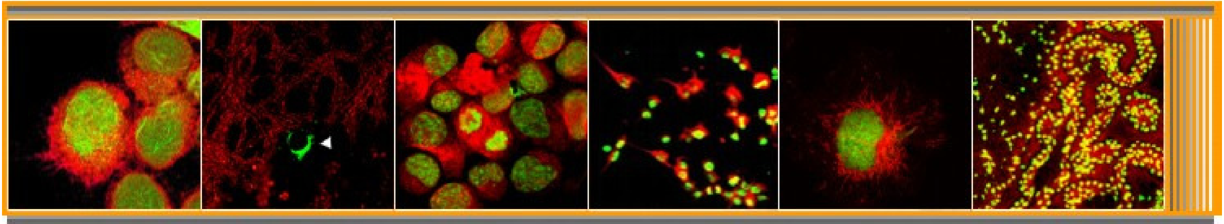

International Conference

Molecular pathways in health and disease of the nervous system

Gesellschaft für Biochemie und Molekularbiologie,
Study Group Neurochemistry

14.-15.09.2007
Hamburg
Germany

- ABSTRACT BOOK -

This meeting is supported by:

Dear colleagues, dear friends,

it is a great pleasure for us to invite you to participate in the Annual Meeting of the Study Group Neurochemistry which is scheduled to take place in Hamburg, September 14 - 15, 2007.

In keeping with the traditions of these annual meetings, the 2007 meeting will bring together those actively engaged in research for the purpose of reviewing and discussing recent progress in neurochemistry/neurobiology and medicine in general, exchanging information, and relating research from in vitro studies up to investigations in the human brain.

The particular theme selected for the 2007 meeting is

Molecular pathways in health and disease of the nervous system

Deficiencies in neural signaling play a role in a number of disorders in the central nervous system. Regardless of whether they appear as a primary defect or whether they are consequences of other pathogenic insults, detailed knowledge of the molecular mechanisms of particular signaling cascades should allow for the derivation of therapeutic strategies to selectively intervene in pathogenic signaling processing.

However, contributions are not restricted to the main topic of the meeting. There will be large poster sessions in which all aspects and subfields of neurochemistry and neuroscience may be presented and may further provide a forum for extensive discussions of the latest developments as well as to meet colleagues and experts.

The conference will take place in Hamburg and provides a lovely place to continue further scientific debates and discussions after the sessions in a more relaxed atmosphere.

We are cordially inviting you to come and to experience this meeting with us, and are looking forward to welcoming you in Hamburg this autumn.

Christian Kaltschmidt
(on behalf of the Organizing Committee)
Speaker of the Study Group Neurochemistry

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Friday, 14.9.

16:00 - 18:00	*Registrierung*
18:00 - 18:30	**Mathias Kneussel**: Transport and dynamics of neurotransmitter receptors to and from inhibitory synapses
18:30 - 19:00	**Hans-Georg Breitinger**: Mechanism-based characterisation of multiple drug receptor interaction and search of new ion channel modulators
19:00 - 19:30	**Thomas Schmitt-John**: Vesicle traffic is critical for the motor neuron disease of *VPS54* mutant wobbler mice
19:30 - 20:00	**Roland Brand**: O-Glycosylation of neurofilaments in amyotrophic lateral sclerosis and Alzheimer's disease
20:00 - 20:45	**Eva M. Mandelkow** (key note lecture): Tau protein in neurite outgrowth, axional transport, and Alzheimer neurodegeneration
20:45	get together meeting

Saturday, 15.9.

8:30 - 9:00	**Ralf Enz**: Protein phosphatase 1 binds to metabotropic glutamate receptors and $GABA_C$ receptor rho subunits at retinal synapses
9:00 - 9:30	**Tim Hucho**: Intracellular Signaling in Pain
9:30 - 10:00	**Rüdiger Schweigreiter**: The dual nature of the nerve growth inhibitor Nogo-A
10:00 - 11:00	**Barbara Kaltschmidt**: A novel role of NF-κB in neural stem cells
11:00 - 11:30	*Coffe Break*
11:30 - 12:00	*Poster Viewing*
12:00 - 12:30	**Thomas Tilling**: A role for nuclear factor I-A (NFI-A) in early postnatal brain maturation? Implications of a gene expression study
12:30 - 13:00	**Rolf Heumann**: Molecular physiology of Ras and Ras homologue enriched in brain (Rheb)
13:00 - 13:30	*Lunch and Poster Viewing*
13:30 - 14:00	*Lunch and Poster Viewing*
14:00 - 14:30	Bussiness Meeting
14:30 - 15:00	**Gerald Thiel**: Expression of synapsin I, synaptophysin, and synaptotagmins II, IV, and VII in human neural stem cells: Chromatin accessibility, histone code and role of the transcription factor REST
15:00 - 15:30	**Ypeng Wang**: Stepwise proteolysis liberates tau fragments that nucleate the aggregation of tau in neural cell model
15:30 - 16:00	**Dieter G. Weiss**: Neuronal networks grown in microelectrode arrays and their use for studies on pyhsiological and biochemical influences of neuro-active substances
16:00 - 16:30	*Coffe Break*
16:30 - 17:00	**Diego Walter**: Protein monoaminylation, a mechanism going far beyond G protein modulation
17:00 - 17:30	**Chandan Goswami**: TRPV1 expression-dependent initiation and regulation of filopodial
17:30 - 18:00	*Poster Viewing*
18:00 - 18:30	**Paul Heppenstall**: Transient Receptor Channels in Pain Sensation
18:30	Get together for CLOSING with a very nice **BOATTRIP** all inclusive BEGINNING: **20:00** p.m. from “Landungsbrücken 1-6”

POSTER ABSTRACTS

1.

Lipofection of GFP-fusion-proteins as a tool to investigate the dynamics and plasticity of dendritic spines in primary mouse frontal cortex cultures

Benjamin M. Bader, Dieter G. Weiss

University of Rostock, Institute of Biological Sciences, Cell Biology and Biosystems Technology, Albert-Einstein-Str. 3, D-18059 Rostock, Germany

Abstract:

Dendritic spines are cellular structures believed to play a major role in synaptic plasticity. For visualizing these sub cellular components in living cells it is crucial to have optimal conditions for the transfer of GFP-fusion-proteins like pEGFP-Actin into these cells. Primary mouse neuronal networks from frontal cortex (E15) cultured for up to 4 weeks were used for transfection. After comparing different non-viral transfection protocols we show here that lipofection is the most efficient way to transfect these cells. Additionally we show a culture-age-depency for the transfection reagents used. During the first two weeks in culture we found Metafectene-Pro™ to be the most efficient in contrast to Lipofectamin2000™ which is more efficient after four weeks in culture. This optimized tansfection assay is used to study the dynamics and morphology of dendritic spines in living neurons. Our present work focuses on the motor enzyme myosin-V and its role in spine plasticity and organelle localization. For this study we introduce an overexpression vector which results in a dilute-lethal mutation of myosin-V. Preliminary data show changes in dendritic tree complexity of the neurons.

2.

