (aged 37.9 ± 9.6 years). All patients satisfied criteria of IHS for the diagnosis of migraine. The control group was represented by 34 healthy subjects (aged 28.9 ±7.0 years). Serum VEGF, Macrophage Inflammatory Protein-1 (MIP-1) and tumor necrosis factor (TNF) were analyze by means of ELISA. Results: The level of circulating VEGF was decreased (<0.05) during interictal period of migraine patients (281.53 ± 180.37 pg/ml) comparing to controls (441.80 ± 295.93 pg/ml). There were no differences (P>0.05) between TNF concentration in migraineurs and controls (median, min.–max.: 2.04; 0.49–18.6 and 2.14; 0.49–8.97, respectively). Similarly, serum MIP-1 did not showed differences (P>0.05) between both groups (migraineurs: 0.0; 0.0–285.25 and controls: 0.0; 0.0–333.54 pg/ml). Serum VEGF correlated with MIP-1 (rS=0.7684, P<0.05) and TNF level (rS=0.4791, P<0.05). Conclusion: Migraineurs have decreased serum VEGF concentration, which may be related endothelial dysfunction and responsible for increased risk of stroke. Both TNF and MIP may be involved in regulatory processes of VEGF production.

Session II

EXPERIMENTAL APPROACHES IN ACUTE AND PROGRESSIVE CNS INJURY

SII-L1

Microglia cells protect neurons by direct engulfment of invading neutrophil granulocytes – a new mechanism of CNS immune privilege

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The central nervous system is an immune-privileged site, where immune responses are suppressed. Mechanisms described so far include the blood-brain barrier as well as, absence of co-stimulatory molecules and the expression of ligands inducing immune cell apoptosis in CNS tissue. On the other site, during ischemic tissue-damage such

as stroke peripheral immune cells acutely infiltrate the brain and may exacerbate neurodegeneration. In this study we investigate the impact of polymorphonuclear neutrophil granulocytes (PMN) on the viability of neurons in a situation of ischemic stress, where the blood-brain barrier loses its integrity. In this situation the major early cellular infiltrate of ischemic lesions are PMNs. Here we demonstrate, that PMN as such are not toxic for neurons. However, under conditions of transient ischemia they potently enhance neuronal death. This, however, is efficiently counteracted against by microglia, the resident phagocytic immune cells of the brain. By time-lapse imaging we demonstrate that this protection is mediated by direct phagocytosis of PMNs by microglia cells. Phagocytosis is not limited to apoptotic or dead PMNs but equally frequent and efficient with fully viable and highly motile PMNs. In order to obtain phagocytosis microglial cells express highly motile cellular protrusions and even chasing behaviour, both, in intact hippocampal slices as well as in disseminated cell cultures. This phagocytosis is mediated by lektin- and integrin-based interaction. Importantly, specifically interfering with this function of microglia totally inhibits their neuroprotective function. While phagocytosis of apoptotic cells by macrophage-like cells is a common process, to the best of our knowledge the phagocytosis of live pro-inflammatory immune cells by other immune cells of the body has never been observed before. Our demonstration that this can mediate a strong neuroprotective function suggests that this represents a previously not recognized pathway of the CNS immune privilege.

SII-L2
Signal transduction underlying stroke-induced gliosis and inflammation as therapeutic target

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Excessive, uncontrolled inflammation and gliosis contribute to a majority of neurologic disorders despite of their different etiology. Activated microglial cells release pro-inflammatory cytokines, inflammation mediators, matrix proteinases and toxic factors. Reactive astrogliosis involves astrocyte proliferation, activation and hypertrophy accompanied by production of cytokines, growth factors and metabolic alterations. Inflammatory signalling involves activation of transcription factors NFκB and MAP kinases as critical signal transducers. We demonstrate activation of JAK/STAT (signal transducers and activators of transcription) signaling pathway associated with inflammatory microglia and "reactive astrogliosis" *in vitro* when primary rat astrocyte cultures were stimulated with the pro-inflammatory cytokines (IL1-β, IFN-γ and TNF-α). Global gene expression profiling revealed a coordinated and strong upregulation of inflammatory and immune response genes in inflammatory microglia *in vitro* and after transient focal ischemia. Moreover, we found a large representation of STAT responsive genes in both conditions suggesting a strong contribution of STAT pathway to stroke-induced inflammation. We studied whether inhibition of signal transduction mediated by MAP kinases and JAK/STAT pathway interferes with expression/release of inflammatory cytokines, mediators, matrix proteinases and toxic factors by activated glial cells. Our findings establish immunosuppressants as effective therapeutic candidate for use in the treatment of human neurologic disorders. Identification of STAT dependent events underlying inflammation and development specific inhibitors may facilitate development of innovative strategies blocking hostile microglial responses to control inflammation.

SII-L3
Proteins of GSK3β pathways as targets for AD drugs

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GSK3β is suggested to be a key enzyme for the development of AD. This kinase phosphorylates β-catenin and then labels it for subsequent ubiquitylation and degradation in proteasome 26S. It also indirectly affects stability of β-catenin by presenilin phosphorylation. Inhibition of the GSK3β is known to stabilize β-catenin and induce its translocation to nuclei. Our data show that cytoplasmic/nuclear β-catenin is specifically expressed in thalamic neurons in the adult mouse forebrain (1,2). We have performed an *in silico* screening for β-catenin neuronal targets and identified 432 putative Lef1/β-catenin genes. Among them there are those encoding 23 proteins involved in cell adhesion, 12 voltage-gated ion channels, 10 proteins involved in synaptic vesicle organization and transport, and 13 neurotransmitter receptors. Using low density custom arrays with putative genes we established potential β-catenin targets in post-mitotic neurons. We plan to find out which gene promoters are sensitive to β-catenin in neurons and to check, which drugs that inhibit GSK3β, affect the expression of β-catenin dependent gene targets. This might identify the downstream target(s) of the GSK3β pathways in neurons as potential sites for novel AD drugs with higher specificity than the inhibitors of GSK3β.
1. Misztal et al. 2007, Acta Neurobiol Exp 67(3): 293, abstract; 2. Wisniewska et al. 2007, Acta Neurobiol Exp 2007, 67(3): 306.

SII-L4
Matrix metalloproteinase-9 – a culprit in epilepsy?

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Temporal lobe epilepsy (TLE) is a chronic devastating disease in which aberrant synaptic plasticity plays a major role. Recently, MMP-9, a matrix metalloproteinase, has been implicated synaptic

plasticity, long-term potentiation and learning and memory formation. Therefore, MMP-9 might play a pathogenic role in epileptogenesis. Indeed, the recent study revealed MMP-9 as a novel synaptic enzyme, and a key pathogenic factor in two distinct animal models of TLE: kainate-evoked-epilepsy and pentylenetetrazole (PTZ) kindling-induced epilepsy. In particular, sensitivity to PTZ-induced epileptogenesis is decreased in MMP-9 knockout (KO) mice, whereas it is increased in MMP-9-overexpressing rats. Moreover, confocal- and immunoelectron-microscopic analyses demonstrated that MMP-9 associates with hippocampal dendritic spines bearing asymmetric (excitatory) synapses. In addition, both MMP-9 protein levels as well as its enzymatic activity become strongly increased upon seizures. Furthermore, MMP-9-deficiency diminishes seizure-evoked pruning of dendritic spines and, most importantly (with regards to epileptogenesis), it decreases aberrant synapse formation following mossy-fibers sprouting. Taken together, the aforementioned results suggest that the synaptic pool of MMP-9 is critically involved in the sequence of events that underlie epileptogenesis in two commonly used models of TLE.

SII-O1

Comparison of developmental composition pattern of perineuronal nets in the visual and the barrel cortex

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The barrel and visual cortices are primary model systems to study cortical plasticity. The ability to undergo experience dependent plastic changes depends on brain region, cortical layers, pattern of activity and is altered during development. For instance in mice, a critical period for barrels formation occurs during the first postnatal days whereas in the visual cortex a critical period for ocular dominance plasticity ends about one month after birth. It has been suggested that formation of perineuronal nets (PNNs) during postnatal development may limit cortical plasticity. The aim of this study was to compare developmental patterns of distribution and composition of PNNs in the visual and somatosensory cortex of mice. For this purpose we used monoclonal antibody against chondroitin sulfate proteoglycans to visualized aggrecan (a major component of PNNs) and Wisteria floribunda agglutinin (WFA), which selectively recognizes N-acetylo-galactosamine residues within PNNs. At all time points examined in both cortices the highest density (cells/mm^2) of WFA stained cells occurred in the layer IV. In the barrel cortex, WFA staining first appeared at P10 whereas in the visual cortex WFA stained cells were seen not before P20. In both cortices, the adult like pattern was evident by P30. A composition of PNNs differed between the visual and barrel cortex. Number of aggrecan expressing cells was significantly higher in the visual cortex than in the barrel cortex. Moreover, in the visual cortex aggrecan expressing cells were seen earlier during development than WFA staining. These data seemed to support a view that PNNs are involved in determination of specific critical period.

