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LECTURE PRESENTATIONS

L1

MicroRNA-target interactions in neurodegenerative diseases

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SpringerPlus 2015, **4(Suppl 1):**L1

MicroRNAs (miRNAs) are short, 22–25 nucleotide long transcripts that may suppress entire signaling pathways by interacting with the 3′-untranslated region (3′-UTR) of coding mRNA targets, interrupting translation and inducing degradation of these targets. The long 3′-UTRs of brain transcripts compared to other tissues predict important roles for brain miRNAs. Supporting this notion, we found that brain miRNAs co-evolved with their target transcripts, that non-coding pseudogenes with miRNA recognition elements compete with brain coding mRNAs on their miRNA interactions, and that Single Nucleotide Polymorphisms (SNPs) on such pseudogenes are enriched in mental diseases including autism and schizophrenia, but not Alzheimer's disease (AD). Focusing on evolutionarily conserved and primate-specific miRNA controllers of cholinergic signaling ('CholinomiRs'), we find modified CholinomiR levels in the brain and/or nucleated blood cells of patients with AD and Parkinson's disease, with treatment-related differences in their levels and prominent impact on the cognitive and anti-inflammatory consequences of cholinergic signals. Examples include the acetylcholinesterase (AChE)-targeted evolutionarily conserved miR-132, whose levels decline drastically in the AD brain. Furthermore, we found that interruption of AChE mRNA's interaction with the primate-specific CholinomiR-608 in carriers of a SNP in the AChE's miR-608 binding site induces domino-like effects that reduce the levels of many other miR-608 targets. Young, healthy carriers of this SNP express 40% higher brain AChE activity than others, potentially affecting the responsiveness to AD's anti-AChE therapeutics, and show elevated trait anxiety, inflammation and hypertension. Non-coding regions affecting miRNA-target interactions in neurodegenerative brains thus merit special attention.

Keywords Alzheimer's disease, microRNAs, pseudogenes

L2

Reacquisition of cocaine conditioned place preference and its inhibition by previous social interaction: Neurochemical and electrophysiological correlates in the nucleus accumbens corridor

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SpringerPlus 2015, **4(Suppl 1):**L2

The nucleus accumbens has long been a major target for studies on the rewarding effects of drugs of abuse or physiological reinforcers, whereas the brain regions medial of the medial accumbens shell have received less attention. We investigated if counterconditioning with dyadic (i.e., one-to-one) social interaction, a strong inhibitor of the subsequent reacquisition of cocaine conditioned place preference (CPP), differentially modulates the activity of the diverse brain regions oriented along a mediolateral corridor reaching from the interhemispheric sulcus to the anterior commissure, i.e., the nucleus of the vertical limb of the diagonal band, the medial septal nucleus, the major island of Calleja, the intermediate part of the lateral septal nucleus, and the medial accumbens shell and core. EGR1 activation was predominantly found in dynorphin-labeled cells, i.e., presumably D1 receptor-expressing medium spiny neurons (D1-MSNs), with D2-MSNs (immunolabeled with an anti-DRD2 antibody) being less affected. Cholinergic interneurons or GABAergic interneurons positive for parvalbumin, neuropeptide Y or calretinin were not involved in these CPP-related EGR1 changes. Glial cells did not show any EGR1 expression either. Cocaine conditioning increased the spike frequency of neurons in the septal nuclei, whereas social interaction conditioning increased the spike frequency in the nucleus accumbens compared to saline control animals. In addition, social interaction conditioning decreased the amount of active neuron clusters in the nucleus accumbens.The present findings could be of relevance for the therapy of impaired social interaction in substance use disorders, depression, psychosis, and autism spectrum disorders.

Acknowledgements This work was supported by the Austrian Science Fund (FWF) grants W1206-B18 (to AS), P26248-B24 (to GZ) and P23824-B18 (to RER)

Keywords Addiction, medium spiny neurons, behaviour

L3

Copper metabolism of astrocytes

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SpringerPlus 2015, **4(Suppl 1):**L3

Copper is an essential trace element which is involved in many important cellular functions [1]. However, excess of copper can impair cellular functions by copper-induced oxidative stress. In brain, astrocytes are considered to play a prominent role in antioxidative defence as well as in the copper homeostasis [1,2]. To investigate uptake, toxicity, storage and export of copper in astrocytes, we used primary rat astrocyte cultures as model system. Cultured astrocytes efficiently take up copper ions predominantly by the copper transporter Ctr1 and the divalent metal transporter DMT1. In addition, copper oxide nanoparticles are rapidly accumulated by astrocytes, most likely by endocytotic processes. Astrocytes tolerate moderate increases in intracellular copper contents very well. However, if the specific cellular copper content exceeds after exposure to copper or copper oxide nanoparticles a threshold level of around 10 nmol copper/mg protein, accelerated production of reactive oxygen species and compromised cell viability were observed. Upon exposure to sub-toxic concentrations of copper ions or copper oxide nanoparticles, astrocytes increase their copper storage capacity by upregulating the cellular contents of glutathione and metallothioneins. In addition, cultured astrocytes have the capacity to export copper ions which is likely to involve the copper-transporting ATPase 7A. The ability of astrocytes to efficiently accumulate, store and export copper ions suggests that astrocytes play a key role in brain copper homeostasis and that an impairment of astrocytic functions may be involved in diseases which are connected with disturbances in brain copper metabolism.

Keywords Astrocytes, metals, oxidative stress

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L4

Altered developmental neuroplasticity due to polysialyltransferase ST8SiaII deficiency in mice leads to schizophrenia-like phenotype

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SpringerPlus 2015, **4(Suppl 1):**L4

Post-translational modification of the neural cell adhesion molecule (NCAM) by polysialic acid (PSA) is crucial for nervous system development and brain plasticity. PSA attachment is catalyzed by the two polysialyltransferases, ST8SiaII and ST8SiaIV. ST8SiaII dominates during embryonic and early postnatal development while ST8SiaIV is the prevailing enzyme of the juvenile and adult brain. A growing body of evidence links aberrant levels of NCAM and PSA to neuropsychiatric disorders, including schizophrenia. To investigate whether polysialyltransferase deficiency might cause a schizophrenia-like phenotype, ST8SiaII–/– mice, ST8SiaIV–/– mice and their wild-type littermates were assessed neuroanatomically and subjected to tests of cognition and sensory functions. ST8SiaII–/– but not ST8SiaIV–/– mice displayed enlarged lateral ventricles and a size reduction of the thalamus accompanied by a smaller internal capsule and a highly disorganized pattern of thalamocortical and corticothalamic fibers. Loss of ST8SiaII was associated with reduced phosphorylation of fibroblast growth factor receptor 1 in the frontal cortex of newborn mice and retarded neuronal differentiation of newly generated cells in the dentate gyrus of adults. ST8SiaII–/– and ST8SiaIV–/– mice were both impaired in short- and long-term recognition memory, but only ST8SiaII–/– mice displayed impaired working memory and a deficit in prepulse inhibition, which could be attenuated by clozapine treatment. Furthermore, ST8SiaII–/– mice exhibited anhedonic behavior and increased sensitivity to amphetamine-induced hyperlocomotion. These data reveal that reduced polysialylation in ST8SiaII–/– mice leads to pathological brain development and schizophrenia-like behavior. We therefore propose that ST8SiaII deficiency has the potential to cause a neurodevelopmental predisposition to schizophrenia.