Characterizing the Wnt-pathway components and their subcellular localization in differentiating human neural progenitor cells

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Abstract:

In the differentiation of neural progenitor cells of different origins (regions) and species the canonical Wnt-pathway is known to be involved. In order to investigate its complex regulatory network, we have conducted biochemical and microscopy analyses in a parallel and complementary fashion. We focussed on the major proteins involved in the canonical pathway: Frizzled / LRP5/6, ß-catenin, Axin, APC and GSK-3ß. Wnt-pathway components were determined at different time points throughout the differentiation process of the cells. The experiments were performed using the human progenitor cell line ReNcell VM197 (ReNeuron, Guildford, UK) derived from 10 weeks old foetal ventral mesencephalon. These cells have the capacity to differentiate into different glial and neural cell types, for example the dopaminergic phenotype. To achieve this goal we performed subcellular fractionation into the major cell compartments consisting of – cytoplasmic, membrane, nuclear and cytoskeletal fractions. By Western blot studies for protein quantification we could show that the cells undergo Wnt-pathway mediated differentiation. Confocal laser scanning microscopy and live cell imaging studies further confirmed the Wnt-protein translocation and the resulting morphological changes observed during neural differentiation. Our preliminary data demonstrate a correlation between cytoskeletal changes and organelle transport with respect to Wnt signalling. Our present work focuses on the integration of these findings with computational modelling which may provide novel insights into Wnt-pathway mediated regulation of protein translocation (transport) through subcellular compartments.

Supported by DFG Graduiertenkolleg 1387 dIEM oSiRiS

3.

Mechanism-based characterization of multiple drug-receptor interactions and search of new ion channel modulators

Hans-Georg Breitinger[*1)], Karim Raafat[1)], Ulrike Breitinger[1)], Diaa Youssef[2]

1) The German University in Cairo, New Cairo City, Egypt
2) Suez Canal University, Ismailia, Egypt

Abstract:

Ligand-gated ion channels mediate rapid synaptic signal transmission in the central nervous system and at neuromuscular junctions. 5-HT_3 receptors are mainly located in CNS regions that are involved in physiological processes such as the vomiting reflex, pain processing, the brain reward system, and anxiety control. Strychnine-sensitive glycine receptors are the predominant mediators of inhibitory transmission in the mammalian spinal cord and brainstem.

Extracts from Red Sea organisms were tested for their activity on strychnine-sensitive glycine receptors on HEK 293 cells. Inhibitory activity could be detected in two extracts, where glycine-mediated currents near EC_{25} and EC_{75} were reversibly inhibited. Another sample activated the glycine receptor if applied alone. Upon co-application with glycine, currents were potentiated, both effects were reversible. Thin-layer chromatography indicated that the active component in the extract was not glycine itself, while the kinetics of activation were different from the action of ivermectin on glycine receptors. These results indicate that the observed receptor activation is indeed mediated by a new glycinergic agonist.

A test system was developed for assaying activation of the serotonin 5-HT_3 receptor and its inhibition by the simultaneous application of more than one drug. The addictive substances nicotine and cocaine were both found to inhibit the receptor, as did the serotonin reuptake inhibitor fluoxetine. Inhibition constants K_i were 25 ± 7 µM for nicotine and 7.5 ± 0.5 µM for Cocaine, both compounds were competitive inhibitors of the receptor. In contrast, Fluoxetine behaved as a non-competitive inhibitor, most likely targeting a low-affinity site of the receptor, with an inhibition constant of 244 ± 24 µM.

Mixed inhibition experiments, where two inhibitors were simultaneously applied to the receptor, confirmed that nicotine and cocaine target the same site on the receptor, which was different from the fluoxetine site. These results are consistent with the different physiological effects of the substances studied. The general mechanisms of interaction of several drugs with one target are discussed.

Simultaneous application of two or more ion channel inhibitors is an important tool in the identification of common drug target sites on ion channel receptors. The technique establishes a basis for the assessment of side effects and altered therapeutic activities that are frequently observed in the use of drug combinations.

4.

Protein monoaminylation, a mechanism going far beyond G protein Modulation

Diego J. Walther[1], Jakob Vowinckel, Maik Grohmann, and Nils Paulmann

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Abstract:

Intracellular organization is the basis of eukaryotic life. Protein translocation is one of the essential organizing mechanisms, which largely depends on vesicular transport. Small G proteins regulate the majority of the vesicular transport within eukaryotic cells (Zahn et al., 2006). A recently discovered posttranslational modification, protein serotonylation, or more general, protein monoaminylation, is now to be considered as a broad eukaryotic modulating mechanism for vesicular transport, cell proliferation, differentiation, and apoptosis (Guilluy et al., 2007; Walther et al., 2003a; Walther et al., 2007). Protein monoaminylation was first identified as a mechanism regulating G protein activity in platelets of serotonin-deficient mice and was implicated to play a crucial role in other (patho-) physiological processes, such as primary pulmonary hypertension and diabetes (Morecroft et al., 2007; Walther et al., 2003a, 2003b). The reaction is committed by transglutaminases that use primary monoaminergic neurotransmitters/hormones, such as serotonin, dopamine, norepinephrine, and histamine and leads to a highly specific transamidation of glutamine residues in the target proteins. In the case of G proteins, the transamidated glutamine belongs to the catalytic core and the monoaminylation results in a blockade of the GTP hydrolyzing activity (Walther et al., 2003a). Thus, monoaminylated G proteins remain in the signaling-active conformation once they have bound GTP. A major challenge is the identification of potentially monoaminylated proteins. Nine genes encoding transglutaminases are known today. Furthermore, there is increasing evidence that also the large protein disulfide isomerase and thioredoxin families possess transglutaminase activity (Blasko et al., 2004). Our efforts so far allow stating that not only G proteins, but also structural proteins, transcription factors, and a broad variety of enzymes are substrates for these posttranslational modifications. Furthermore, in analogy to protein phosphorylation, all investigated monoaminylated proteins exhibit distinct biological activities before and after their modification (Walther et al., 2007). Hence, protein monoaminylation is a versatile mechanism regulating protein functions that, compared with protein phosphorylation, provide cells with much broader and exquisite fine-tuning capabilities to respond to monoaminergic neurotransmitter/hormone stimulation.

5.

Protein phosphatase 1 binds to metabotropic glutamate receptors and $GABA_C$ receptor rho subunits at retinal synapses

Ralf Enz, Heinrich Sticht, Johann Helmut Brandstätter[1],Nadja Schröder and Melanie Rose

Institut für Biochemie, [1]Institut für Biologie, Friedrich-Alexander-Universität Erlangen-Nürnberg, 91054 Erlangen

Abstract:

An important mechanism to control synaptic signal transduction is the modulation of neurotransmitter receptors by kinases and phosphatases. However, molecular mechanisms that specify the interaction between these proteins are largely unknown. Here, we identified protein phosphatase 1 (PP1) as a binding partner of the metabotropic glutamate receptors mGluR1a, mGluR5a/b and mGluR7b. Confocal laser-scanning microscopy showed that PP1 is present in processes of retinal bipolar cells expressing the interacting glutamate receptors. We mapped interacting domains and identified a conserved amino acid motif (KSV[ST]W) in the intracellular C-termini of mGluR1a, mGluR5a/b and mGluR7b. Using site-directed mutagenesis, pull-down experiments and bioinformatic structure determination, we propose a consensus sequence for PP1 binding proteins. A database search with this sequence yielded several potential PP1 interactors, among them PNUTS, a nuclear targeting subunit of PP1. PNUTS uses different domains to bind $GABA_C$ receptor rhosubunits and PP1, and consequently these three proteins were able to form a ternary complex. Furthermore, we analyzed the structure of PNUTS in contact with the phosphatase by homology-based molecular modeling. In the retina, PNUTS and PP1 are expressed in different cellular compartments, while $GABA_C$ receptors are present at synapses. Because PP1 and $GABA_C$ receptors are co-expressed in axon terminals of rod bipolar cells that express PNUTS, we suggest that PNUTS could act as a temporary bridge between rho subunits and the phosphatase. In summary, this study describes a new consensus sequence for a PP1 binding motif and identifies neurotransmitter receptors as binding partners of this enzyme.