SII-O2

Relationships between acetyl-CoA level and function of fenotypically modified N9 murine microglial cells

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Neurodegenerative lesions in cholinergic encephalopathies include an excessive activation of microglia, which may aggravate this process. Inflamatory cytokines were reported to affect viability of cultured cholinergic cells, through the influence on their acetyl-CoA metabolism. The aim of this work was to investigate whether phenotypic modifications of N9 microglia by common neuron-differentiating stimuli can alter its energy metabolism, viability and biological activity in neurodegenerative conditions. In basal conditions lipopolysaccharide (LPS) slightly decreased PDH activity and acetyl-CoA content and activated NO synthesis (600%). The cyclic AMP alone (0.25 mM), caused 15% increase of nonviable cells at 300% rise in NO synthesis and 50 and 30% decreases in acetyl-CoA and ATP contents, respectively. In such conditions LPS resulted in further increase of NO accumulation and aggravated loss of cell viability and their acetyl-CoA content. In basic conditions retinoic acid (RA) alone did not alter viability and NO synthesizing capacity but increased acetyl-CoA and blunted cytotoxic potencies of cAMP and LPS to N9. RA-evoked restoration of N9 cell viability was accompanied by the increase in their acetyl-CoA content. These data indicate that activation of microglia depletes their acetyl-CoA, making them more vulnerable to cytotoxic insults. On the contrary, rescue of microglia by RA-signaling pathways is connected with restoration their acetyl-CoA pool.
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SII-P1

Aβ mediated diminution of MTT reduction — An artefact of single cell culture?

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The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide (MTT) reduction assay is a frequently used and easily repro-

ducible method to measure beta-amyloid (Aβ) toxicity in different types of single cell culture. To our knowledge, the influence of Aβ on MTT reduction has never been tested in more complex tissue. Initially, we reproduced the disturbed MTT reduction in neuron and astroglia primary cell cultures from rats as well as in the BV2 microglia cell line, utilizing four different Aβ species, namely freshly dissolved Aβ (25-35), fibrillar Aβ (1-40), oligomeric Aβ (1-42) and oligomeric Aβ (1-40). In contrast to the findings in single cell cultures, none of these Aβ species altered MTT reduction in rat organotypic hippocampal slice cultures (OHC). Moreover, application of Aβ to acutely isolated hippocampal slices from adult rats and *in vivo* intracerebroventricular injection of Aβ also did not influence the MTT reduction in the respective tissue. Failure of Aβ penetration into the tissue cannot explain the differences between single cells and the more complex brain tissue. Thus electrophysiological investigations disclosed an impairment of long-term potentiation (LTP) in the CA1 region of hippocampal slices from rat by application of oligomeric Aβ (1-40), but not by freshly dissolved Aβ (25-35) or fibrillar Aβ (1-40). In conclusion, the experiments revealed a glaring discrepancy between single cell cultures and complex brain tissue regarding the effect of different Aβ species on MTT reduction. Particularly, the differential effect of oligomeric versus other Aβ forms on LTP was not reflected in the MTT reduction assay. This may indicate that the Aβ oligomer effect on synaptic function reflected by LTP impairment precedes changes in formazane formation rate or that cells embedded in a more natural environment in the tissue are less susceptible to damage by Aβ, raising cautions against the consideration of single cell MTT reduction activity as a reliable assay in Alzheimer's drug discovery studies.

SII-P2

Delayed activation of metalloproteinase 9 after photothrombotic stroke in aged mice

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Age-related brain injuries, including ischemia, can lead to neurological disabilities, functional decline of the brain and poor recovery. Metalloproteinase-2 (MMP-2) and metalloproteinase-9 (MMP-9) belonging to a large family of zinc-dependent proteinases, play an important role in development and neuronal plasticity, cell migration, angiogenesis and wound healing. After stroke, activation of MMPs is strongly up-regulated. The aim of this study was to compare the time course of activation of MMP- 9 and MMP-2 in the cerebral cortex of young (3–4 months old) and aged (12–13 months old) mice following focal photothrombotic stroke. To reveal metalloproteinases MMP-9 and MMP-2 activity *in situ* zymography and SDS-PAGE zymography were used. In young mice, pronounced induction of MMP-9 activity was found one day after stroke and remained at high level 7 and 14 days after infarct. In aged mice, however, MMP-9 activity could be seen not earlier than 14 days post-stroke. Photothrombotic stroke did not affect MMP-2 activity, which remained at stable, constitutive level during whole examined post stroke time period. In both young and aged mice, MMP-2 activity could be detected in sham operated animals, while constitutive MMP-9 activity was not observed. We speculate that the delayed activation of MMP-9 in aged mice may be one of the reasons of lesser ability to recover by old patients.

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SII-P3

Inhibitors of poly(ADP-ribose)polymerase protect hippocampal neurons against genotoxic stress *via* PI-3K/AKT regulated pathway and by suppression of AIF translocation

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Poly(ADP-ribose)polymerase (PARP-1) plays a key role in DNA repair but its over activation has been proposed to be important in pathogenesis of brain ischemia and in neurodegenerative diseases. PARP catalyzes the conversion of bNAD+ to polymers of poly(ADP-ribose) (PAR) and is fully responsible for producing of PAR polymers during genotoxic stress. The last data indicated that PAR act at the mitochondria to induce cell death through stimulation of apoptosis inducing factor (AIF) release. However, the role of PAR in cell death seems to be complex and not fully elucidated. To better understand the role and relationship between AIF and PARP/PAR in death signaling the hippocampal neuronal (HT22) cells in culture were subjected to different concentration of DNA alkylating agent, 1-methyl-3-nitro-1-nitrosoguanidine (MNNG). The immunochemical and spectrophotometrical methods were applied. Consequently, HT22 cells treated with MNNG at 50–500 mM demonstrated concentration dependent mitochondria failure and death. 24 h after 500 mM MNNG treatment only 15% of cells survive. PARP-1 inhibitors: 3-aminobenzamide (3AB) and PJ34 at 5 mM and 20 mM, respectively, protect most of the cells against MNNG induced death signaling. At lethal MNNG concentration PARP/PAR dependent AIF translocation from mitochondria is observed and the caspase independent death signaling is activated. Concomitantly PARP inhibitors affect the endogenous pathway regulated by PI-3K/AKT PKB/GSK-3 and influence the level of GSK-3β active form phosphorylated on Tyrosine 216. Summarizing our data indicated that inhibitors of PARP have positive effect on neuroprotective pathway regulated by PI-3K/AKT and on mitochondria function.

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SII-P4

Key role of acetyl-CoA in life and death of brain cholinergic neurons

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Preferential loss of brain cholinergic neurons in course of Alzheimer's disease (AD) and other encephalopathies might result from the fact that they utilize acetyl-CoA, not only for energy and N-acetylaspartate production but also for acetylcholine (ACh) synthesis. Therefore, acetyl-CoA metabolism might be a likely target for both cytotoxic signals and therapeutic procedures. The shortage of acetyl-CoA in cholinergic cell mitochondria caused their high susceptibility to amyloid-beta, NO, Al and Zn. They caused dose-dependent increase of nonviable cell fraction and cytoplasmic cytochrome c levels, decreases in mitochondrial enzyme and ChAT activities, intramitochondrial and cytoplasmic acetyl-CoA and ACh levels, with loss of morphologic differentiation. The expression of cholinergic phenotype positively correlated with compound-evoked alterations in cytoplasmic acetyl-CoA levels (r=0.90, P=0.002). On the other hand, cytoprotective properties correlated with their ability to maintain high level of acetyl-CoA in mitochondria. Accordingly nonviable cell fraction inversely correlated with pyruvate dehydrogenase activity (r=−0.79, P=0.002) and content of mitochondrial acetyl-CoA (r=−0.92, P=0.0002). These data indicate the existence in cholinergic neurons two independent pools of cytoplasmic and mitochondrial acetyl-CoA, that under pathologic conditions affect expression of cholinergic phenotype and their viability, respectively.
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SII-P5

Accumulation of zinc in cholinergic SN56 neuroblastoma cells in acute and chronic neurotoxicity paradigms