Acknowledgements The study was supported by the EU 6FWP grant LSHM-CT-2005-512012.
Keywords Neural cell adhesion molecule, sialyltransferase, neuroplasticity

L5

Two steps forward, one step back: successes and failures in structure-based discovery of GPCR ligands

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SpringerPlus 2015, **4(Suppl 1):**L5

G protein–coupled receptors (GPCRs) are intensely studied as drug targets and for their role in signaling. High-resolution crystal structures of GPCRs capturing different receptor conformations are now available, which have provided insights into the mechanism of activation and ligand selectivity for this important class of drug targets. I will first present a series of structure-based screens for novel ligands of the A2A adenosine receptor (A2AAR), which is a drug target for Parkinson's disease (antagonists) and ischemia (agonists). As crystal structures for both inactive- and active-like receptor conformations of the A2AAR have now been determined, molecular docking screens for novel ligands can be performed. Virtual screens against different conformations of the A2AAR were carried out to investigate if structure-based methods can be used to identify agonists and antagonists. Our results shed light on the importance of access to crystal structures and the role of the chemical library in screens for ligands with specific pharmacological properties. For most GPCRs, no experimental coordinates are available and structure-based screens are forced to rely on homology models. However, it is still unclear if models of GPCRs are sufficiently accurate to be used in ligand discovery. The determination of crystal structures for dopamine and serotonin receptors, and the challenges to the community to predict these in the GPCR Dock competitions, have enabled us to carry out comparisons of ligand discovery from models versus crystal structures. Our results from these challenges reveal opportunities and limitations of the use of homology models in ligand discovery and design of selective lead candidates.

Acknowledgements Symposium organized by COST action CM1207 – GLISTEN.
Keywords G protein-coupled receptors, chemical biology, virtual screening

L6

Comparison of A1 and A2A receptor dynamics using FRET based receptor sensors

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SpringerPlus 2015, **4(Suppl 1):**L6

Recently published crystal structures of the adenosine A2A receptor in the active and inactive state have revealed static endpoints of the conformational changes associated with the activation process. To investigate the activation dynamics of different adenosine receptor subtypes we used fluorescence resonance energy transfer (FRET) measurements of a modified A1 and A2A receptor construct (A1R, A2AR). Those optical probes where designed by fusion of the cyan fluorescent protein (CFP) to the C-terminus of the receptor and insertion of the fluorescein arsenical hairpin binder (FlAsH) motif into the 3rd intracellular loop. Based on the ligand binding pocket revealed from the crystal structure 10 optical probes including individual mutations were created for each receptor. To compare A1R and A2AR dynamics, we established HEK293 cell lines stably expressing these optical probes and investigated the signal amplitude and the receptor activation kinetics in living cells. We indentified 3 different effects of these mutations. One class causes problems in membrane localization of the A1R but not the A2AR. The 2nd group is involved in binding of the ribose moiety and has stronger effects in the A1R compared to the

A2AR. The 3rd class consists of the mutants that are involved in binding of the adenine moiety and have similar effects for adenosine and theophylline binding for the A2AR. Thus, our study provides evidence that amino acids serve different functions within the A1R and A2AR ligand binding pocket. In summary the different signal amplitudes and different activation kinetics are indicative for a different activation behavior of the A1R and A2AR and the data from the receptor mutants support these findings and gives new insight into the A1R- structure.
Acknowledgements Symposium organized by COST action CM1207 – GLISTEN.
Keywords Receptor dynamics, adenosine receptor, FRET

L7

Defining the organizational structure of dopamine and muscarninic acetylcholine receptors

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SpringerPlus 2015, **4(Suppl 1):**L7

G protein-coupled receptors, including the M3 muscarinic acetylcholine receptor and the dopamine D3 receptor, can form homo-oligomers. However, the basis of these interactions and the overall organizational structure of such oligomers are poorly understood. Combinations of site-directed mutagenesis and homogenous time-resolved FRET studies that assessed interactions between receptor protomers at the surface of transfected cells indicated important contributions of regions of transmembrane domains I, IV, V, VI and VII, as well as intracellular helix VIII, to the overall organization. Molecular modelling studies based on both these results and an X-ray structure of the inactive state of the M3 receptor bound by the antagonist/inverse agonist tiotropium were then employed. The results could be accommodated fully by models in which a proportion of the cell surface M3 receptor population is a tetramer with rhombic, but not linear, orientation. This is consistent with previous studies based on spectrally-resolved, multi-photon FRET. Modelling studies suggest, furthermore, an important role for molecules of cholesterol at the dimer + dimer interface of the tetramer, consistent with the presence of cholesterol at key locations in many G protein-coupled receptor crystal structures. Mutants that displayed disrupted quaternary organization were often poorly expressed and showed immature N-glycosylation. Sustained treatment of cells expressing such mutants with the muscarinic receptor inverse agonist atropine increased cellular levels and restored both cell surface delivery and quaternary organization to many of the mutants. These observations suggest that organization as a tetramer may occur before plasma membrane delivery and may be a key step in cellular quality control assessment.
Acknowledgements Symposium organized by COST action CM1207 – GLISTEN.
Keywords Dopmaine, acetylcholine receptor structure

L8

Investigation of GPCR allosterism and dimerization in single living cells using fluorescent ligands

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SpringerPlus 2015, **4(Suppl 1):**L7

Previous work, using fluorescent adenosine receptor agonists and antagonists, has provided novel insights into the allosteric regulation of adenosine A3 (A3AR) and A1 (A1AR) receptors by allosteric ligands and receptor dimerization in single living cells [1,2]. We have also used a fluorescent analogue of CGP12177 to investigate ligand binding to the human β1-adrenoceptor. This work has demonstrated that there is negative cooperativity between the two different ligand-binding conformations of the β1-adrenoceptor activated by catecholamines and CGP12177 respectively [3]. Finally, we have used fluorescence correlation spectroscopy (FCS) to investigate ligand binding to A1AR and A3AR in small 0.2 μm^2 microdomains of single living cells [4]. FCS studies with a fluorescent A3-agonist have enabled high affinity labeling of the active conformation (R*) of the receptor [4]. We have also used a fluorescent adenosine A3-antagonist (CA200645) to study the binding characteristics of antagonist-occupied receptor conformations (R) in membrane microdomains of single cells [5]. Investigation of the dissociation kinetics of CA200645 provided further support for allosteric regulation of this receptor by homodimerization [5].
Acknowledgements Symposium organized by COST action CM1207 – GLISTEN.
Keywords Ligand binding, fluorescence, cooperativity
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L9