Supported by the Deutsche Forschungsgemeinschaft

6.

Development of culture conditions for live imaging analysis of neuronal survival of aged hippocampal slices.

A. Ghori, C. Tackenberg, R.Brandt

Department of Neurobiology, University of Osnabrueck, Germany

Abstract:

Alzheimer's disease (AD) is a neurodegenerative disease, characterized in the brain by the presence of neurofibillary tangles (NFTs) and amyloid plaques. It is the most common form of dementia in older people above the age of 65. Mouse models in combination with *ex vivo* cultures of organotypic tissue slices have become a very useful way to understand the mechanism of AD. However, in current studies cultures were prepared only from young mice, whereas it may provide better insights to AD by using aged cultures or cultures prepared from aged mice.
A goal of this study was to develop culture conditions to analyze neuronal survival using aged hippocampal slices. Organotypic hippocampal slices were prepared from Naval Medical Research Institute (NMRI) mice of different ages and cultured for different times. Slices were then infected with Sindbis virus to express fluorescent-tagged human wt tau. The droplet method was used to apply the virus and the amount of the virus was varied from 1 µl to 6 µl on each hippocampal slice (corresponding to 2.2×10^5 to 1.3×10^6 infectious particles). The effect of the virus was than studied using confocal laser scan microscopy (cLSM). Our data show that organotypic slices prepared from 7 to 14 days old (P07-P14) mice exhibited sufficient neuronal viability. In contrast, neurons did not survive in mice aged above P14. In the second part of our experiments we prepared hippocampal slices from 7 days old mice (P07). These slices were then cultured for longer periods *in vitro* to prepare aged cultures. The culture period varied from 12- 30 days. Long-term hippocampal slices that were cultured between 12-17 days *in vitro* (DIV) exhibited sufficient neuronal survival. In contrast, slices cultured above 17 days *in vitro* did not survive the infections. Furthermore, our data demonstrated that toxic effects of virus were stronger on hippocampal slices aged *in vitro*.
The data indicate the presence of an optimal time window for preparing aged hippocampal slices useful for virus mediated gene transfer which is between 7-14 days with respect to the age of the mice and a maximum of 17 days *in vitro* culture.

7.

TRPV1 expression-dependent initiation and regulation of filopodial

B. Goswami*, T. Hucho

Signal Transduction in Pain and Mental Retardation, Department of Human Molecular Genetics, Max Planck Institute for Molecular Genetics, Ihnestraße. 73, 14195, Berlin, Germany
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Abstract:

Transient receptor potential vanilloid subtype 1 (TRPV1), a non-selective cation channel, is present endogenously in dorsal root ganglia (DRG) neurons. It is involved in the recognition of various pain producing physical and chemical stimuli. In this work we demonstrate that expression of TRPV1 induces neurite-like structures and filopodia and that the expressed protein is localized at the filopodial tips. Exogenous expression of TRPV1 induces filopodia both in DRG neuron-derived F11 cells and in non-neuronal cells, such as HeLa and HEK cells. We find that some of the TRPV1 expression-induced filopodia contain microtubules and microtubule-associated components, and establish cell-to-cell extensions. Using live cell microscopy we demonstrate that the filopodia are responsive to TRPV1-specific ligands. But both, initiation and subsequent cell-to-cell extension formation, is independent of TRPV1 channel activity. The N-terminal intracellular domain of TRPV1 is sufficient for filopodial structure initiation while the C-terminal cytoplasmic domain is involved in the stabilization of microtubules within these structures. In addition, exogenous expression of TRPV1 results in altered cellular distribution and in enhanced endogenous expression of non-conventional myosin motors, namely myosin IIa and myosin IIIa. These data indicate a novel role of TRPV1 ion channels in regulating the cellular morphology and cellular contact formation.

8.

A transgenic tau hyperphosphorylation model reveals gender-specific neuroprotective effects of Alzheimer's disease-like modified tau

Hundelt, M.[1], Fath, T.[2], Selle, K.[1], Kosfeld, A.[1], Oesterwind, K.[1], Schultz, C.[3], Götz, J.[4], von Engelhardt, J.[5], Monyer, H.[5], Lewejohann, L.[6], Sachser, N.[6], and Brandt, R.[1]

[1]Department of Neurobiology, University of Osnabrück, Germany
[2]The Children's Hospital at Westmead, Australia
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[4]Alzheimer's and Parkinson's Disease Laboratory, Brain and Mind Research Institute, University of Sydney, Australia
[5]Department of Clinical Neurobiology, University of Heidelberg, Germany
[6]Department of Behavioural Biology, University of Münster, Germany

Abstract:

Many mouse models that develop β-amyloid plaques or tau tangles composed of hyperphosphorylated tau exist to study Alzheimer's disease (AD). However, the role of hyperphosphorylation of tau in the progression of the disease is still unsolved. Here we describe a novel transgenic mouse model that was generated by expressing a pseudohyperphosphorylated (PHP) variant of the largest CNS tau isoform (441 aa) at moderate levels in forebrain neurons. The transgenic mice did not show any evidence for neurodegeneration such as activated caspase-3 as a marker for apoptosis, differences in the thickness of the neocortex between the genotypes, or mitochondrial dysfunction measured by the content of ATP-synthase. Furthermore they did not develop highly aggregated states of tau nor did they exhibit differences in behavior or neurologic status. This indicates that moderate levels of modified tau alone are not sufficient to induce neurodegeneration in transgenic mice. However, PHP-tau induced an increased spine density in the hippocampus and neocortex of young male mice. Furthermore, extracellular regulated kinases (ERK1/2) were activated but did not induce increased phosphorylation of tau at phosphorylation sites for ERK (T205, T212). Increased activation of c-Jun N-terminal kinase (JNK) was also detected. ERK1/2 and JNK activation and spine density was increased only in young male transgenic animals, but not in young females. The data suggest a possible protective effect of tau hyperphosphorylation which is gender-specific. It is known that more women than men suffer from AD (1) which would be consistent with a male-specific neuroprotective effect of hyperphosphorylated tau. Previously, a postmortem study has shown activation of ERK or JNK in some cases with Braak stage 0 (non-demented cases lacking any signs of dementia) (2). In Braak stages I and II (non-demented cases with limited pathology) simultaneous activation of ERK and JNK was found. The activation of ERK1/2 that we observed in young male mice expressing PHP-tau together with a less distinct activation of JNK could suggest that these transgenic animals are in an early or pre-disease state corresponding to Braak stages 0-II. Thus with this model it becomes possible to study the effects of (pseudo-)hyperphosphorylation independently from aggregation at conditions which may prevail in an early preaggregation state of the disease.