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Excessive activation of glutamatergic neurons in course of different encephalopathies is accompanied by marked increase of zinc concentration in the synaptic cleft. This cation is co-released with glutamate and subsequently taken up by cholinergic and other postsynaptic elements through ZIP transporters, NMDA and other voltage dependent Ca-channels. On the other hand, Zn distribution and clearance from cellular compartments is executed by multiple Zn-transporters (ZnT). The aim of this work was to investigate how variable levels of Zn in extracellular space affect its accumulation in cholinergic cells and their functions. Acute, 30 min exposure of differentiated and nondifferentiated SN56 cells to increasing concentrations of Zn yielded concave up, non saturable, super imposable accumulation plots. At 0.15 mM extracellular concentration, intracellular accumulation of Zn was about 60 nmol/mg protein. On the other hand, after 24 h cell culture with same Zn concentration its intracellular level was found to be equal to 6 nmol/mg protein, only. Atypical shape of concentration-dependent plots of Zn accumulation might be explained by the coexistence in cholinergic cell plasma membranes low density, high-affinity and high density low affinity Zn-transporting sites. On the other hand, time-dependent decrease of Zn accumulation might result from an adaptative increase of density of one of ZnT proteins, presumably ZnT4, thereby protecting cells against Zn overload.
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SII-P6

Acute cytotoxicity of zinc on SN56 cholinergic cells

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Zinc is a trace element essential for living organisms. However, its excess in the aging human brain is claimed to contribute to patomechanisms Alzheimer's disease. The aim of this work was to find out whether acute effects of Zn on neurons may be caused by alterations in their acetyl-CoA metabolism. Zn quickly accumulated in cholinergic SN56 cells in concentration-dependent fashion. In cell homogenates Zn caused, inhibition of pyruvate dehydrogenase (PDH), aconitase, isocitrate dehydrogenase and ketoglutarate dehydrogenase (KDH) activities, with Ki values equal to 0.058, 0.010, 0.005 and 0.0015 mM, respectively. For choline acetyltransferase [IC 0.5] for Zn was above 0.3 mM. No inhibition of succinate dehydrogenase activity was found. It also decreased cytoplasmic acetyl-CoA and ACh levels ([IC 0.5] 0.15 mM), and inhibited ACh release ([IC 0.5] 0.10 mM). Lipoamide (LA) or EDTA, added before or simultaneously with Zn prevented these inhibitions. When LA or EDTA were added 10 min after Zn, they did not reverse aconitase inhibition, partially restored KDH activity and totally reversed inhibition of PDH. Activities of PDH and KDH but not aconitase suppressed by 24 hour cell culture with Zn, were also restored by post culture additions of LA and EDTA to harvested cell homogenates. It indicates that, Zn could exert its acute effects on cholinergic cells through inhibitory-binding to crucial enzymes of energy metabolism, yielding acute depletion of cytoplasmic acetyl-CoA and suppression of cholinergic transmitter functions.
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SII-P7

A 2DG mapping of functional activity in the mouse barrel after local removal of synaptic zinc *in vivo*

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Synaptic zinc is co-released with glutamate from a subpopulation of glutamatergic terminals in the neocortex and modulates neurotransmission. Using histochemical detection we showed that the level of synaptic zinc was regulated by sensory experience in the barrel cortex of mice and this suggested involvement of synaptic zinc in cortical plasticity. We sought to establish a new experimental approach to directly investigate the role of synaptic zinc in neuronal plasticity by its local removal (chelation) *in vivo* in the barrel cortex. The aim of present study was also to investigate whether long-lasting chelation has any effect on mapping of functional changes by 2-deoxyglucose (2DG) uptake. We used highly specific zinc chelator, tetrakis-(2-pyridylmethyl)-ethylenediamine (TPEN; 5 mM). We examined different implants stereotaxically placed directly on the barrel cortex or on the dura. No sufficient chelation was observed when Elvax polimer was used to release TPEN. Implantation of spongostan saturated with TPEN solution resulted in a complete loss of zinc staining in the entire cortical depth under the implant for more than 24 h but not more than 48 h. After unilateral spongostan implantation, TPEN was added daily during 3 days to maintain the continuous chelation. Then, the 2DG study was performed with two-sided sensory stimulation of selected vibrissae. The 2DG uptake within cortical representation of stimulated vibrissae was increased under the implant in comparison to control side. However the width of labeled region remained unchanged in all cortical layers, and therefore this method is useful to study the role of zinc in the cortex.

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SII-P8

Interaction of aryl hydrocarbon receptor-mediated apoptosis of neuronal cells with estrogen receptor signaling

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Aryl hydrocarbon receptor (AhR) may be responsible for dioxin intoxication, which creates severe clinical problems, such as behavioural and cognitive impairments and an increased number of newborns with deformed brains. Thus, activation of AhRs induces neuronal damage, but the mechanism by which this occurs is largely unknown. Because beta-naphthoflavone is an AhR agonist, we evaluated its impact on apoptotic processes in the mouse primary neuronal cell cultures. In order to verify whether AhR-mediated activation of caspase-3 and lactate dehydrogenase (LDH) release were tissue- and age-dependent, we related them to neocortical and hippocampal tissues, both on 1 and 7 days *in vitro*. In addition to the effects of estrogen receptor (ER) antagonists and selective estrogen receptor modulators (SERMs), the interaction between AhR-induced apoptosis and ER signaling was evaluated by determining the levels and cellular distribution of AhR and ERbeta. beta-naphtoflavone (0.1–100 mM) enhanced caspase-3 activity and LDH release in neocortical and hippocampal cells. A high-affinity ER antagonist, ICI 182,780, and SERM, tamoxifen, enhanced beta-naphtoflavone-mediated apoptosis. Another SERM, raloxifene, and an ERalpha antagonist, methyl-piperidino-pyrazole, did not affect beta-naphtoflavone-induced caspase-3 activity. However, they inhibited beta-naphtoflavone-induced LDH release at late hour post-treatment, thus suggesting delayed control of AhR-mediated neuronal cell death. The apoptotic effects of beta-naphtoflavone were accompanied by increased levels of AhRs, and these receptors colocalized with ERbeta as demonstrated by confocal microscopy. These data provide evidence for direct interaction of the AhR-mediated apoptotic pathway with estrogen receptor signaling, which gives insight into new strategies to treat or prevent AhR-mediated neurotoxicity.

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SII-P9

FK506 prevents pro-inflammatory and cytotoxic activation of cytokine-stimulated rat astrocytes

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Reactive astrogliosis is implicated in many acute and chronic neuropathological conditions and involves astrocyte proliferation, activation and hypertrophy accompanied by production of cytokines, growth factors and metabolic alterations. Astrocyte activation may exert both beneficial and detrimental effects on nervous system cells, therefore its modulation is an attractive target for neuroprotective therapies. We have demonstrated that a widely used immunosuppressant and calcineurin inhibitior FK506 potently reduced gliosis *in vivo* and improved recovery in a rat stroke model (Zawadzka and Kaminska 2004, Glia 49: 36–51). To dissect the mechanism of FK506 action on activated

astrocytes, we employed a model of "reactive astrogliosis *in vitro*" based on primary rat astrocyte cultures stimulated with the mixture of pro-inflammatory cytokines: IL1-beta, IFN-gamma and TNF-alpha. Cytokine cocktail induced activation of NFkappaB, p38 MAPK and JNK signaling pathways followed by cellular hypertrophy, rearrangement of astrocyte cytoskeleton, nitric oxide production and expression of mRNA for IL-6 and trail. FK506, as well as another calcineurin inhibitor cyclosporin A, reduced the astrocyte hypertrophy. FK506 decreased the level of activated p38 MAPK, as well as down-regulated trail mRNA. Interestingly, FK506 was also able to reduce the activation of p38 MAPK in astrocytes exposed to hydrogen peroxide implicating potential of this drug in counteracting some effects of oxidative stress observed during ischemic reperfusion or neuroinflammation. Our data suggest that FK506 may exert its neuroprotective effect partially *via* inhibition of the pro-inflammatory astroglia activation and implicate a calcineurin as a new candidate for triggering of astrogliosis.

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SII-P10

A novel voltage-gated potassium channel in hippocampal mitochondria

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Transient cerebral ischemia is known to induce endogenous adaptive mechanisms such as the activation of mitochondrial ATP-sensitive potassium channels or calcium-sensitive large-conductance potassium channels that can prevent or delay neuronal injury. In this study a single channel activity was measured after patch-clamp of the mitoplasts isolated from control gerbil hippocampus. In 70% of the all patches, a potassium selective current was recorded with mean conductance 109 ± 6 pS in symmetrical 150 mM KCl solution. Patch-clamp single channel studies protein showed properties of the voltage-gated potassium channel (Kv channel): it was blocked by negative voltage and margatoxin (MgTx) a specific Kv1.3 inhibitor. The inhibition by MgTx was no reversed. The channel was not affected by the other Kv1.3 channel blocker agitoxin-2 at nanomolar range. Additionally, we showed that ATP/Mg^{2+} complex and low or high concentration of Ca^{2+} ions have no effects on observed activity of ion channel. Mitochondrial localization of Kv1.3 was verified by western blots using antibodies recognizing peptides located in N- or C-terminal of plasma membrane Kv1.3 protein. N-terminal specific antibodies recognized proteins with molecular masses around 95, 62 and 40 kDa in plasma membrane and 60 kDa in mitochondria while C-terminal specific antibodies recognized 40 kDa protein in mitochondria. Mitochondrial localization of Kv1.3 protein was also shown by immunohistochemistry. We conclude that gerbil hippocampus mitochondria contain voltage-gated potassium channel (mitoKv) with properties similar to the surface membrane Kv1.3 channel which can play an important regulatory role for protection with respect of ischemia-reperfusion phenomena.