Gliomas and epilepsy: insights from neuropathological studies in humans

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SpringerPlus 2015, **4(Suppl 1):**L8

Brain tumors represent a recognized cause of epilepsy in both children and adults. In principle, any tumor (extra-axial, intra-axial, benign or malignant, common or uncommon) can cause seizures. However, patients with supratentorial low-grade glial tumors are more likely to develop epilepsy. Several clinical studies emphasize that pharmacologically intractable epilepsy critically affects the daily life of patients with brain tumors, even if the tumor is under control. Recently, the term of long-term epilepsy associated tumour (LEAT) has been introduced. LEATs are low grade, slowly growing, cortically-based tumours which predominantly occur in young patients with long histories (often 2 years or more) of drug-resistant epilepsy. Glioneuronal tumors (GNT), including gangliogliomas (GG) and dysembryoplastic neuroepithelial tumors (DNTs), represent the most common tumor within the spectrum of LEAT. The advent of the neurosurgical treatment of epilepsy-associated brain lesions confirmed the strong epileptogenicity of these tumor entities. Understanding the mechanisms that underlie epileptogenesis in LEATs is essential to identify new treatment targets and to develop an effective therapy. Mechanisms such as alterations of the balance between excitation and inhibition and alterations in neuron-glia interactions might be involved. Astroglial cells express functional receptors for a variety of neurotransmitters and may critically modulate synaptic transmission. In addition, an increasing number of observations indicate that pro-epileptogenic inflammatory pathways are activated in GNT and may contribute to the onset and progression of seizures. The recent advances and likely candidate mechanisms and molecules involved in tumor-associated epileptogenesis will be discussed.
Acknowledgements EA is supported by the National Epilepsy Fund – "Power of the Small", the Hersenstichting Nederland (NEF 013-1) and KIKA (Stichting Kinderen Kankervrij).
Keywords Glioma, glioneuronal tumor, epilepsy

L10

Purinergic transmission in brain tumors and its impact on drug development

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SpringerPlus 2015, **4(Suppl 1):**L10

Extracellular nucleoside and nucleotides acting via adenosine/P1 and nucleotide P2 (P2X/P2Y) purinoceptors are fundamental signaling molecules controlling the survival and proliferation of astrocytes and oligodendrocytes (Ceruti and Abbracchio, Adv Exp Med Biol. 2013;986:13). The malignant transformation of these cells to

progressively more aggressive tumors (respectively, astrocytomas and anaplastic glioblastoma, and glioblastoma multiforme, containing proliferating cells resembling Oligodendrocytes Precursor Cells, OPCs) confers growth advantage and chemoresistance. Characterization of the specific P1 and P2 receptors on these tumors may unveil new strategies to reduce cancer growth and/or promote differentiation to non-cancerous glial phenotypes. The adenosine A3 receptor (A3AR) has emerged as a potential target. Under hypoxia, a condition typical of gliomas' core, A3AR mediates chemoresistance via the PKB/Akt pathway (leading to inactivation of the pro-apopototic Bad protein) and by upregulating matrix metalloproteinase-9, that degrades extracellular matrix and promotes migration of glioma cells towards healthy brain regions (Ceruti and Abbracchio, and ref therein). Thus, inhibition of A3AR with selective antagonists could represent an appealing therapeutic approach. More recently, the P2X7 receptor has been recently found to be over-expressed in grade IV human gliomas (Monif et al., J Inflammation. 2014;11:25) and its blockade with the synthetic antagonist Brilliant Blue G decreased tumour cell number. Finally, treatment of human glioblastoma multiforme cells with UDP, UDP-glucose or LTD4, that act as agonists at the new P2Y-like GPR17 receptor, reduced the formation of glioma spheres, suggesting that GPR17 stimulation on highly proliferating tumor OPCs may drive their differentiation to the oligodendroglial fate, negatively affecting both tumor proliferation and self-renewal (Dougherty et al., Cancer Res. 2012;72:4856).

Keywords Cell differentiation, purinoceptors, new targets in glioma

L11

Cannabinoid signalling in glioma cells

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SpringerPlus 2015, **4(Suppl 1):**L11

Cannabinoids, originally derived from Cannabis sativa, as well as their endogenous and synthetic counterparts, were shown to induce apoptosis of glioma cells in vitro and tumour regression in vivo via their specific receptors, cannabinoid receptors CB1 and/or CB2. CB2 are abnormally expressed in human gliomas and glioma cell lines. Most of the analysed gliomas expressed significant levels of CB2 receptor and the extent of CB2 expression in the tumour specimens was related to tumour malignancy. A synthetic cannabinoid, WIN 55,212-2, down-regulated the Akt and Erk signalling pathways in C6 glioma cells that resulted in reduction of phosphorylated Bad levels, mitochondrial depolarization and activation of caspase cascade leading to apoptosis. We examined whether synthetic cannabinoids with different receptor specificity: WIN55,212-2 (a non-selective CB1/CB2 agonist) and JWH133 (a CB2-selective agonist) affect survival of four human glioma cell lines and three primary human glioma cell lines. WIN-55,212-2 decreased cell viability in all examined cell lines and induced cell death. Susceptibility of the cells to JWH133 treatment correlated with the CB2 expression. Cannabinoids triggered a decrease of mitochondrial membrane potential, cleavage of caspase-9 and effector caspases. Induction of cell death by cannabinoid treatment led to the generation of a pro-apoptotic sphingolipid ceramide and disruption of signalling pathways crucial for regulation of proliferation and survival. Increased ceramide levels induced ER-stress and autophagy in drug-treated glioblastoma cells. We conclude that cannabinoids are efficient inhibitors of human glioma cells growth, once the cells express specific type of cannabinoid receptor.