9.

FRET analysis of the conformation of tau in solution

Sadasivam Jeganathan, Martin von Bergen, Eckhard Mandelkow

Max-Planck-Institute, Dept. Structural Molecular Biology, Hamburg, Germany.

Abstract:

The aggregation of the microtubules associated protein tau into Paired helical Filaments (PHFs) is one of the hallmarks of Alzheimers disease. The physiological function is to bind to microtubules and to stabilize them. Tau belongs to the group of natively unfolded proteins and is largely devoid of secondary structure in the soluble state, as judged by several spectroscopic and biochemical criteria. Nevertheless, the reaction of certain antibodies with discontinuous epitopes has suggested the possibility that tau shows some global conformations. We therefore searched for residual structural elements, using fluorescence resonance energy transfer (FRET) between tryptophan residues and cysteine residues (labeled by IAEDANS) introduced into tau by site directed mutagenesis. We found high FRET between the position 310-Tryptophan and both 291-IAEDANS and 322-IAEDANS. The calculated distances were ~2.2 nm and ~1.9 nm, respectively. Theoretical values, based on a pure random coil model were 36 Å and 28 Å. These differences indicate a local compaction within the second and third repeat of the microtubule binding domain. The results also showed that the repeat domain of tau comes into close vicinity of the C-terminal tail of tau (1.8-2.3 nm), but not to the N-terminus. Surprisingly the N- and the C-terminal ends showed FRET, corresponding to an average distance of ~2.1 nm. We conclude that the natively unfolded protein tau contains preferred long range interactions between the repeat domain and the C-terminus and between the N- and the C-terminus. This arrangement of tau domains and especially the residual structure of the repeat region might provide a docking site which facilitates interaction with microtubules and disturbance of the folding might play a role in the pathological aggregation into Paired Helical Filaments. - Supported by MPG and DFG.

10.

High Constitutive NF-κB Expression in a Novel Adult Neural Stem Cell Line Independent from Exogenous Growth Factors: development into a potential tumour stem cell?

Aljoscha Kaus[1], Darius Widera[1], Susannah Kassmer[2], Christian Kaltschmidt[*3], Barbara Kaltschmidt[*1],

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*: equal contribution

Abstract:

The environmental and genetic signals regulating progressive lineage elaboration in the mammalian brain are yet poorly understood. Moreover, developmental characteristics and profiles of the central nervous system (CNS) stem cells regarding mechanisms of their nearly unlimited self-renewal, lineage restriction, and cellular maturation remain fragmentary. Those properties are characteristical for cancerous cells as well.
We differentiated adult neural stem cells (NSCs) derived from the subventricular zone of adult rats in the absense of growth factors on poly-d-lysin/laminin coated substrates. This procedure normally yields an adherent culture of neurons and glial cells. To our surprise, we were able to harvest non adherent suspension cells maintainable in culture without growth factor supplementation. Detailed analysis by typing of the re-aggregated clusters by Immunofluorescence as well as RT-PCR for expression of stemness markers, such as nestin, musashi, notch1 and notch2 yielded a profile analogous to neural stem cells. Moreover, the cells exerted a proliferative doubling time of about 26h and migration activity in response to MCP-1. We were able to detect a high constitutive NF-κB activity via a Luciferase reporter assay and an increased, aberrant, polyploid DNA content via FACS analysis. Hence, we termed this culture exogene **G**rowth factor **i**ndependent **N**eural **S**tem **C**ells (GiNSC). 9 Single cell derived clones were obtained and cultured for at least 30 passages.
Although they are autonomous of exogenous growth factor supplementation, our GiNSCs bear stemness characteristics and general properties of pluripotent adult neural stem cells. In this regard, GiNSCs may provide a potent tool in cancer research, as their exogene cytokine independent proliferation and their constitutively high NF-κB expression presumes cancerous properties.

11.

Functional neurotoxicity of amyloid beta peptides in cortical network cultures

Evgeni Kirazov[a], Ludmil Kirazov[a], Olaf H.-U. Schroeder[b], Alexandra Gramowski[c], Dieter G. Weiss[c]

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[b] NeuroProof GmbH, Rostock, Germany
[c] Universität Rostock, Institut für Zellbiologie und Biosystemtechnik, Rostock, Germany

Abstract:

Amyloid beta peptides play an essential role in the generation of Alzheimer’s disease. However, there is no clear evidence how these peptides contribute to the pathological genesis. Although there are many reports on neurotoxic effects of soluble amyloid beta peptides, some questions remain still open. There are scarce data describing acute neuro-active effects and quantifying the potency of the neurotoxic effects.
We describe the acute effects of two amyloid beta peptides (1-40 and 1-42) and the biological active fragment 25-35 on the electrical activity of cortical network cultures on microelectrode arrays (MEA-neurochips) characterized by 31 quantitative parameters. With this approach a precise assessment of the influence of the amyloid beta peptides is possible. Our data showed a more sensitive activity decline to fragment 25-35 and 1-42, with a significant decrease at 25nM as the lowest concentration tested. However, 1-40 reveals the stronger and biphasic neuroactive effect at lower concentrations with an EC_{50} at 15µM. All effects were observable within 10 min after application and were reversible upon medium change.
These results lead us to the hypothesis that the rapid neurotoxic effect of increased amyloid beta peptide concentrations on the synaptic function could cause the early memory loss in Alzheimer’s disease, preceding the synaptic loss and neuron degeneration.

12.

The Novel Estrogen Receptor GPR30 Mediates Estrogen-Induced And PKCε - Dependent Mechanical Hyperalgesia In Vitro And In Vivo

Julia A. Kuhn[1], Olayinka A. Dina[2], Jon D. Levine[2], Tim Hucho[1]

[1]Max Planck Institute for molecular Genetics, Ihnestr. 63-73, 14195 Berlin
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Abstract:

The epsilon isoform of protein kinase C (PKCε) is an important second messenger in models of acute as well as chronic mechanical sensitization. Only few receptors have been identified to lead to activation of PKCε (β2-adrenergic receptor, bradykinin receptor). Recently, we reported estrogen to activate PKCε in primary sensory neurons as well as induce PKCε-dependent sensitization in male rats. Which of the known estrogen receptors (estrogen receptor α (ERα), estrogen receptor β (ERβ), G-protein coupled receptor 30 (GPR30)) mediates this effect remained unknown. Here we show, that the agonist of the novel estrogen receptor GPR30, G-1, induces rapid PKCε-translocation in primary nociceptive neurons. Also, ICI 182 780, which acts as an agonist of GPR30 while blocking the classical estrogen receptors ERα and ERβ, leads to fast activation of PKCε. In contrast, the specific agonists of estrogen receptor α, PPT, and estrogen receptor β, DPN, do not have any effect. Western Blot analysis using a polyclonal antiserum directed against the human C-terminus of GPR30 and RT-PCR studies show, indeed, GPR30 to be expressed in rat dorsal root ganglia. Also, in behavioural experiments intradermal injection of G-1 into the dorsum of the paw of male rats induces strong mechanical hyperalgesia in a concentration dependent manner. These data indicate an involvement of the novel estrogen receptor GPR30 in the modulation of nociceptive signaling pathways by sex hormones.