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SII-P11

Ammonia alters the expression of the inwardly rectifying potassium channel kir 4.1 *in vivo* and *in vitro*

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Ammonia is a major pathogenic factor in hepatic encephalopathy (HE). Cerebral edema resulting from astrocytic swelling is a major complication of HE. This study addresses a hypothesis linking brain edema to the dysfunction of inwardly rectifying potassium channels – the major route of potassium clearance by astrocytes. We measured the effect of thioacetamide (TAA)-induced HE and *in vitro* treatment of cultured astrocytes with ammonia, on the expression of Kir 4.1, the most common of the channels. Three TAA administrations (250 mg/kg, ip) at 24 h intervals induce liver failure associated with edematous changes in the cerebral cortex. Real-time PCR and Western-blot analysis revealed a markedly decreased expression of Kir4.1 mRNA and protein, respectively, in the cerebral cortex of the TAA-treated rats. Treatment of cerebral cortical astrocytes with 5mM ammonium chloride for 72 h, which induced astrocytic swelling as measured with the [3H]OMG (Kletzien method for cell volume measurement), likewise decreased Kir4.1 expression at the mRNA and protein level. However, a considerable variation has been observed with different preparations used, and the degree of correlation between cell swelling and Kir4.1 expression remains to be established. Treatment of cultured astrocytes with 5 mM glutamine for 72 h reproduced the effects of ammonia on Kir4.1 mRNA expression, adding credence to the current view that the cell swelling-inducing effect of ammonia is mediated by glutamine (Albrecht and Norenberg 2006, Hepatology).

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SII-P12

The role of reverse mode of Na^+/Ca^{2+} exchanger in vascular response to acute hyponatremia

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In a previous study we have found that dilation of the middle cerebral artery (MCA) of the rat during acute hyponatremia depends on endothelium and NO/cGMP signalling. The production of NO in the endothelium is stimulated by the increase of intracellular concentration of Ca^{2+} which can occur either as a result of the release of calcium ions from intracellular stores or their influx from the extracellular space. The latter requires opening of specific Ca^{2+} channels of the cell membrane and/or operation of Na^+/Ca^{2+} exchanger (NCX) in the reverse mode. In the present study we sought the participation of the reverse mode of NCX in the response of MCA to acute hyponatremia. Experiments were performed on MCAs harvested from the brains of male Wistar rats and mounted in the organ chamber under the inverted microscope equipped with video camera and monitor. The diameter of the vessel was measured directly from the screen and recorded. The vessel was perfused at a transmural pressure of 80 mm Hg, temperature of 37°C and pH 7.4. Hyponatremia was induced by decreasing the intra- and extraluminal concentration of Na^+ from 144 to 120 mM. In some experiments hyponatremia was preceded by the administration to the chamber of the inhibitor of the reverse mode of NCX (KB-R7943, 10 microM) or the inhibitor of L-type Ca^{2+} channel (Nimodipine, 0.1 microM). In the last experiment endothelium was removed before administration of KB-R7943 (10 microM) and lowering of Na^+. Hyponatremia resulted in the dilation of the MCA by 17% ($P<0.05$). This response was abolished by the pretreatment with KB-R7943 but not with Nimodipine. The results show that small decrease in the concentration of extracellular Na^+ may stimulate reverse mode of NCX in the endothelium but not in the smooth muscle cells.

SII-P13

The role of astroglia and purinergic signaling in the early stages of EAE

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In the CNS an intensive communication between neurons and glial cells occurs. Activation of astrocytes is observed under different pathological conditions, including multiple sclerosis, which results in overexpression of number of proteins, like GFAP and S-100β, which is involved in development of inflammatory reaction. There is a growing number of evidence that different brain pathologies are characterized by very early active contribution of astrocytes to neurodegenerative axonal damage. Our study presents time-dependent analysis of astroglia-specific protein expression in different phases of EAE (from 4 to 25 days after immunization). The biphasic response of astroglia was observed – upregulation of both proteins, GFAP and S-100β, in the early stages of the disease and in the peak of the neurological deficits in animals (10 dpi). Astrocytes build a network within the CNS and are connected by gap junctions, formed by connexins, mostly Cx43 which was shown to induce ATP release *via* hemichannels. In the cross-talk between astrocytes and neurons may be involved purinergic receptor P2X7, ATP-gated ion channel activated in pathological conditions and participating in regulation of inflammatory response. In the EAE rats, in the early stages of the disease, we observed the enhanced level of Cx43 protein and P2X7R protein which was accompanied by changes in mRNA profile. We conclude that early activation of astroglia in the inductive phase of EAE occurs which is connected with the overexpression of purinergic receptor P2X7. The results suggest that in MS/EAE pathology activation of astroglia in the preclinical stage, may contribute to the axonal damage and subsequent inflammation, and that the purinergic signaling may play a role in both these phenomena.

SII-P14

Effects of hypocretin 1 on cyclic AMP formation in primary neuronal cell cultures from rat cerebral cortex

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Hypocretin 1 and hypocretin 2 (Hcrt; also called orexin-A and orexin-B, respectively) are two newly discovered neuropeptides synthesized in the hypothalamus. Besides playing a role in the control of arousal and wake-sleep cycle, both hypocretins have been reported to exert diverse physiological actions, including central regulation of feeding and energy homeostasis, regulation of cardiovascular and autonomic functions, and of the neuroendocrine system. The physiological effects of hypocretins are mediated *via* two G-protein coupled receptors, Hcrtr-1 and Hcrtr-2. Hcrtr-2 receptor has equal affinity for both Hcrt1 and Hcrt2, whist Hcrtr-2 receptor has ten-fold greater affinity for Hcrt1 than for Hcrt2. Despite of several studies, relatively little is known about hypocretin receptors signaling in different tissues. Here we show effects of Hcrt1 on cyclic AMP formation in primary neuronal cultures from rat cerebral cortex. Hcrt1 used at 0.01–1 μM concentrations did not exert significant actions on the basal cyclic AMP formation in primary cultures from rat cerebral cortex. Hctr1 inhibited, in a concentration-dependent manner, increases in cyclic AMP accumulation evoked by the incubation of neuronal cultures with forskolin (a direct activator of adenylyl cyclase; 1 and 3 μM), pituitary adenylate cyclase-activating polypeptide (PACAP27; 0.1 micromolar) or with vasoactive intestinal peptide (VIP; 3 μM). The obtained results indicate that Hcrtr in rat cortical neurons couples through Gi subclass

of G-proteins, and suggest the presence of a neuron-linked functional interaction between hypocretins and neuropeptides PACAP/VIP.
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SII-P15
The interpretation of oligoclonal bands based on artificial neuronal networks

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The examination of oligoclonal bands (OCB) in cerebrospinal fluid (CSF) is important for the diagnosis of multiple sclerosis, however the correlation between quality, number and disease progression is uncertain. The aim of this study was to test the automatic system, for detection and identification of OCB. The patterns of OCB, obtained from isoelectrofocusing of CSF proteins, were scanned with the use of flatbed scanner. Next, for each of the scanned images, the selected features of the pattern were extracted by the means of the image processing algorithms, and arranged into the feature vector. That created the "signature" of the image that was subsequently analyzed by classifier based on the Artificial Neural Networks'. The result was the positive (P), negative (N) or "uncertain" (U) classification of the bands' pattern. We have used database of the 225 samples, manually classified by the expert, that formed the training set for the classifier with the equal number of positive and negative results. Among 20 samples (10P/10N) used in the testing phase of the system 17 were classified correctly, 3 were "uncertain", no false results was obtained. The system is implemented in the MATLAB environment. The future work focus on designing the system that would be able not only to classify the OCB patterns, but also to possibly cluster the images into the groups with common parameters.