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Keywords Cannabinoids, signal transduction, cell death

L12

The role of integrins in glioma biology and anti-glioma therapies

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SpringerPlus 2015, **4(Suppl 1):**L12

Integrins are a group of molecules expressed by various cells including glioma cells and endothelial cells within the tumor. There are 18 known alpha and beta integrin subunits which form a heterodimer. Integrins regulate different cellular processes such as proliferation, adhesion, motility and survival as shown in numerous preclinical models. Furthermore, integrins control the activity of the transforming growth factor (TGF)-beta pathway and are involved in the process of angiogenesis which is indispensable for continued tumor growth. Because of the high expression levels of some integrins on glioma cells and their numerous functions, inhibition of integrin signaling has been considered a promising strategy for the treatment of glioma patients. Besides blocking antibodies which are currently under clinical investigation in other cancer entities, the integrin inhibitor cilengitide has been tested within several trials in glioblastoma patients over the last years. Cilengitide is a cyclic RDG peptide which targets integrins alphvbeta3 and alphavbeta5. Based on the results of smaller, initial trials suggesting an activity of cilengitide against glioblastoma, 2 larger trials were subsequently performed. However, both trials, which combined temozolomide-based chemoradiation with cilengitide failed to demonstrate an improved outcome with the addition of cilengitide. Ongoing translational analyses suggest that integrin levels in the tumor tissue are neither prognostic nor predict response to cilengitide. While the clinical development of cilengitide has been stopped, integrin inhibition with more effective agents may still be a promising approach in clinical neurooncology.

Keywords Glioma, integrin, TGF-beta

L13

Drosophila modelling axonal transport in the face of tau pathology

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SpringerPlus 2015, **4(Suppl 1):**L13

Alzheimer's disease (AD) is a devastating neurodegenerative disease causing irreversible cognitive decline in the elderly. There is no disease-modifying therapy for this condition and the mechanisms underpinning neuronal dysfunction and neurodegeneration are unclear. Compromised cytoskeletal integrity within neurons is reported in AD. This is believed to result from loss-of-function of the microtubule-associated protein tau, which becomes hyper-phosphorylated and deposits into neurofibrillary tangles in AD. We have developed a Drosophila model of tauopathy in which abnormal human tau mediates neuronal dysfunction characterised by microtubule destabilisation, axonal transport disruption, synaptic defects and behavioural impairments. Here we show that a microtubule-stabilising drug, NAPVSIPQ (NAP), prevents as well as reverses these phenotypes even after they have become established. Moreover, it does not alter abnormal tau levels indicating that it by-passes toxic tau altogether. Thus, microtubule stabilisation is a disease-modifying therapeutic strategy protecting against tau-mediated neuronal dysfunction, which holds great promise for tauopathies like AD.

Keywords Microtubule stabilisation, tau, NAP

L14

Recent developments in tau-based therapeutics for Alzheimer's disease and related dementsia

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Current therapies for Alzheimer's disease (AD) and related disorders have demonstrated very modest, symptomatic efficacy, leaving an unmet medical need for new, more effective therapies. While drug development efforts in the last two decades have primarily focused on the amyloid cascade hypothesis, with disappointing results so far, tau-based strategies have received little attention until recently despite that the presence of extensive tau pathology is central to the disease. The discovery at the turn of the century of mutations within the tau gene that cause fronto-temporal dementia demonstrated that tau dysfunction was per se sufficient to cause neuronal loss and clinical dementia. Development of tau pathology is associated with progressive neuronal loss and cognitive decline and is the common

underlying cause of a group of neurodegenerative disorders collectively known as "tauopathies". Tauopathies are clinically, morphologically and biochemically heterogeneous neurodegenerative diseases characterized by the deposition of abnormal tau protein in the brain. The neuropathological phenotypes are distinguished based on the involvement of different anatomical areas, cell types and presence of distinct isoforms of tau in the pathological deposits. Thus, the spectrum of tauopathy entities expands beyond the traditionally discussed disease forms. Emerging evidence strongly suggests that accumulation of abnormal tau is mediated through spreading of seeds of the protein from cell to cell. This prion-like mechanism would support the concept that in AD brains, tau pathology iinitiates in a very small part of the brain many years before becoming symptomatic, spreading slowly and progressively to the whole brain following an anatomically defined pattern. Emerging therapeutic strategies aimed at treating the underlying causes of the tau pathology will be discussed, including some novel therapeutic approaches on the verge of providing new treatment paradigms in upcoming years.
Keywords Tau, Alzheimer, dementia

L15

The oligodendroglia cytoskeleton in health and disease

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SpringerPlus 2015, **4(Suppl 1):**L15

Oligodendrocytes, the myelin forming cells of the CNS, enwrap neuronal axons and form multilamellar myelin sheets. They are derived from oligodendrocyte precursor cells which migrate from the subventricular zone into the different regions of the brain. Differentiation from the early progenitor to the mature multiprocessed oligodendrocyte is characterized by different morphological stages. To support cell morphology and establish and maintain the myelin membrane, an intact, spatially organized cytoskeleton with dynamic properties is essential. In particular microtubules and their associated proteins play an important role. A variety of microtubule binding proteins, including tau, are present in oligodendrocytes. Oligodendrocytes in culture express all six isoforms of tau which are developmentally regulated. Tau proteins are present in immature and mature oligodendrocytes and specifically prominent in the branching points of the cellular processes. Downregulation of tau impairs cell differentiation and the process of early myelination. In neurodegenerative diseases collectively termed tauopathies, fibrillary tau accumulations occur not only in neurons but also in glia. Tau positive coiled bodies originating in oligodendrocytes are characteristic for the brains of patients with frontotemporal dementias, such as corticobasal degeneration and progressive supranuclear palsy. These aggregates are further characterized by the presence of heat shock proteins and ubiquitin, indicating that stress situations are causally related to the pathogenesis. In this respect, proteasomal dysfunctions have been discussed to be involved in neurodegenerative disorders and the aging process. Data on the consequences of proteolytic stress in oligodendrocytes and the protein aggregation process will be presented. Our data demonstrate that an intact cytoskeleton is essential for cellular defense mechanisms.
Keywords Oligodendrocytes, microtubules, neurodegenerative diseases

L16

Tau regulates the localization and function of End Binding proteins in neuronal cells

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SpringerPlus 2015, **4(Suppl 1):**L16

Tau is a classical microtubule-associated protein known to regulate microtubule stability in neurons. In our study, we have addressed the putative crosstalk between tau and End binding proteins 1 and 3 (EB1/3), the core microtubule plus-end tracking proteins (+TIPs), in differentiating neuronal cells. We show that tau and EB proteins interact directly and that the cellular distribution and mobility of EB proteins depends on tau localization and expression levels. Moreover, our data reveal that tau is essential for the proper localization of EB1 at the medial-distal region of the axon shaft in developing neurons. In summary, we provide evidence for a new function of tau protein as a direct regulator of EB1/3 proteins. This further indicates that the interplay between classical MAPs and core +TIPs may be important for the fine-tuned regulation of microtubule dynamics and stability during neuronal differentiation.
Keywords Tau, end binding proteins, microtubule dynamics

L17

Multiple signaling pathways of orexin receptors

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SpringerPlus 2015, **4(Suppl 1):**L17