13.

Antisense and RNAi approaches to study the function of TRPV1

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Abstract:

The vanilloid receptor TRPV1 is a central integrator of various noxious stimuli, but its functional relevance in neuropathic pain is still under controversial debate. We compared different approaches for gene silencing to inhibit TRPV1 expression *in vitro* and *in vivo*. In cell culture, siRNAs were found to be up to 1000fold more efficient than phosphorothioate oligonucleotides [1]. Accordingly, repeated intrathecal injections of (unmodified) antisense oligonucleotides were required to reduce pain sensitivity of mononeuropathic rats [2]. In contrast, a single bolus injection of a TRPV1-specific siRNA was sufficient to achieve a significant analgesic effect [3]. A second independent siRNA was used to confirm the biological effect. Mice treated with siRNAs against TRPV1 showed a drastically diminished response to capsaicin given intraperitoneally. Subsequently, transgenic mice expressing shRNAs against TRPV1 were generated [4]. Continuous knockdown of the vanilloid receptor allowed detailed functional investigations, which revealed a lack of capsaicin-induced hypothermia and drastically diminished nocifensive response to capsaicin injected into the hind paw as well as reduced sensitivity towards noxious heat. These results demonstrate the suitability of RNAi approaches for target validation in pain research [5].

14.

Activated Ras in neurons mimics antidepressant activity

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Abstract:

Brain-derived neurotrophic factor (BDNF) is implicated in clinical depression and its treatment. Repeated administrations of antidepressants has been shown to enhance the BDNF expression and phosphorylation of its cognate TrkB receptor [1]. In contrast, stress exposure and depression is associated with downregulation of BDNF [2]. The Ras-mediated extracellular signal-regulated cascade (ERK) pathway is considered as a major BDNF/TrkB intracellular signalling pathway. To investigate the possible contribution of the Ras/ERK-pathway on antidepressant activity we utilized a synRas transgenic mouse model expressing constitutively activated human Ha-Ras in differentiated neurons via the synapsin I promotor [3]. The synRas mice show an elevated level of activated Ras and activating phosphorylation levels of $ERK_{1/2}$ in the cortex and hippocampus. This is associated with an increased density of cortical spines and synapses as well as increased spontaneous synaptic release frequencies and enhancement of specific types of synaptic long-term potentiation [4]. Immunoblotting analysis revealed that chronic fluoxetine administration to wild type mice led to an increased Ras activation followed with subsequent elevation of ERK1/2 phosphorylation thus mimicking the synRas phenotype. Consistently, our results obtained in two animal models of depression show an antidepressant-like behavior of the synRas transgenic mice compared to their wild type littermates. Interestingly, the synRas mice displayed a dramatic reduction of the number of new born cells within the dentate gyrus of the hippocampus [5], indicating that the antidepressive-like behaviour is not linked to increased neural progenitor proliferation. Taken together our data suggests, that neuronal Ras activation results in an antidepressant-like behaviour in mice, with no direct correlation to hippocampal neurogenesis.

15.

Null-mutation of the motorneurodegeneration-associated gene Vps54 leads to embryonic lethatity in mice

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Abstract:

The wobbler mouse is an intensively investigated animal-model for human motor neuron diseases such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). Besides motor neuron degeneration the *wobbler* mutation causes a spermiogenesis defect similar to human globozoospermia. Recently, we identified a point mutation in the vesicle traffic factor Vps54, which is responsible for the motoneurodegenerative phenotype of the wobbler mouse (1). The wobbler point mutation is believed to be a hypomorphic allele of Vps54, thus we generated a Vps54 knock out mouse. Here we demonstrate the effects of the Vps54 null-mutation. Homozygous Vps54 knock out individuals die around day 11.5 of the embryonic development (E11.5). E9.5 mutant embryos are indistinguishable from wild type, while E12.5 Vps54 null embryos are nearly completely resorbed. E11.5 Vps54 null embryos show a retarded development, nearly absent dorsal root ganglia and an abnormal membrane blebbing seen at the luminal surface of the ependyma. Heterozygous Vps54 null mice are normally healthy and fertile, but in about 10 % of the cases they develop a motor neuron disease and males among these show a spermiogenesis defect similar to wobbler males. Interestingly, affected Vps54 null heterozygotes show muscle atrophy predominantly beginning in hind legs, while in case of wobbler mice the muscle atrophy starts in the forelegs.

16.

Expression of the multifunctional protein CAD among cultured glial cells

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Abstract:

The de novo biosynthetic pathway of pyrimidine nucleotides in mammals is initiated by the multifunctional enzyme CAD. CAD has three enzymatic activities: i) Glutamine-dependent carbamoyl phosphate synthetase; ii) aspartate transcarbamoylase and iii) dihydroorotase. To study the capability of glial cells to initiate the de novo synthesis of pyrimidine nucleotides the distribution of CAD among cultured glial cells was investigated immunocytochemically. Astroglia- and ependymocyte-rich primary cultures were prepared from new-born rat brains; oligodendroglia- and microglia-rich secondary cultures were derived from astroglia-rich primary cultres. Double immunofluorescence labelling of cultured cells was carried out with a rabbit anti-CAD serum in combination with antibodies against glial cell type specific marker molecules. In each of these cultures the majority of the cells showed CAD-specific immunofluorescence in the cytosol. Since, these cells could also be stained with fluorescence labelled isolectine B_4 from Griffonia simplicifolia or with antibodies against glial fibrillary acidic protein, or antibodies against ciliar alpha-tubulin, or antibodies against galactocerebroside, they were identified as microglial cells, astrocytes, ependymocytes or oligodendrocytes, respectively. These results indicate that astrocytes, ependymocytes, oligodendrocytes and microglia in culture are expressing CAD. The presence of CAD, in turn, strongly suggest that these glial cells are capable of carrying out the initial steps of de novo biosynthesis of pyrimidine nucleotides.

17.

Neurofibrillary tangles and neuronal loss in an inducible transgenic mouse model expressing a mutant repeat domain of tau

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Abstract:

Accumulations of tau intracellular deposits are characteristic for tauopathies and Alzheimer's disease. In order to understand the toxicity of tau aggregation and the reversibility of the pathologic processes we generated inducible transgenic mouse lines, which express either the 4-repeat tau domain with the FTDP-17 mutation Delta-K280 (TauRD/DeltaK280 - "pro-aggregation mutant") or the 4-repeat tau domain with Delta-K280 deletion and two proline mutations in the hexapeptide motifs (TauRD/DeltaK280/I277P/I308 - "anti-aggregation mutant"). The DeltaK280 mutation is known to accelerate the aggregation of tau, but the inserted proline residues inhibit the tau aggregation in vitro and in cell models. Inducible transgene expression in mice was driven by a forebrain-specific CaMKII promoter in a Tet-Off system and can be suppressed by doxycycline. The pro-aggregation mutant showed aggregated tau in sarkosyl insoluble fractions and Gallyas silver stained neurofibrillary tangles from 3 months onwards, even though the level of the human tau protein was low (less than endogenous mouse tau). Tau preparations from pro-aggregation mutant mice revealed PHFs by electron microscopy. Consistent with the tau pathology the neuronal loss was age-dependent and visible in the dentate gyrus as early as 5 months. The immunohistochemisty results showed phosphorylated tau at S262 missorted into the somatodendritic compartment of cortical and hippocampal neurons. By contrast, the anti-aggregation mutant with a similar expression level as the pro-aggregation mutant did not show aggregated tau or neuronal loss. The level of synaptophysin, a presynaptic marker and the number of spine-synapses were reduced in the stratum radiatum of the pro-aggregation mutant, but not of the anti-aggregation mutant. Six weeks of switching off the tau transgene in the pro-aggregation mutant lead to ~90% reduction in the level of soluble human tau protein, to complete reversal of the pathological somatodendritic localization and of phosphorylation in the repeat domain, but the aggregation was only partly reversed. - Supported by MPG and DFG.