SII-P16
Open-chain half bastadins mimic the effects of cyclic bastadins on calcium homeostasis in cultured neurons

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Controlled fluctuations in intracellular calcium concentrations play an important role in many cellular processes such as muscle contraction, secretion, metabolism, and neuronal function. Ryanodine receptors (RyR) are intracellular ion channels that mediate the release of calcium ions from internal stores and have been suggested as pharmacological targets for heart disease and neurodegenerative diseases. Bastadins are an ever-growing family of marine natural products isolated from sponges. Seemingly subtle differences in their substitution pattern suffice to alter the response observed. To characterize bastadin-induced changes in calcium homeostasis we measured, using FLUO-3, a calcium-sensitive fluorescent probe, the ability of bastadin 5 (positive control) and the acyclic analogues 2a, b and 4 to increase the intracellular calcium concentration in cultured rat cerebellar granule cells. We monitored also the influx of extracellular calcium to neurons employing 45Ca isotope. The observed effect of the acyclic analogues on calcium accumulation decreased in order: 4>2b>2a. Addition of ryanodine to medium before application of bastadin abolished analyzed effects. This observation indicates that RyR are the primary targets for their pharmacological activity. Thus, the influx of extracellular calcium to bastadin-treated neurons appears to be a secondary effect resulting from the release of calcium from the ryanodine-sensitive pool. Constraining the catechol aryl ether moiety of bastadins by incorporation into a macrocyle is not necessary in order to mimic the effects of these marine natural products on neuronal calcium homeostasis. Simple, acyclic analogues that embody the "western" or "eastern" parts of bastadins were found to evoke comparable responses with bastadin 5.

SII-P17
Alzheimer's mechanisms in ischemic delayed neuronal death in hippocampus

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Ischemia is well known for its ability to influence the activity of the blood-brain barrier (BBB). We investigated BBB changes by examining the leakage of horseradish peroxidase (HRP) and amyloid precursor protein (APP) from the circulation into ischemic hippocampus. Using Wistar rats BBB and APP changes were studied by light microscope following 10 min brain ischemia with 6 months survival. As controls sham-operated animals were sacrificed in due time. Rats were perfusion fixed for these investigations. HRP was introduced i.v. and circulated for 30 min as an indicator of BBB changes. Five brains were cut at 60 µm slices in the frontal plane by a vibratome for HRP staining. Paraf-

fin sections from other 5 brains were selected for APP staining and structural observations. Control brains went through the same procedures as ischemic. The areas of BBB damage were associated with increased expression of HRP and C-terminal of APP/β-amyloid peptide in perivascular space suggesting, respectively, an additional response to ischemia and neuronal death. These results suggest that the events associated with delayed neuronal death in hippocampus change pathologically BBB function. Additionally these data suggest that the leakage of cytotoxic APP parts in the CA1 and other sectors of hippocampus may play a role in the development of creepy delayed neuronal death after the ischemia. These findings also suggest that the BBB vessels along the hippocampal fissure especially in the medial part of the hippocampus are more vulnerable to ischemic episodes than those in other hippocampal sectors.

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SII-P18

Glycogen synthase kinase 3β (GSK-3β) and its phosphorylated form on tyrosine 216 in animal model of parkinsonism

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Parkinson's disease is progressing disease, due to a lesion of neuromelanin-containing dopaminergic neurons in the substantia nigra and a dramatic loss of dopamine in the striatum. It was demonstrated that systemic paraquat administration had neurotoxic effect on dopaminergic neurons. Till now nothing is known about the role of glycogen synthase kinase-3β (GSK-3β) in paraquat toxicity. The aim of the study was to examine whether the long-term (37 weeks) administration of paraquat in rats influence total GSK-3β and its active form phosphorylated on tyrosine 216 (pY216) in brain. Stereological counting of TH-ir neurons in the region of the substantia nigra pars compacta revealed a statistically significant decrease in their number and density in rats treated with paraquat. The DOPAC/dopamine ratio was slightly, but significantly decreased. Total protein level of GSK-3β and GSK-3β pY216 significantly decreased in nuclear fractions of all investigated brain parts. The immunoreactivity of GSK-3β and its active form was also lower after paraquat treatment in the mitochondrial fraction in the striatum. Moreover in cytosolic fraction a significant decrease of GSK-3β and GSK3β pY216 immunoreactivity was observed in the hippocampus and striatum of paraquat treated rats. However the protein level of both GSK-3β forms significantly increased in cerebral cortex of animals treated with paraquat comparing to control. These data indicate for the first time that systemic paraquat administration significantly affected GSK-3β pY216 in the brain parts. A decrease of GSK-3β pY216 in nuclear fraction of all investigated brain parts may through Fyn kinase and transcription factor Nrf2 influence the function of antioxidative gene

SII-P19

Involvement of multiple protein kinases in cPLA2 phosphorylation, AA release and cell death in astrocyte treated with 1-methyl-4-phenylpyridinium – the possible key role of PKG

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Cytosolic phospholipase A2 (cPLA2) demonstrates selective affinity to arachidonic acid (AA) liberation, which is known to be elevated in PD. We indicated that NO/GC/cGMP pathway was upregulated in the primary astrocyte culture treated with MPP+. We investigated if the cGMP/cGMP-dependent protein kinase (PKG) signaling pathway was involved in 1-methyl-4-phenylpyridinium (MPP+)-induced cPLA2 activation of the primary astrocyte culture. We found increased levels of total and phosphorylated cPLA2 and increased AA release in the primary astrocyte culture exposed to MPP+. We used cPLA2-specific inhibitors and Ca^{2+}-independent PLA2 (iPLA2), and we found that cPLA2 released more AA after stimulation with MPP+ than iPLA2 and that there was a time-dependent delay of AA release by iPLA2 compared to cPLA2. The PKG inhibitor KT5823 decreased MPP-induced AA release in the primary astrocyte culture. KT5823, in addition to PKC and ERK1/2 inhibitors, decreased cPLA2 activity as well as total and phosphorylated cPLA2 protein levels in the astrocyte treated with MPP+. Dual treatment with PKG and PKC or ERK1/2 inhibitors had the same effect on cPLA2 activity and protein levels. PKG is involved in the enhancement of cPLA2 phosphorylation at Serine-505 and in AA release in the astrocyte exposed to MPP+. Our results indicate that the nNOS/cGMP/PKG pathway stimulates cPLA2 phosphorylation at Ser-505 by activation of PKC or ERK1/2. These results suggest that activation of cPLA2 by upregulation nNOS/cGMP pathway may play important role in MPP+-induced astrocyte activation, neurotoxicity and oxidative stress in the nigrostriatal system.

SII-P20

cGMP-dependent protein kinase is involved in cPLA2 phosphorylation, AA release and cell death in *in vivo* and *in vitro* models of 1-methyl-4-phenylpyridinium-induced parkinsonism

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We investigated if the cGMP/cCGP-dependent protein kinase (PKG) signaling pathway was involved in 1-methyl-4-phenylpyridinium (MPP+)-induced cPLA2 activation of dopaminergic neuronal cells (PC12 cells). We found increased levels of total and phosphorylated cPLA2 and increased AA release in the nigrostriatal system of MPTP-induced parkinsonism mice and in PC12 cells exposed to MPP+. We used cPLA2-specific inhibitors and Ca^{2+}-independent PLA2 (iPLA2), and we found that cPLA2 released more AA after stimulation with MPTP/MPP+ than iPLA2 and that there was a time-dependent delay of AA release by iPLA2 compared to cPLA2. The PKG inhibitor KT5823 decreased MPTP-induced AA release in the nigrostriatal pathway. KT5823, in addition to PKC and ERK1/2 inhibitors, decreased cPLA2 activity as well as total and phosphorlyated cPLA2 protein levels in the midbrain and striatum of MPTP-induced parkinsonism mice. Inhibition occurred within 30 minutes and persisted for up to 24 hours. Similar results were also observed in MPP+-treated PC12 cells. Dual treatment with PKG and PKC inhibitors had the same effect on cPLA2 activity and protein levels. PKG is involved in the enhancement of cPLA2 phosphorylation at Serine-505 and in AA release in PC12 cells exposed to MPP+. In PC12 cells, inhibitors of cPLA2 and PKG increased viability and prevented MPP+-induced apoptosis. Our results indicate that the nNOS/cGMP/PKG pathway stimulates cPLA2 phosphorylation at Ser-505 by activation of PKC or ERK1/2. Our results also suggest that upregulation of the nNOS/cGMP pathway observed in experimental models of PD may mediate dopaminergic neuron degeneration and death through activation of cPLA2.
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SII-P21