Orexin/hypocretin peptides are central for the regulation of the sleep/arousal states, and have, in addition, a role in the regulation of e.g. metabolism, addiction, stress response and pain gating. Orexin responses are mediated by G-protein-coupled OX1 and OX2 orexin receptors. Orexin signaling (in different cell types) is very versatile, ranging from excitation to induction of cell death. Orexin receptor coupling is promiscuous, engaging members of at least three different families of G-proteins, namely Gi, Gs and Gq, and some non-G-protein mediators as well. Preferred G-protein-coupling of the receptors appears different in different tissues, but the mechanism determining this are unknown. The primary signal transducers of orexin receptors very effectively activate phosholipase cascades, including PLA2, PLC and PLD, and also PLC-diacylglycerol lipase-mediated endocannabinoid generation. These cascades may play a significant role in the regulation of K+ and non-selective cation channels, which are the effectors for the orexin-mediated neuronal excitation. In some cell types, orexin receptor stimulation induces programmed cell death. Some different mechanisms for his have been proposed, but the picture is still incomplete. Orexin receptors have been a target for multiple drug discovery projects. Main focus has been on the antagonists, with insomnia as the indication. For agonist drugs there are some ongoing smaller academic and semi-academic projects. Use of orexin receptor agonists is suggested to be beneficial in narcolepsy (as peptide replacement therapy) and putatively in other sleep/wakefulness disturbances, metabolic disorders and cancer.
Keywords Orexin, hypocretin, phospholipase

L18

Galanin receptor ligands

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SpringerPlus 2015, **4(Suppl 1):**L18

The effect of galanin is mediated through three GPCR subtypes, GalR1-3. The limited number of specific ligands to the galanin receptor subtypes has hindered the understanding of the individual effects of each receptor subtype. This review summarizes the current data of the importance of the galanin receptor subtypes and receptor subtype specific agonists and antagonists and their involvement in different biological and pathological functions.
Keywords Galanin, neuropeptide, ligand

L19

Application of fluorescence methods for characterization of ligand binding to G protein coupled receptors

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SpringerPlus 2015, **4(Suppl 1):**L19

G protein coupled receptors (GPCR) comprise a large family of trans-membrane proteins involved in the regulation of signal transduction

through the cell membrane in response to various extracellular stimuli. GPCR have become important targets of many drugs for treatments of very different diseases. During the last decade several fluorescence-based methods have been implemented for the characterization of signal transduction via GPCRs, starting from ligand binding and including several steps leading up to a response on the level of gene regulation. We have proposed the fluorescence anisotropy (FA) and fluorescence intensity (FI) assay to investigate fluorescent ligand binding properties to different GPCRs (Veiksina et al., 2010). The implementations of budded baculoviruses that display G protein-coupled receptors on their surfaces have significantly increased sensitivity and applicability of these assays (Veiksina et al., 2014). The developed novel assay systems opened new possibilities for real-time monitoring of ligand binding to their receptors for understanding their particular kinetic properties. These assays are also compatible for homogenous HTS suitable fo ligand screening. There has been implemented assay systems for receptors of peptides like melanocortin (MC4R) and neuropeptide Y (NPY1R) as well as for receptors of monoamines like dopamine (D1DAR) and serotonin (5-HT1AR).

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Keywords GPCR, Fluorescence anisotropy, budded baculoviruses

L20

Imaging of α2C-adrenoceptors in the living brain: a method to monitor noradrenaline release?

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Objectives: The PET tracer [11C]ORM-13070 was recently validated for receptor occupancy analysis of brain α2C-adrenoceptors, and PET experiments in monkeys and humans indicated that tracer uptake into the caudate and putamen was reduced by interventions that increased synaptic noradrenaline concentrations in the brain [1-3]. This study aimed to confirm the sensitivity of [11C]ORM-13070 binding to increased levels of synaptic noradrenaline.

Methods: PET imaging of the brain was performed with a 3D High Resolution Research Tomograph. Eight subjects underwent a control [11C]ORM-13070 PET scan and two PET scans after two different noradrenaline challenges, i.e. a sub-anaesthetic infusion of ketamine and oral intake of atomoxetine combined with cold stimulation. Tracer uptake in the caudate nucleus and putamen was described with AUC values in scan time windows of 10-20 min and 5-30 min post injection, and quantified with the ratio method. Voxel-based analysis was performed with average B/F images. Both challenges caused small but statistically significant (10–20%, $p < 0.05$) reductions in tracer uptake in both target regions. Voxel-based analysis revealed significant clusters in the dorsal putamen with both challenges. Ketamine was associated with significant elevations in circulating noradrenaline and adrenaline levels, while the atomoxetine + cold treatment was not. Strong experimental support was gained for the feasibility of [11C]ORM-13070 PET imaging of brain noradrenergic neurotransmission.

Keywords Adrenoceptor, noradrenaline, positron emission tomography

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L21

The other side of opioid receptor signaling: regulation by protein–protein interaction

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Opioid receptors (OR) are widely known as mediators of the analgesic effects of opioids and also contribute to the development of tolerance and dependence. Moreover, opioids are implicated in cell proliferation and survival. How opioids modulate these downstream signaling pathways is a research receiving a lot of attention. Our group aims to define the signaling pathways through which opioid receptors participate in these physiological processes. Emphasis is given to unconventional interacting partners of the μ and δ-opioid receptors such as the Regulators of G protein signalling (RGS) proteins and STAT5B (Georgoussi et al. 2012). Evidence will be presented with which RGS proteins opioid receptors interact, how RGS members confer selectivity to receptors to choose a specific subset of G proteins, how activation of opioid receptors result in recruitment of RGS proteins to the plasma membrane and exert a differential modulatory effect in ERK1,2 phosphorylation, agonist-driven adenylyl cyclase inhibition and internalization of the opioid receptors (Papakonstantinou et al., 2015). Moreover evidence will be presented on how STAT5B associates with the δ-opioid receptor and forms selective pairs with selective Gα, Gβγ subunits and RGS proteins, and how activation of the δ-opioid receptor with selective agonists promotes a multi-component signaling complex involving the STAT5B transcription factor and other signaling intermediates to mediate neuronal survival and neurite outgrowth (Georganta et al., 2013). Understanding the mechanism that control OR signaling is important to address problems related to phenomena such as pain perception, tolerance and dependence that occur upon chronic opiate administration and define whether disruption of such interactions may contribute to the development of novel therapeutic strategies.