18.

Prevention of tau aggregation in vitro and in cells by low MW compounds derived from a phenylthiazolyl-hydrazide core.

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Abstract:

Tau protein forms anomalous aggregates ("paired helical filaments") in Alzheimers disease and other brain diseases ("tauopathies"). This is considered to be a possible cause of neurodegeneration. We are therefore searching for compounds which are capable to inhibit and reverse the aggregation of tau protein. The search involves screening of libraries of chemical compounds for their effects on the assembly and disassembly of paired helical filaments (characterized by the assembly-inhibition and disassembly-inducing half-maximal concentrations IC50 and DC50). Previous screens have revealed several active compounds which fall into different chemical groups. Based on these results and after performing an in silico screen we have now developed a new lead compound by defining a phenylthiazolyl-hydrazide-based core structure and then modifying it. The phenylthiazolyl-hydrazide (PTH) derivatives were synthesized and tested for their activities of inhibiting PHF assembly or inducing PHF disassembly. About 26% of the tested compounds showed an improvement of the inhibitory potential. The results were used to build up structure-activity relationships and to optimize the reagents with regard to PHF inhibition and cell viability. Analysis via saturation transfer difference NMR revealed strong epitope interactions of this compounds with the tau protein. In addition we used a cell model of tau pathology, based on the inducible aggregation of tau protein, which allows one to test whether compounds which inhibit tau polymerisation in vitro are also beneficial for cells. The results show that cellular "tau amyloidosis" can be reduced by inhibitor compounds and that the viability of the neurons can be improved. - Supported by MPG, DFG and ISOA.

19.

Spatial-temporal differences of the expression profiles of the GCAP isoforms in the zebrafish retina

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Abstract:

Guanylate cyclase-activating proteins (GCAPs) are small neuronal calcium sensor proteins that regulate guanylate cyclase (GC) activity in vertebrate photoreceptor cells. An unexpected diversity of GCAP isoforms has recently been discovered in the zebrafish retina (1). In particular zebrafish cones express a large variety of several isoforms designated as *zGCAP3, zGCAP4, zGCAP5* and *zGCAP7*. However, their biochemical properties and their physiological function are not well understood. In order to understand the role of zGCAP isoforms in cone function we first investigated the spatio-temporal expression patterns of these isoforms in the developing zebrafish retina. RT-PCR analyses were performed with isoform specific primers in eye preparations from larvae at different developmental stages and demonstrated the early appearance of the mRNA transcripts from *zGCAP3, 4* and *7*. Localization of mRNA transcripts by *in situ* hybridizations showed a dynamic pattern for *zGCAP3*, which is first detectable around 72 hours post fertilization, *zGCAP4* was detected in a few photoreceptor cells on 4 days post fertilization (dpf) and *zGCAP7* transcripts were detected in a *zGCAP4* related pattern on day 6. Transcripts of the *zGCAP5* isoform were detected in young larval stages till day 12 neither by RT-PCR nor by *in situ* hybridization. Furthermore, cone specific zGCAP isoforms exhibited differences in their spatio-temporal expression profiles during development of the retina. We examined developmental stages of zebrafish larvae between 3 and 15 dpf with respect to the spatial exression of zGCAP isoforms. Within this time window cone photoreceptors develop and mature, so the function of the visual system is attributed to cones only. In the present study the expression profiles of zGCAPs is compared with the expression profiles of other cone specific proteins involved in the phototransduction process. Finally, biochemical analyses with heterologously expressed and purified zGCAP4 showed that it strongly activates the membrane bound bovine photoceceptor GC in a calcium-dependent fashion and that it undergoes a calcium-induced conformational change in the physiological range of cytoplasmic calcium. Our results indicate that zGCAP isoforms exhibit an isoform specific expression pattern. Properties of zGCAP4 are consistent with a key role of this calcium sensor in cone light adaptation.

20.

Synaptic transmission and electrical activity in cortical neuronal networks in vitro following inhibition of axonal transport by nocodazole

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Abstract:

It is the aim of this study to clarify the consequences on synaptic transmission of axonal transport blockage over several hours. The questions to be answered are: is transmission inhibited or modified by an inhibition of synaptic vesicle supply; what is the time scale of the changes; are microtubule or actin filament-based mechanisms at work? The study was performed on neuronal networks in vitro grown from dissociated murine frontal cortex cells (day E15) on micro-electrode arrays. Cells organize into a neuronal network that shows spontaneous electrical activity and that is amenable to video-microscopic analysis of organelle movement in the axons. The cytoskeleton was influenced by dissociating microtubules by nocodazol or actin filaments by cytochalasin.
The following results were obtained:

1. AVEC-DIC video-microscopy allowed observation and quantification of organelle movements in cell processes.
2. Nocodazol destroys the native microtubule organization and causes the appearance of MAP2-containing dendritic and axonal swellings.
3. Nocodazol abolishes within minutes the movements of all organelles except some mitochondria movements.
4. Cytochalasin has no obvious direct influence on axonal transport.
5. The inhibition of axonal transport causes little if any changes of the overall electrical activity of the neuronal networks within the first 8 to 10 hours.

The results are discussed in terms of the possibility of sufficient local transmitter synthesis, the degree of involvement of electrical synapses, and a potential later breakdown of electrical activity. Whether the fine details of the electrical activity patterns are changed during transport inhibition is presently analyzed. Long term studies to clarify the further time course are the next steps to be performed.

21.