Anxiolytic effects of mild hypobaric hypoxia in mice

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Mild hypobaric hypoxia (MHH) which is known to induce tolerance to brain hypoxia/ischemia, appeared to prevent development of anxiety and depressive-like behavior in rats submitted to the unavoidable adversive stress. The latter effect of unclear mechanism so far wasn't confirmed in other animal models. Since enhanced brain expression of the neuropeptide Y (NPY) mimics effects of anxiolytic and antidepressant drugs, and we noticed that MHH increases the number of NPY-positive neurons in the gerbil hippocampus, we propose possible involvement of NPY in the positive behavioral effects of MHH. The aim of present study was to reproduce behavioral effects of MHH in balb/c57 mice using the tail suspension test and the elevated plus maze, and to evaluate immunohistochemically in these animals the number of NPY-positive neurons in the hippocampus. We confirmed that intermittent MHH (360 Torr, 2 h for 3 consecutive days) in mice induces anxiolytic and weak antidepressant-like effects. The elevated plus maze trials performed 48 h after MHH revealed a significant increase in frequency of the open arm entries, reduced duration of the closed arm occupancy, and 7-fold increased time spent on the open arms in comparison to sham animals. In the tail suspension a significant decrease of the duration of immobility was observed 24 h, but not 48 h after MHH, when we detected a modest but significant increase in the number of NPY-positive neurons in the hippocampus. Thus, although our preliminary data confirm anxiolytic and antidepressant-like effects of MHH in mice, further studies are needed to characterize better these effects and to learn its mechanism, particularly to verify the role of NPY.
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Session III

STEM CELLS FOR THE TREATMENT OF CNS DISEASES

SIII-L1

Umbilical Cord Blood – models for neurodegeneration and drug testing

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Taking tissue engineering applications into clinical trials requires the development of efficient, effective and safe protocols incorporated with effective 3-dimentional cell culturing and differentiation systems in order to develop transplantable tissues that may offer a life-line for many patients in the future. Umbilical cord blood, which is perhaps the most abundant world stem cell source, has shown previously practical and ethical advantages over other stem cells sources in many research and clinical applications including regenerative medicine. We developed a three

step protocol for isolation, expansion and sequential neuronal differentiation and maturation of cord blood pluripotent stem cells (characterized with our unique triple immunocytochemisty scheme for Oct-4, Sox-2 and Nanog expression) in serum-free defined culturing conditions. We incorporated this protocol with 3-dimentional culturing system which produced properly organized neuronal tissues expressing Nestin and NF-200. We showed that umbilical cord blood pluripotent stem cells are a potential and promising candidate for future neural tissue engineering and regenerative medicine applications.

SIII-L2
Human cord blood cells in neurotoxicology: Advantage of emerging technologies

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Stem cell technology provides a new tool for better understanding the mechanisms involved in compound-induced adverse reactions of the organism, which particularly applies to the field of developmental neurotoxicity. Human Umbilical Cord Blood Neural Stem Cell (HUCB-NSC) line is a model system where key neurodevelopmental processes were investigated by conventional and emerging techniques. The advantage of the HUCB-NSC line is that cells are of human origin, non-transformed and can be cultured/harvested at different developmental stages. In this report emerging nano/micro-technologies were used to create biofunctional micropatterns and multi electrode array chips for the detection of cell behaviour and vulnerability to toxic compounds. Micropatterned surfaces were produced by a spatial arrangement of different functional domains. This included a nano/micro-fabrication technique like contact printing in order to create a pattern of separated or interconnected polypeptide spots directing cell growth and differentiation. Another approach was to create protein microarrays by piezoelectric (non contact) deposition of extracellular matrix proteins. Such a method allows defined active areas to be produced on the same platform and enables unambiguous access to cell behavior on different protein types and concentrations. HUCB-NSC were shown to adhere and differentiate on microarray platforms in a protein type, concentration and cell density dependent manner. Sensor and omics techniques applied to HUCB-NSC included measurements of electrical activity using multielectrode array chips and metabolite profiling by mass spectrometry. Spontaneous electrical field potentials and the protein composition of tested cells were sensitive to neurotoxic treatments in a developmental stage specific manner.

SIII-L3
Cell-based therapies and functional recovery in experimental stroke rats

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Stroke is the third leading cause of deaths in Western countries, and more importantly, it is a leading cause of adult disability. Restorative approaches such as cell-based therapies are clinically appealing as it might be possible to help stroke patients even when treatment is initiated days or weeks after the ischemic insult. Noninvasive intravascular administration of cells, which provides a broad distribution of cells to the close proximity of ischemic tissue, has perhaps the most immediate access to clinical applications. For example, intravenous infusion of human umbilical cord blood (HUCB) cells and bone marrow stromal (BMS) cells has been shown to improve sensorimotor functions in rats subjected to focal cerebral ischemia. Interestingly, entry of cells into the central nervous system is not needed for the beneficial effect indicating that peripheral mechanisms or trophic factors may play a role. Another approach is a stereotaxic transplantation of cells into the brain. Our recent data suggest, that subventricular zone (SVZ)-derived mouse neural stem cells or human embryonic stem cell (hESC)-derived neural precursors, when transplanted close to the infarct, do provide some improvement in sensorimotor function after focal cerebral ischemia in rats, but do not restore more complicated sensorimotor function such as skilled reaching. The major problem seems to be a marginal long-term survival rate of transplanted cells. Thus, more work is needed to define the optimal cell type, route of administration, and timing of administration after cerebral ischemia to support cell survival. This would also ensure safe and effective translation of experimental results into clinical practice.

SIII-L4
Stem cell-based therapy in Parkinson disease

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The use of stem cells has been proven an attractive strategy for restoring lost cell or tissue functions. Stem cell therapy is nowadays routinely used in many clinical applications. The complexity of the tissue in the central nervous system however often limits the use of stem cells yet to reconstructions of defined well-known neurotransmitter deficits, for example the reconstitution of the nigro-striatal system in Parkinson's disease (PD). However, the selective loss of a specific type of dopaminergic cells in PD makes the prospect of replacing the missing or damaged cells very attractive. Indeed, first controlled clinical trials of

intrastriatal transplantation of primary dopaminergic tissue in PD provide the "proof-of-principle" of cell replacement strategies in PD. On the other hand, those trials also demonstrated several scientic limitations. Recent developments in stem cell research however provide new cell types for cell replacement strategies in PD. Furthermore, the ongoing developments during the recent years showed alternative applications of stem cells in neurological diseases such as the use of neural stem cells as neuroprotective agents or as vectors for transferring genes of interest. Due to the discovery of neural stem cell also in the adult brain, the questions concerning their role in physiological and pathophysiological conditions as well as their potential for endogenous regeneration has to be further investigated. Cultivating and characterizing these cells aims to understand mechanisms of neuronal degeneration and regeneration and could help to develop therapeutic strategies, as for example the stimulation of endogenous stem cells by pharmacological compounds. In this presentation, the different strategies for the use of stem cells to treat PD will be summarized and discussed.

SIII-O1

The crucial role of the local microenvironment in fate-decision of neonatal rat NG2 progenitors

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The fate choice of neural progenitors could be dictated by local cellular environment of adult CNS. The aim of our study was to investigate the effect of hippocampal tissue on the differentiation and maturation of the oligodendrocyte NG2 precursors. Methods: The hippocampal slice culture was established from the brains of 7-day old rats. The NG2 precursors, obtained from a 12-day old mixed primary culture of neonatal rat hemispheres, were labeled with CMFDA and seeded on hippocampal slices. After 7–14 days in co-culture, the cells were stained with neural markers. Results: The NG2 cells differentiated predominantly into oligodendrocytes, presenting various stages of maturation: progenitors NG2+, O4+ and finally mature Galc-positive cells. However, except for a few cells with astrocyte-specific S100β staining, a considerable number of these cells differentiated into TUJ+ and MAP-2+ neurons. Moreover, a certain population of these cells preserved proliferative properties of primary precursors, as revealed by the Ki67 expression. Conclusions: Neuronal microenvironment provided by the culture of hippocampal slices is potent to induce neurogenesis from oligodendrocyte NG2+/PDGFRα+/CNP+ progenitors and promotes their differentiation not only into macroglia but also into neurons. It also sustains their proliferative capacity. The results indicate a crucial role of the local cellular environment in fate-decision of primary NG2+ multipotent neural progenitors, which may affect their behavior after transplantation into CNS.
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SIII-O2

Inducing efficient catecholaminergic differentiation of GFAP, SOX2-positive neural progenitors (NHA) by means of kinetic factors

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Extensive research has been performed to control differentiation of neural stem cells. For last three years we have strived to obtain stabilized protocol of catecholaminergic differentiation of GFAP, SOX2- positive neural progenitors (NHA) In 2007 we published first results presenting differentiation of GFAP positive neural progenitors (NHA) in accordance to model of discordant phenotypes suppression (Rieske 2007, Eur J Neurosci). Since the beginning of 2008 we are able to differentiate uncommitted GFAP and SOX2 positive neural progenitors (NHA) in different environmental conditions to: only neural cells consisted of neuronal and glial cells, or fibroblast-like cells, or mixture of neural and fibroblast-like cells (Witusik 2007, Brain Res; Witusik 2008 BMC Biotechnol). In spite of successful blockade of fibroblast-like differentiation by means of environmental changes, we were able to barely increase the percentage of neuronal (GABA-ergic and catecholaminergic) over glial cells under several different cell culture testing conditions. It was so far also impossible to alter radically ratio catecholaminergic /GABA-ergic cells by means of changing environmental factors (SHH, GDNF, bFGF, EGF, BMPs). It strongly suggested influence of stochastic events or so called continuum processes during neuronal versus glial, and catecholaminergic versus GABA-ergic differentiation of described neural progenitors. Nevertheless including kinetic factors to our differentiation protocols allowed to increase percentage of catecholaminergic cells from 10 to 45% of neuronal cells.