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Keywords Opioid receptors, RGS proteins, STAT5

L22

Autism as a disease of the synapse: search for mechanistic insight

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Autism spectrum disorders (ASD) are heterogeneous, heritable neurodevelopmental conditions, affecting ~0.5% of the population across cultures, with a ~4:1 male/female ratio. ASD are characterized by social interaction and communication deficits, restricted interests, repetitive behaviors, and reduced cognitive flexibility. Causes likely converge at the synapse, as shown by mutations of synaptic genes or mutations causing quantitative alterations in synaptic protein expression. Neuroligin4 (NLGN4X) mutations are among the most frequent causes of heritable ASD. But monogenetic forms altogether account for 1200 schizophrenic subjects and validated it in Asperger autists. We hypothesized that a coincidence of unfortunate normal variation in synaptic or synapse-regulating genes rather than mutations underlies most autistic phenotypes. We identified 'proautistic' variants in synaptic genes, which in aggregate are associated with high autism severity. A transcranial magnetic stimulation study on respective individuals revealed enhanced glutamatergic and GABAergic activity. IPS-derived cortical neurons from these subjects are now functionally characterized.

Keywords Autism, synapse, ambra1

L23

Elucidating the mechanisms of TDP-43 aggregation in a cellular model of motor neuron disease

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Motor neuron disease (MND) is a neurodegenerative disease of mid-life, with average survival of 3-5 years after diagnosis. The only approved treatment, riluzole, is only moderately effective and so there is a great need for novel therapeutics. TAR DNA binding protein 43 (TDP-43) has held a particular centrality in MND research since its discovery as the principal protein component of the characteristic protein aggregates found in the disease. Subsequent work has investigated its role as an RNA-binding protein and regulator of approximately 3000 RNA transcripts. In the majority of disease cases, both familial and sporadic, normally nuclear TDP-43 is sequestered in the cytoplasm in protein aggregates. Using differentiated motor neuron-like cells (NSC-34) and primary motor neurons, we have treated cells with the disease-relevant ER stressor tunicamycin. Overnight treatment with a low concentration of tunicamycin resulted in the formation of aggregates immunoreactive for endogenous TDP-43, as visualised by immunocytochemistry and Western blotting of the RIPA-insoluble fraction. We have found that these aggregates do not co-stain for TIAR, a well-known marker of stress granules. However, using the oxidative stressor sodium arsenite, we are able to induce formation of stress granules, though they do not co-stain for TDP-43. The tunicamycin treatment also led to a moderate decrease in cell viability, as shown by MTT and Trypan Blue Exclusion assays. In this study, we have recapitulated a known pathological process (ER stress) of MND in vitro, which resulted in the formation of TDP-43 aggregates. The signalling pathways driving TDP-43 aggregation are unknown, hence work detailing these mechanisms is likely to present wide scope for therapeutic intervention.
Keywords TDP-43, MND, aggregation

L24

The role of dynein mediated transport in the clearance of misfolded proteins responsible for motoneuron diseases

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Spinal and bulbar muscular atrophy (SBMA) and amyotrophic lateral sclerosis (ALS) are motoneuron diseases. A mutation in the androgen receptor (ARpolyQ) gene is responsible for SBMA. Mutations in the SOD1, in the TDP-43, in the FUS-TLS or in the C9ORF72 genes are responsible for familiar form of ALS. The mutated coded proteins misfold and aggregates. Efficient protein quality control (PQC) is required for the maintenance of physiological and soluble protein pool in affected motoneuron. The balance between autophagy and ubiquitin-proteasome system (UPS) prevents protein aggregation and increases degradation of SBMA and ALS misfolded proteins. Dynein binds the co-chaperone BAG3 and transports the mutant proteins at microtubule organization center where misfolded proteins interact, aggregate and can be degraded by autophagy. However, here misfolded proteins may blocks autophagy flux. In NSC34 cells, dynein is sequestered into ARpolyQ aggregates suggesting the role of dynein into aggregates formation process. Unexpectedly, the silencing of dynein heavy chain resulted in a drastic reduction of ARpolyQ retained in filter retardation assay (FRA). Moreover, dynein silencing drastically altered autophagic markers localization (LC3 and p62) by immunofluorescence. Notably, treatment with a dynein inhibitor (EHNA) drastically reduced the retention of ARpolyQ, mutSOD1 and mutTDP43 aggregates in FRA, even when autophagy was inhibit with 3-MA. Conversely UPS blockage with MG132 counteracted the reduction induced by altered dynein transports. RTq-PCR on NSC34 cells treated with EHNA showed an increased BAG1:BAG3 ratio that can targeting the misfolded proteins to UPS. Moreover, in NSC34 cells, EHNA increased the degradation of proteasome reporter GFPu, while BAG1 overexpression reduced the level of aggregates retained in FTA. These data suggest that, when autophagy is overload, by misfolded proteins, dynein inhibition restores the physiological and soluble protein pool via UPS.
Keywords MNDs, PQC, dynein

L25

Effects of novel synthetic microneurotrophins in diabetic retinopathy

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Diabetic retinopathy (DR) is a neovascular, inflammatory and neurodegenerative disease. The neovascular component is treatable but no therapeutic agents are available for the other two components. Early changes in the diabetic retina include neuronal death of amacrine and retinal ganglion cells (RGC) (Barber et al., J Clin Invest, 1998), and elevated levels of inflammatory mediators (Yoshimura et al., Plos One, 2009). Nerve Growth factor (NGF) receptors, namely TrkA and p75NTR, are expressed in RGC. The TrkA receptor activates prosurvival, while p75NTR activates inflammatory and apoptotic pathways (Mysona et al., Expert Rev Ophthalmol, 2014). Dehydroepiandrosterone (DHEA) binds to both receptors, mimicking NGF. It affords anti-apoptotic, neuroprotective and anti-inflammatory effects in the retina (Kokona et al., Neuro-pharmacol, 2012, Straub et al., J Clin Endocrinol, 1998). The therapeutic use of DHEA is restricted due to its metabolic products. The main objective of this study was to investigate and compare the neuroprotective and anti-inflammatory effects of DHEA and its spiro-epoxy derivatives BNN27 and BNN20 (Calogeropoulou et al., J Med Chem, 2009) (not metabolized to estrogens and androgens), in the rat streptozotocin model of DR. BNN27, via TrkA activation, protected in a dose-dependent manner (2, 10, 50 mg/kg, ip) bNOS (brain nitric oxide synthetase) and TH (tyrosine hydroxylase) expressing amacrine cells and ganglion axons (NFL immunoreactivity) similar to DHEA's actions, while BNN20 was less effective. BNN27 activated the TrkA prosurvival signaling pathway ERK1/2 kinase. It reduced the activation of SAPK/JNK kinase and the expression of p75NTR. BNN27 also increased the expression of anti-inflammatory cytokines (IL10). These results suggest that NGF TrkA receptor is involved in the neuroprotective and anti-inflammatory effects of BNN27 and is a valuable target via which BNN27 could afford efficacious therapeutics for the treatment of DR.
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Keywords Diabetic retinopathy, Microneurotrophins, neuroprotection