Transcriptional response to muscarinic acetylcholine receptor stimulation: Regulation of Egr-1 biosynthesis by Elk-1, MKP-1 and calcineurin in carbachol-stimulated human neuroblastoma cells

Oliver G. Rössler, Isabell Henß, and Gerald Thiel

Abstract:

Carbachol-mediated activation of type M3 muscarinic acetylcholine receptors induces the biosynthesis of the transcription factor Egr-1 in human SH-SY5Y neuroblastoma cells involving elevated Ca2+ levels, protein kinase C and extracellular signal-regulated protein kinase. Using a lentivirus-based technique to implant an Egr-1-responsive reporter gene reporter gene into the chromatin of SH-SY5Y cells we show that biologically active Egr-1 is synthesized in carbachol stimulated neuroblastoma cells. Carbachol triggered the phosphorylation of the ternary complex factor Elk-1, a key transcriptional regulator of serum response element-driven gene transcription, and strikingly enhanced the transcriptional activation potential of Elk-1. Chromatin immunoprecipitation experiments revealed that Elk-1 binds in vivo to the 5´-upstream region of the Egr-1 gene in carbachol-stimulated neuroblastoma cells. Together, these data indicate
that Elk-1 connects the intracellular signaling cascade elicited by activation of M3 muscarinic acetylcholine receptors with the transcription of the Egr-1 gene. Lentiviral-mediated expression of either MAP kinase phosphatase-1 (MKP-1) or a constitutively active mutant of calcineurin A inhibited Egr-1 biosynthesis following carbachol stimulation, indicating that these phosphatases function as shut-off devices of muscarinic acetylcholine receptor signaling. Additionally, carbachol stimulation increased transcription of a chromatin-embedded collagenase promoter/reporter gene, showing that AP-1 activity is enhanced in carbachol-stimulated neuroblastoma. Expression experiments revealed that both MKP-1 and a constitutively active mutant of calcineurin A impaired carbachol-induced upregulation of AP-1 activity. The fact that carbachol stimulation activates the transcription factors Egr-1 and AP-1 in neuroblastoma cells suggests that changes in the gene expression pattern are an integral part of muscarinic acetylcholine receptor signaling.

22.

Toxicity by soluble oligomers of β-amyloid(1–42) on cholinergic SN56.B5.G4 cells is mediated through mitogen-activated protein kinase (MAPK) signaling

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Abstract:

Impairments in central cholinergic transmission represent a consistent feature of Alzheimer´s disease. To reveal whether β-amyloid (Aβ) displays a particular toxicity for cholinergic neurons, the cholinergic cell line SN56.B5.G4 created by fusion of N18TG2 neuroblastoma cells with cholinergic neurons derived from septal regions of 21-day-old C57/Bl6 mice (a kind gift by Dr. Bruce H. Wainer, Atlanta, USA) was used as a model. The influence of Aβ(1-42) on cholinergic cell viability was determined by MTT reduction assay, demonstrating a significantly reduced cell survival by about 68% (compared to vehicle-treated control cells) following exposure of differentiated SN56.B5.G4 cells by 50 µM fresh-made Aβ (1-42) for 24h. Using Western analysis, a time-dependent transient phosphorylation of c-Jun was observed, being highest at two hours of incubation by Aβ(1-42), while phosphorylation of JNK, ERK, and p38 were not affected, at least up to 24h of Aβ exposure. The protein expression levels of non-phosphorylated JNK and p38 were not altered by Aβ, but that of ERK expression which steadily increased with exposure time. To further disclose whether Aβ-induced cell toxicity is mediated through activation of MAPK signaling, cholinergic cells were exposed to Aβ(1-42) in the presence of selective MAPK inhibitors. While inhibition of ERK signaling by PD98059, and of JNK by SP600125 hardly affected Aβ-induced cell toxicity, inhibition of p38 by PD169316 prevented Aβ-induced cholinergic cell death. The data suggest a major role of p38 signaling in mediating Aβ-induced loss of cholinergic SN56.B5.G4 cells, and should be considered as a potential target for neuroprotective measures.
Supported by Interdisciplinary Center for Clinical Research (IZKF), University of Leipzig (TPC29) to R.S.

23.

Vesicle traffic is critical for the motor neuron disease of Vps54 mutant wobbler mice

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Abstract:

The wobbler mouse is an intensively investigated animal-model for human motor neuron diseases such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). In the course of positional cloning of the *wobbler* mutation we identified a pointmutation in *Vps54* gene. A transgenic rescue experiment proved *Vps54* to be the *wobbler* gene and thus, critical for motor neuron degeneration (1). The *Vps54* gene encodes a highly conserved vesicle traffic factor. Vps54 protein is a component of the "GARP" (Golgi-associated retrograde Protein) complex, a vesicle tethering factor involved in the retrograde vesicle transport from early and late endosomes to the trans Golgi network. In wobbler mice a single amino acid exchange in the c-terminus of Vps54 leads, besides the signs of neurodegeneration, to enlarged endosomal structures in the degenerating motor neurons. In addition, we observed a functional impairment of the retrograde vesicle traffic in cells derived from wobbler- and Vps54 knock out embryos by analyzing choleratoxin uptake and transport. Furthermore, we show the effects of vesicle traffic impairment on the sorting of mannose-6-phosphate receptors and on the accumulation of intermediate filaments and amyloid precursor protein.

24.

The CXCL16/CXCR6 axis in normal and malignant astroglial cells

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Abstract:

Chemokines have been identified as chemotactic molecules for leukocytes. Nevertheless, it has been recognized that they also play a role in the physiology and pathology of the brain. CXCL16 is a transmembrane chemokine that mediates chemotaxis and cell- cell adhesion by interaction with its receptor CXCR6/Bonzo. Here we report on the expression and shedding of this chemokine in the brain *in situ* and *in vitro*. By quantitative RT-PCR and immunohistochemistry we show that CXCL16 is highly upregulated in human glioma tissue, while its expression in normal brain is low and mainly restricted to brain endothelial cells. In cultivated human glioma cells as well as in activated mouse astroglial cells, CXCL16 mRNA and protein are constitutively expressed and can be further upregulated by tumour necrosis factor α and interferon γ. CXCL16 is continuously released from glial cells by proteolytic cleavage which is rapidly enhanced by stimulation with phorbol-12-myristate-13-acetate (PMA). As shown by the use of characterized inhibitors, two distinct members of the disintegrin-like metalloproteinase family, ADAM10 and 17, are involved in the constitutive and PMA-induced shedding of glial CXCL16. In addition to the chemokine, its receptor CXCR6 could be detected by quantitative RT-PCR in human glioma tissue, cultivated murine astrocytes and in microglial cells. Interestingly, its expression can be upregulated by its own ligand, CXCL16. Functionally, recombinant soluble CXCL16 enhanced proliferation of CXCR6-positive murine glial cells, an effect probably mediated by signalling via the Akt/ PI3K- pathway. Thus, the transmembrane chemokine CXCL16 is expressed in the brain by malignant and inflamed astroglial cells, shed to a soluble form and acts as autocrine and paracrine growth factor for glial cells themselves.

25.

The Dual Nature of the Nerve Growth Inhibitor Nogo-A

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Abstract:

Reticulons (RTN) are a large family of transmembrane proteins present throughout the eukaryotic kingdom in virtually every cell type. Despite their wide distribution, their cellular function is still mostly unknown. RTN4, also termed Nogo, comes in three isoforms, Nogo-A, -B, and -C. Nogo-A is expressed in glia and neurons of the central nervous system (CNS) and localizes to the plasma membrane and, predominantly, to the tubular endoplasmic reticulum (ER). When translocated to the glial surface Nogo-A acts as major inhibitor of nerve growth in the adult CNS. ER-associated neuronal Nogo-A, however, promotes dendritic growth and arborization, as we have shown recently, indicating a dual functionality of Nogo-A based on differential subcellular localization. We mapped this effect of ER-associated Nogo-A to a central domain, called NiG. In order to elucidate the underlying mechanisms, we use NiG as bait and screen for ER-specific interaction partners from CNS tissue in a proteomics based approach.