SIII-P1

Neurogenesis in gerbil hippocampus following brain ischemia – involvement of MMPs

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Accumulating evidence indicate that global cerebral ischemia enhanced neurogenesis in adult brain. The mechanism(s) responsible for the arising a new neurons from progenitor cells are poorly understood. Recent *in vitro* studies indicate the involvement of metalloproteinases (MMPs) in the regulation of proliferation and differentiation of neural progenitor cells, by providing an environment, which is instructive and/or permissive to stem cells activation. To elucidate if MMPs participate in neurogenesis-associated

processes after ischemic insult *in vivo*, we aimed to establish spatial and temporal relationships between neural stem cells development and the modulations of MMPs enzymatic activity in the adult hippocampal dentate gyrus as well as in the ischemia damaged CA1 sector. Our results show that postischemic acceleration of progenitor proliferation in dentate gyrus is accompanied by the increase of MMPs activity. It may indicate that activation of MMPs is likely to be involved in neurogenesis-associated processes. Contrary, the endogenous neurogenesis in the ischemia damaged CA1 pyramidal layer seems to be rather elusive. Despite the appearance of BrdU-positive cells and the newborn neurons they did not attain maturity. Simultaneously, the activity of MMPs in this area was markedly reduced. The increase of MMPs activity seen in the astrocytes in the neighboring structures – stratum oriens and stratum radiatum, probably plays a role in delayed tissue remodeling and delayed repair processes. In conclusion, our results show that MMPs may, at least in part, contribute to ischemia-induced neurogenesis in the dentate gyrus of the adult brain, along with other previously reported factors.

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SIII-P2

Endothelial progenitor cell in newly-formed capillaries following surgical brain injury of rat cerebral cortex

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Several lines of evidence suggests that neovascularization in adult organism may be mediated by circulating progenitor cells. During ischemia and brain injury, populations of endothelial progenitor cells are mobilized and recruited to ischemic and injured areas, accelerating the neovascularization process. Surgical brain injury causes neovascularization in the disrupted brain parenchyma, which occurs with participation of endothelial-like cells. The aim of study was comparison of ultrastructural and immunohistochemical features of endothelial progenitor cells participated in new vessel formation following surgical brain injury in non-diabetic and diabetic rats. We investigated subcellular localization of protein markers: Flk-1, AC133 and vimentin. We detected the presence of immature endothelial cells showing positive immunostaining for all investigated markers in the proximity to the injured brain area in diabetic rats. Only few these cells were observed in the brains of non-diabetic animals. Ultrastructural studies showed many morphological changes within capillaries in the injured brain derived from diabetic animals. Our results point to the diabetes related dysfunction of the brain capillaries. The number of progenitors and, probably, their abnormal differentiation contribute to disorders in the process of repair following the injury in diabetes-affected rats.

SIII-P3

Controlling of HUCB-NSC culture by micropatterned biofunctionized surfaces

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Stem cells have potential to maintain in organism by self-renewal division and ability to multilineage differentiation. Cell-cell contacts, paracrine signals and extracellular matrix proteins occurring in the neurogenic niches are main determinants of neural stem cells fate and dynamic of their differentiation. Aim of this study is to investigate whether could nano/micro-patterned, biofunctionalized surface guides human cord blood derived neural stem cell to growth and differentiation. Methods: To investigate the influence of the cell plating density we used the microcontact printed patterns of adhesive substrate (poly-L-lysine) on cell-repellent poly-ethylene glycol (PEG) substrate. Two different geometries of the patterning have been applied: to test proliferative response the cells were seeded in different densities on the pattern with separated pitches, while to verify their ability to differentiation the culture medium was supplemented with cAMP and cells seeded on the surface patterned with interconnecting lines. After 2, 4 and 7 days the cells were fixed and immunostained for Ki67 (proliferation marker) and beta III tubulin/ S100β markers for neuronal/astrocytic lineage. Results: The low cell density of HUCB-NSC (104 cell/cm^2) and the presence of neuromorphogenes (cAMP) supports neural stem cell differentiation, while enhanced initial cell density promotes the growth rate (increase of the cell number falling on biofunctionalized unit per 100 μm^2 surface). Conclusions: Micropatterned platforms with biofunctionalized surface can be used for screening of the plethora of extracellular signals directing neural stem cell to growth and differentiation.

SIII-P4

Activation of astrocytes and microglia provides the critical cues for HUCB-NSC proliferation and differentiation *in vitro*

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Brain inflammation contributes to the propagation of neuropathological events that involves activation of astrocytes and microglia. It remains obscure how activated glial cells affect the survival and differentiation of neural stem cells (NSC). The aim of the study was to analyze neuronal commitment of Human Umbilical Cord Blood derived Neural Stem Cells (HUCB-NSC) cultured in the presence of normal and LPS- or TMT-activated glial cells. Methods: HUCB-

NSC (5 × 104/cm^2) were co-cultured with normal or LPS (0.1 μg/ml) and TMT (1μM)-stimulated astrocytes and microglial cells isolated from neonatal rat brain for proliferation and cell phenotype assessment. Pro-inflammatory cytokines were estimated (ELISA). Results: Normal rat astrocytes induce HUCB-NSC to differentiate mostly into neurones but microglia stimulate HUCB-NSC to differentiate into neurons as well as into astrocytes. LPS- and TMT-induced astrocytes diminish neurogenesis of HUCB-NSC and increase astrocyte differentiation in comparison to non-stimulated astrocytes. Microglia activation by LPS and TMT decreases HUCB-NSC differentiation into neurons but enhances oligodendrogenesis compared to normal microglia. Stimulation of astrocytes and microglia by LPS and TMT declines HUCB-NSC proliferation co-cultured with astrocytes or with microglia. The presence of IL-1β, IL-6, TNF-α and NO was observed in glia cell culture supernatants after LPS and TMT implementation. Conclusion: Activation of astrocytes and microglia induced by LPS and TMT attenuate pro-neural effect of non-stimulated (resting) glia and suppress proliferation of HUCB-NSC *in vitro*. The release of pro-inflammatory cytokines and NO might be partly responsible for this effect.
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SIII-P5
Fibronectin – the most potent factor in neural stem cells of HUCB-NSC line development is associated with MMPs activity

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Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are considered the main regulators of the cell microenvironment which governs NSC development. The aim of our study was to investigate whether the selected ECM components (laminin, fibronectin, collagen) influence the neural stem cells proliferation and differentiation. In addition we checked if MMPs are engaged in the above developmental processes. Methods: The cells of HUCB-NSC line were seeded on the ECM components-coated plates. The serum-free medium was applied 48 h before experiments. On the 4th, 8th and 14th day in culture, cell proliferation assay and in situ zymography were performed, followed by the immunocytochemistry with the specific neural markers. Results: Our results showed that fibronectin stimulated cell proliferation as well as MMPs activity most intensively (~20% increase in the 2-week cultured cells). It was also the most potent factor in promoting the cell differentiation, mostly toward neurons. We also checked the involvement of the MMPs in cell development. For this purpose we used MMPs inhibitors: GM6001 and TIMPs as well as inhibitors for serine and furin proteases – Pefabloc and Dec-RVKR-CMK. We found that only inhibitors of MMPs influence stem cells developmental processes. GM6001 down-regulated cell proliferation (~30%) and differentiation into neurons (~20%). The decrease of cell proliferation was also observed in the presence of TIMP2. In contrast, TIMP1 accelerated cell divisions. Conclusion: Our results demonstrate that fibronectin is the potent factor in promoting the cell proliferation, differentiation, and support the participation of MMPs in the mechanism(s) responsible for neural stem cells maturation.
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SIII-P6
Examination of the microscopic imaging of neural stem cells for counting a number of cells

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The goal of the investigation is to examine cells imaging with the brightfield and confocal microscopic imaging using two techniques: with and without fluorescence. The investigations are done as an introducing part of the long term goal to develop methods of monitoring a number of cells in culture in time. Several types of events in the monitored cells' culture, e. g. cell's division and death, cells' fusion and its changes in shape and/or position should be detected and quantified. Because of immanent features of each microscopic technique some of events are easier to be observed in one of the microscopic techniques than in others. Other events are detectable in all microscopic imaging. To detect them, the area covered by the cells in the image should be selected in the first step of the image processing. So the method of cells' area detection, described by Korzynska and Iwanowski (2008) [In: Pietka, Kawa (Eds), Information Technique in Biomedicine, ASC 47, pp. 365–376], is applied to all tested types of the microscopic images of HUCB-NSC transfected by GFP (from Medical Research Center PAS) and the results of area detection are examined and compared. It was found, that the proposed method detects cells' area more precisely in the fluorescent images, than in the brightfield or confocal microscopy. Comparing results of cells' area detection of the fluorescent image of confocal microscopy with the integrated fluorescent signal in epifluorescent images of conventional optical microscopy the better result of detection cells in the image plane were observed for flattened, large cells in confocal images while for rounded, convergent ones for the brightfield microscopic techniques.