L26

Unravelling carbon monoxide protection in cerebral ischemia: from the organelle to the organism

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Perinatal complications are a serious clinical problem, in particular hypoxic-ischemic (HI) episodes, caused by birth asphyxia or uterine and fetal blood flow interruption. HI corresponds to 23% of neonatal deaths, being one of the top 20 leading causes of disease burden. Preconditioning (PC) is a stimulation below the injury threshold that activates endogenous protective mechanisms to prevent damage. Low doses of carbon monoxide (CO) play a beneficial role through PC induction. Herein, CO cytoprotection was explored in distinct brain models. The used experimental models range from monoculture of astrocytes, co-cultures of neurons and astrocytes, to the whole organism with the rat model of perinatal ischemia. In primary cultures of astrocytic cells, CO not only impairs mitochondrial membrane permeabilization, by ANT glutathionylation, but also strengths mitochondrial oxidative metabolism, by modulating COX activity, increasing mitochondrial biogenesis and ATP amounts. Also, CO reinforces astrocytes-neurons

communication towards neuronal survival. Purinergic molecules are the main mediators for this non-cell autonomous effect. Our results seem to indicate that the main pathway involved includes ATP release from astrocytes, its metabolization and A2A receptor binding to initiate protective mechanisms within the neurons. Rat pups were exposed to CO before hypoxia-ischemia induction (Vannucci model). 24 h after HI the brains were collected for cell death and tissue protection assessment (histological and immunohistochemical analysis). It was found limited apoptosis in hippocampus following cerebral ischemia: lower cytochrome c release and caspase-3 activation yielding an increased Bcl-2 expression. Altogether, one can conclude that there is not just a unique pathway for the CO-induced endogenous protection, brain tolerance is the result of a complex cellular change in response to injury. Indeed, CO regulates cell death pathways and modulates cellular metabolism.

Keywords Carbon monoxide, preconditioning, ischemia

L27

The effect of different modes of hypobaric hypoxia on the expression of transcription factor pCREB and pro-survival proteins BDNF and BCL-2 in rat neocortex and hippocampus

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Severe hypobaric hypoxia (180 mm Hg) is a harmful stimulus that induces structural and functional injures of susceptible brain neurons in the neocortex and hippocampus. In contrast, moderate hypobaric hypoxia (360 mm Hg) activates endogenous cellular defensive mechanisms. The rate of neuroprotection afforded by the mild hypoxia depends on the quantity of hypoxic sessions. In particular, preconditioning (pre-exposure) by three trials of mild hypoxia protects from deleterious effects of subsequent severe hypoxia whereas preconditioning with one mild hypoxic trial does not. The molecular mechanisms induced by mild hypoxic preconditioning are unclear. Pro-survival proteins, such as neurotrophic factor BDNF and anti-apoptotic factor Bcl-2 are supposed to be involved in this process. In the present study, the effects of three-trial and one-trial hypoxic preconditioning on the expression of pro-survival proteins BDNF and Bcl-2 as well as their up-stream activator pCREB, have been studied in the neocortex and hippocampus of rats. As revealed by quantitative immunocytochemistry, the severe hypobaric hypoxia didn't affect or down-regulated the neuronal levels of pCREB, BDNF and Bcl-2 at 3–24 h after the exposure. The one-trial preconditioning did not change this effect of severe hypoxia. In contrast, preconditioning by three trials of mild hypoxia (360 Torr, 2 h, 24 h intervals, 3 times) significantly enhanced the pCREB, BDNF and Bcl-2 neuronal expression in response to severe hypoxic challenge. Three-trial mild hypoxia alone also up-regulated the expression of molecular factors examined in the neocortex and hippocampus at 24 h whereas one trial of the mild hypoxia did not. The results of the present study indicate that development of the neuronal hypoxic tolerance induced by the three-trial, in contrast to one-trial, mild hypoxic preconditioning is apparently largely associated with the activation of CREB, as well as BDNF and Bcl-2 overexpression.

Keywords Hypoxic preconditioning, brain hypoxic tolerance, pCREB

L28

Ankrd11 is a chromatin regulator involved in autism that is essential for neural development

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Ankrd11 is a potential transcriptional regulator that is implicated in cognitive dysfunction and ASD, but has no known function in the brain. We show that Ankrd11 is expressed in the embryonic cortex, and that when it was knocked-down in murine cortical precursors this caused decreased proliferation, reduced neurogenesis, and aberrant neuronal positioning in developing cortex. Knockdown of Ankrd11 in human forebrain neural precursors phenocopied Ankrd11 knockdown in murine neural precursors. Decreased proliferation of embryonic and adult neural precursors and neuronal mispositioning were also observed in Yoda mice carrying a point mutation in the histone deacetylase (HDAC)-binding domain of Ankrd11. Yoda mice also displayed ASD-like behavioural abnormalities. Consistent with a role for Ankrd11 in histone acetylation, Ankrd11 was associated with chromatin and co-localized with HDAC3. Also, expression and histone acetylation of Ankrd11 target genes were altered in Yoda neural precursors. Finally, the Ankrd11 knockdown-mediated decrease in precursor proliferation was rescued by inhibiting histone acetyltransferase activity or expressing HDAC3. Thus, Ankrd11 is a crucial epigenetic regulator of neural development that regulates histone acetylation and gene expression, thereby providing a likely explanation for its association with cognitive dysfunction and ASD.

Keywords Chromatin, stem cells, brain development

L29

Active endocannabinoids are secreted on the surface of microglial microvesicles

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Endocannabinoids (eCBs) are bioactive lipids which primarily influence synaptic communication in the nervous system. They are synthesized by neurons but also by microglia, especially under inflammation. To exert their function, eCBs travel across the intercellular space. However, how eCBs move extracellularly remains obscure. Our recent evidence indicates that reactive microglia release extracellular vesicles (EVs), which may represent an ideal vehicle for the transport of hydrophobic eCBs. Hence, in this study we investigated whether microglial EVs carry eCBs and may influence neurotransmission. First we analyzed the eCB content of EVs and found a clear enrichment of anandamide (AEA) in EVs relative to parental microglia. This analysis revealed higher AEA levels in EVs shed from the plasma membrane (microvesicles), compared to those which originate from the endocytic compartment (exosomes). To bioassay the activity of vesicular AEA, we used patch clamp analysis of miniature inhibitory post-synaptic currents (mIPSC) on hippocampal neurons in vitro. Exposure of neurons to microvesicles (MVs) induced a significant decrease in mIPSC frequency, mimicking the action of CB1R agonists. The involvement of vesicular AEA in this phenomenon was inferred from the ability of the CB1R antagonist SR141716A to block the reduction of mIPSC frequency evoked by MVs. Western blot analysis showed that MVs induces an increase in ERK phosphorylation, which was completely inhibited by SR141716A. This indicate that CB1R activation by AEA-storing MVs translates into downstream signaling. Finally, consistent with a surface localization of AEA, MVs membranes maintained their capability to decrease mIPSC frequency. Overall, this study shows that microglial MVs carry AEA on their surface to stimulate CB1R on target GABAergic neurons thus playing a crucial role in the modulation of inhibitory transmission.