26.

Functional interaction of Aß and tau pathology in an ex vivo model of the hippocampus

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Abstract:

Amyloid plaques composed of aggregated Aß and neurofibrillary tangles (NFTs) that consist of hyperphosphorylated tau (HP tau) protein are histopathological hallmarks of Alzheimer's disease (AD).
To determine a potential role of HP tau in AD pathology, we constructed a pseudohyperphosphorylated (PHP) tau, which mimics key structural and functional aspects of AD-like HP tau protein (Eidenmüller *et al,* 2000). EGFP tagged tau constructs were cloned into Sindbis virus to allow efficient expression in neurons.
To analyze the role and a potential interaction between tau and Aß pathology, organotypic hippocampal slices were prepared from APP transgenic C57BL6 mice and infected with the Sindbis Virus constructs.
Confocal low resolution live imaging was used to determine Aß and tau mediated neurotoxicity. PHP tau caused a stronger neurodegenerative effect than wt tau on a non transgenic background.
Furthermore, Aß increased toxicity of wt tau more than toxicity of PHP tau, suggesting that Aß leads to wt tau hyperphosphorylation and therewith induces neurotoxicity.
The toxicity of tau hyperphosphorylation on APP background is comparable to toxicity of PHP tau confirming that PHP tau is a good model to study effects due to tau hyperphosphorylation.
High resolution imaging of dendritic spines of hippocampal CA1 and CA3 neurons was used to determine a potential alteration of synaptic integrity by Aß.
Neurons from APP mice showed a significant decreased spine density. This effect was partially abolished when cultures were treated with DAPT, a known gamma-secretase inhibitor. Algorithm based analysis of spine shape (Shahani *et al,* 2006) revealed no alteration of spine morphology by Aß.

27.

Gonadotropin-releasing hormone receptor stimulation upregulates ATF3 biosynthesis in pituitary cells involving a cytosolic Ca^{2+} rise, activation ERK and expression of Egr-1

Sabine I. Mayer, Verena Dexheimer and Gerald Thiel

Abstract:

ATF3 belongs of the family of basic region leucine zipper transcription factors. Here, we show that buserelin, a gonadotropin-releasing hormone (GnRH) receptor-specific ligand, induces a signaling cascade leading to the biosynthesis of ATF3. Buserelin-triggered ATF3 biosynthesis was attenuated by the mitogen-activated protein kinase kinase inhibitor PD98059 and by BAPTA-AM, the acetoxymethylester of the cytosolic Ca^{2+} chelator BAPTA. These results indicate that phosphorylation and activation of extracellular signal-regulated protein kinase ERK and elevated levels of intracellular Ca^{2+} are essential for buserelin-induced upregulation of ATF3 expression. Lentiviral-mediated expression of either MAP kinase phosphatase-1 (MKP-1) or a constitutively active mutant of calcineurin A inhibited ATF3 biosynthesis following buserelin stimulation, indicating that these phosphatases function as shut-off devices of GnRH receptor signaling. Stimulation of pituitary cells with buserelin also induced the biosynthesis of Egr-1. Expression of a dominant-negative mutant of Egr-1 reduced the buserelin-induced upregulation of ATF3 expression, indicating that Egr-1 connects the intracellular signaling cascade elicited by activation of GnRH receptors with the transcription of the ATF3 gene. Moreover, the regulation of ATF3 transcription by Egr-1 suggests that Egr-1 influences cAMP response element mediated transcription via the regulation of ATF3.

28.

Stepwise proteolysis liberates tau fragments that nucleate the aggregation of tau in a neuronal cell model

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Abstract:

Tau is a highly soluble protein, and yet it aggregates abnormally in Alzheimer's disease. This begs the question of what factor(s) cause the pathological aggregation. It is known that in vitro, tau fragments derived from the microtubule-binding repeat domain aggregate more readily than full-length tau. This suggests the possibility that fragmentation of tau by proteolysis could lead to the nucleation and aggregation of full-length tau; however, the mechanism and pathway of such a fragmentation has remained ill-defined. We have addressed this problem in neuronal cell models (N2a cells) expressing the repeat domain of tau or tau mutants in an inducible fashion (Khlistunova et al., JBC 2006). The cells show abnormal aggregation of tau similar to Alzheimer aggregates, but notably the aggregation is preceded by fragmentation. We have followed the fate of the pre-aggregation fragments and determined the cleavage sites. Fragments are generated in a step-wise fashion, starting with a cleavage near the N-terminus of the tau construct, followed by successive cleavages near the C-terminus. The N-terminal cleavage is a prerequisite for further processing, suggesting that it opens up a conformation which becomes more susceptible to further cleavage. Fragments generated by cleavage near both ends are highly competent to aggregate and can nucleate the aggregation of full-length tau, both in vitro and in the cell models. Both the C-terminal truncation and the co-aggregation of fragments with full-length tau depend on the propensity for β-structure. The aggregation is modulated by phosphorylation but does not depend on it. In the cells, aggregation is toxic, but not fragmentation as such; conversely, toxicity can be prevented by inhibiting either aggregation or proteolysis. The results reveal a novel pathway of abnormal tau aggregation in cells.

- Supported by MPG and DFG.

29.

Neuronal networks grown on microelectrode arrays and their use for studies on physiological and biochemical influences of neuro-active substances

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Abstract:

Means allowing a complete study of cell and tissue functions and its morphology and dynamics *in vitro* are required for future attempts in cell physiology, pharmacology and toxicology. This is especially important in neurobiology, where research has to be focussed not only on successfully growing electrically active neuronal ensembles *in vitro* and on their long-term survival, but in addition on gaining electrical contact to the cells and on reliable long-term cell-electrode coupling.

We culture functional neuronal networks from dissociated embryonic mouse spinal cord or brain areas directly on glass/ITO- or silicon-based 64 electrode arrays with stable cell-electrode coupling for many months. This allowed the monitoring of the onset of electrical activity, of bursting activity stabilization and of the development of histiotypic native or drug-modified electrical activity patterns. A multi-parametric live cell imaging approach with state-of-the-art high resolution video, confocal, DIC and digital fluorescence microscopy was established in addition as test battery to monitor fine morphology and its dynamics, to distinguish the different glial and neuronal cell types, their axonal and dendritic processes and to correlate the pharmacological impairment of electrical activity with Ca^{2+} level oscillations. Electrical network activity is classified and characterized at the level of spike and burst patterns yielding dose-response curves of 31 or more activity-describing variables for monitoring the effects of defined network activity states. The system allows highly complex studies of cellular behaviour which give new insight into the molecular mechanisms of drug action. We showed that neuronal networks in vitro are suitable to study network electrical activity maturation (weeks 1 to 4), the long-term functioning of the active networks (many months) and a precise characterization of activity changes caused by different states of altered neurochemistry. Examples will be given for chemicals acting at the $GABA_A$, $GABA_B$ and the serotonin receptors.

The system is used for analyzing the mechanisms of action of neuro-active chemicals and for drug screening. It yields not only a very detailed description of the electrical communication in the network but also allows a distinction between cytotoxicity of neural cells (irreversible damage and cell loss) and functional neurotoxicity of compounds interacting temporarily with receptors and ion channels.

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