SIII-P7
Systemic neurotransplantation – systematic review

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Systemic neurotransplantation (SNT) was introduced to laboratory as a new route of cell delivery in minimal invasive fashion. The aim of the systematic review was to evaluate the effect of SNT and to explore the variables influencing the outcome. PubMed library was searched and 60 articles utilizing SNT approach were found and subjected to analysis. Morphological (lesion size), behavioral and molecular (such as: neurogenesis, immunomodulation) effects were the primary measures of outcome. For comparison between experimental and control groups means, standard deviations and animal numbers were used. It was calculated according to Hedges g formula and expressed as an effect size and standard error (SE). For setting together the effect sizes from particular studies were weighted by the inverse variance. Then the effect size of the group of studies was calculated by method of fixed effect model and expressed as weighted mean and confidence interval (CI) which was set at 95%. SNT exerted large positive influence on outcomes in animal models of neurological disorders. Morphological effect was weaker, and molecular effect was stronger than behavioral effect. The explanation of this phenomenon may be that grafted cells cause not only the reduction of lesion size, but also enhance the function of the remained, macroscopically non-damaged brain by molecular means such as apoptosis inhibition, enhancement of cell proliferation, growth factor expression etc. Species and tissue compatibility between donor and recipient of SNT facilitates neurological recovery. In contrast, immunosuppression decreases therapeutic effect. Among several correlates only cell dosage influences positively outcomes. Our analysis should help to design clinical trials.

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SIII-P8

Analysis of the neurogenic potential of human umbilical cord blood neural stem cells (HUCB-NSC) after their transplantation into rat brain

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Transplantation (tx) of neural stem cells (NSC) is the key strategy of cell replacement therapy in the central nervous system. The goal of the study was to compare survival, migration and differentiation of HUCB-NSC after their tx into the brain of neonatal and adult Wistar rats. Methods: HUCB-NSC (2×10^4) labeled with CMFDA were tx into SVZ of the postnatal day 0 (P0) rats or into intact brain of adult rats. After 1, 3, 7, 14 or 21 days brains were removed, frozen and cut into 20 μm coronary slices, then immunohistochemical studies were performed to visualize HUCB-NSC fate in the brain. Results: In neonatal rats, 3 days after tx most of HUCB-NSC remained in the graft. During the first week HUCB-NSC started to disperse and migrate. HUCB-NSC situated at the periphery of the graft or in migratory stream display proliferation marker (Ki67). After 7–21 days HUCB-NSC survived in the host brain with many cells expressing neuronal or astrocytic phenotypes. Few of HUCB-NSC presented the features of adult neurons (MAP2+ with long protrusions). In adult rats, 3 days after tx, HUCB-NSC form dense deposit with single cells migrating into brain tissue. Most of the HUCB-NSCs stayed undifferentiated with few cells expressing neuronal (NF200) or astrocytic (GFAP) markers. After 7 days numerous HUCB-NSC situated inside the graft underwent degeneration and subsequent depletion. Tx HUCB-NSC induced inflammatory response detected by macrophage/microglia (ED1+) accumulation and astrogliosis (GFAP+). No viable HUCB-NSC were found after 14 days. Conclusions: Host environment dictates the fate of tx neural stem cells derived from human cord blood however immunological response in adult rats limits the time of observation due to short survival of HUCB-NSC.

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SIII-P9

Immune response following grafting of human umbilical cord blood neural stem cells (HUCB-NSC) into adult rats with ouabain (OUA) induced brain lesion

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Many types of neural progenitors from different sources have been tested for experimental therapy in CNS injuries. We have established neural stem cell line from human cord blood (HUCB-NSC). *In vitro* evidence has suggested that HUCB-NSC are not immunogenic however their transplantation (tx) into adult rats led to the graft cell rejection. The question arises what is the nature of the host immune response to transplanted HUCB-NSC? Methods: 2×10^4 HUCB-NSC were tx into corpus callosum of a focal brain injury induced by OUA injection (1 μl/5 mmol) into striatum of adult Wistar rats. At 1, 3, 7 and 14 days thereafter brains were removed and immunocytochemical analysis for T cells (CD5), B cells (CD45), macrophages (ED1) and neutrophils (CD15) was performed. Results: One day after HUCB-NSC tx, most cells remained in the injection site and only few cells migrated to the lesion area. Concomitantly, infiltration of ED1+ and CD15+ cells with occasional appearance of CD5+ and CD45+ cells was seen around the graft and close to the lesion. At 3rd day after HUCB-NSC tx, the infiltrate of ED1+ cells increased however, the number of CD15+, CD5+ and CD45+ cells stayed unchanged. At 7th day after HUCB-NSC tx, the number of ED1+ and CD15+ cells was reduced dramatically compare to the 3rd day and only single CD5+ and CD45+ cells were observed. By 14 days, limited number of ED1+, CD15+, CD5+ and CD45+ cells were found most likely due to the scar formation and rejection of HUCB-NSC. At that time no viable HUCB-NSC have been noticed in brain tissue of the host.

Conclusion: Transfer of HUCB-NSC into ouabain induced brain lesion rats elicits innate (macrophages/neutrophils) and adaptive (T/B lymphocytes) immune response in the acute phase post-transplantation.
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SIII-P10

Freshly isolated and neurally directed human umbilical cord mononuclear cells (HUCB-MNs) exert distinct effects on functional recovery following focal brain damage in the rat

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Focal brain damage following stroke leads to severe functional impairments. The aim of the study was to compare therapeutic effectiveness of intra-arterial infusion of HUCB-MNs at different stages of their neural conversion *in vitro*. Methods: Focal brain damage of dorsolateral striatum was induced in Wistar rats by stereotactic injection of previously established low dose of ouabain (1 µl, 5 mmol). Three days later 10^7 HUCB-MNs cells were infused (during 3 min) into carotid artery. Thirty days following surgery groups of 7–8 rats were housed in large enriched environment cages with various toys. Rats were behaviorally tested for 30 days after lesion. Results: Freshly isolated cells were much more effective in enhancing recovery from motor deficits measured in walking beam task. Rats treated with HUCB-MNs cells presented also tendency to reduce turning bias and apomorphine induced rotations affected by unilateral lesion. This therapy enhances also recovery from impairments visible in object recognition task. However, rats treated with neurally directed HUCB-MNs also showed a significant improvement in this task. The observed effects were much more prominent in T-maze habit learning task where cell treatment attenuated substantially lesion-induced learning deficits. What interesting, the mechanism underlying this improvement seems to be different from this observed spontaneously in non-injured animals. Conclusions: Freshly isolated and neurally directed HUCB-MNs differently enhance recovery from distinct functional deficits induced by focal brain damage. Non-cultured HUCB-MNs seems to be more effective in reducing motor deficits. Neurally directed HUCB-MNs may be more potent in restoration of impaired habit learning processes.
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SIII-P11

EGFR involvement in neural and mesenchymal differentiation of glioblastoma progenitor cells

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We have compared differentiation ability of glioblastoma cells with differentiation ability of neural progenitors. An efficient differentiation arrest was observed in all cell lines isolated from glioblastomas in contrast to normal neural progenitors. However, cells isolated from six glioblastomas showed features of early stages of neural differentiation. Moreover, the cells derived from a majority of glioblastomas (8 out of 10) as well as neural progenitors showed features of non-neural (mesenchymal-like) differentiation. Moreover aggregated cells sustained EGFR amplification, whereas cells grown as a monolayer did not. Cells showing EGFR amplification became apoptotic grown as monolayer. Majority of mesenchymally differentiated glioblastoma cells showed features of senescence. Novel hypotheses which we would like to test are as follows: Neural progenitors could be a potential source of glioblastomas. Glioblastoma presents not only tumor stem cells but also tumor progenitor cells. Stable coexpression of glial and neuronal markers presented by glioblastoma cells results from differentiation arrest. Aggregating glioblastoma cells allows to sustain, EGFR amplification *in vitro*. Moreover aggregated cells show proliferation ability, and differentiation arrest, whereas monolayer cells can be efficiently differentiated and finally senescent. It suggests that simultaneous analysis of differentiation processes altogether with considering status of genes such as EGFR may help in designing new molecular targets for chemotherapy.