Keywords Endocannabinoids, extracellular vesicles, microglia-neuron signaling

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L30

Peripheral nerve implants enriched with chemotactic factors for peripheral nervous tissue engineering

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Peripheral nerve injuries are an important part of everyday medical practice since there are reported over 600,000 cases in Europe and in the United States annually [1]. Many of such nerve injures cause gaps between nerve stumps. Without intervention, they can lead to the formation of a stump neuroma, what can result in functional impairment of the nerve fiber. The current approaches to regeneration of damaged peripheral nerves include: autografting, allografting, and, last but not least, the implantation of polymeric tubes and conduits between nerve stumps. Nerve autografting is considered as the "gold standard" technique for the repair of peripheral nerve discontinuities, but it has a number of limitations, such as the requirement for the second surgical procedure to harvest the graft tissue, the donor site morbidity, additional injuries and scarring as well as the increased recovery time. Allografts (e.g., cadaver nerve grafts) and xenografts (e.g. animal nerve grafts) can be an alternative to autografts, but their main drawback lies in the high possibility of an undesirable immune response. The most promising materials for peripheral nerve conduits preparation are natural biopolymers. They are obtained from natural sources, exhibit similar properties to the tissues they are replacing and reveal good cell adhesion. Furthermore, they tend to accelerate regeneration processes due to specific chemical interactions within the human body, e.g. with extracellular matrix (ECM) molecules. The purpose of our study is to create a conduit with properties that will mimic the ones of the extracellular matrix of the peripheral nervous system. Our focus is put on natural polymers, especially chitosan. In addition, we use chemotactic factors which exhibit properties beneficial in regeneration of the peripheral nervous tissue.

Keywords Biomaterials, hydrogel, nerve regeneration

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L31

Ginkgolic acid specifically potentiates alpha 1 glycine receptors

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Ginkgo biloba extract is a neuroactive agent that is widely used for correction of age-associated impairment of memory, attention deficit and other cognitive functions. It has been shown that ginkgolides and bilobalides, Ginkgo biloba extract components, are potent blockers of glycine receptors [1,2]. However, the effect of ginkgolic acid, the other Ginkgo biloba extract constituent, on ligand-gated ion channels was not investigated. In the present study, using patch-clamp technique and transient transfection of different subunits in CHO cells we have shown that glycine receptors (GlyRs) are modulated by ginkgolic acid in a subunit-specific manner. After pre-application of ginkgolic acid (0.5–2 min), glycine-induced currents mediated by α1 GlyRs were strongly potentiated, while currents mediated by α2 GlyRs exhibited weak inhibition. There was no significant effect of ginkgolic acid on amplitudes of currents mediated by α3 GlyRs or on GABAARs composed of 1α/1β/2γ subunits. In order to further investigate subunit-specific effect of ginkgolic acid we have focused on possible interaction sites for this compound inside different GlyR domains. We found that mutation of three residues (T59A/A261G/A303S) in α2 subunit can convert the inhibitory action of ginkgolic acid into potentiation. Indeed, application of ginkgolic acid to cells expressing α2 T59A/A261G/A303S subunits resulted in an increase of responses to low concentrations of glycine and abolishment of the inhibitory effect, typical for wild type α2 GlyR. Our results suggest that (i) ginkgolic acid selectively enhances the function of α1 GlyRs and attenuates the function of α2 GlyRs, (ii) mutation of α2 subunit converts effect of ginkgolic acid from inhibition to potentiation.

Keywords Glycine receptor, ginkgolic acid, patch-clamp

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L32

Uptake, metabolism and toxicity of iron oxide nanoparticles in cultured microglia, astrocytes and neurons

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Iron oxide nanoparticles (IONPs) are frequently used for biomedical applications including magnetic resonance imaging, drug delivery or tumor treatment by hyperthermia. Since IONPs can reach the brain from periphery or are directly applied into the brain, the investigation of potential adverse effects of IONPs on properties and functions of the different types of brain cells is an important task. In order to directly compare different types of brain cells concerning the uptake and toxicity of IONPs, we synthesized fluorescent IONPs and characterized these BODIPY-labeled particles regarding their physicochemical properties. In the medium used for the cell studies, these particles had a hydrodynamic diameter of around 158 ± 28 nm and a negative surface charge with a zeta-potential of –10 ± 2 mV. Exposure of primary cultures of rat astrocytes, neurons and microglial cells revealed that among these cell types, microglial cells accumulated IONPs most rapidly. However, microglial cells were also most vulnerable towards acute IONP-induced stress while astrocytes and neurons were not acutely damaged by IONPs. In microglial cells, but not in astrocytes or neurons, IONPs were found to be localized in lysosomes and large amounts of reactive oxygen species (ROS) were observed after IONP-exposure. The IONP-induced toxicity in microglial cells was prevented by neutralizing lysosomes or by chelation of intracellular ferrous iron ions, suggesting that the toxic potential of IONPs in microglia involves rapid particle uptake, liberation of ferrous iron from the internalized IONPs in the acidic lysosomal compartment and iron-catalyzed ROS formation. These data suggest that also in brain IONPs may harm microglial cells and compromise microglial functions.

Keywords Toxicity, protection, nanoparticles

L33

Regulation of mitochondrial trafficking, function and quality control by the mitochondrial GTPases Miro1 and Miro2

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SpringerPlus 2015, **4(Suppl 1):**L33

Regulated trafficking of mitochondria in neurons is essential for providing ATP at the correct spatial location to power neural function and computation, and for providing calcium buffering at sites of calcium entry or release. Indeed the regulation of mitochondrial distribution, morphology and function are proposed to play an important role in neuronal development and survival but the regulatory mechanisms remain unclear. Miro family proteins (Miro1 and Miro2 in mammals) contain a transmembrane domain locating them to the outer mitochondrial membrane, along with two GTPase domains and two calcium-sensing EF-hand domains that face into the cytosol, and play a key role in regulating mitochondrial transport. Miro proteins mediate mitochondrial trafficking in neurons by linking mitochondria to kinesin and dynein motor proteins for their transport in axons and dendrites. Miro proteins are also targets for the Parkinson's Disease associated PINK1/Parkin mitophagy pathway and are therefore implicated in altered mitochondrial dynamics during mitophagy. Here I will present our recent results on the role played by Miro proteins in regulating mitochondrial trafficking and quality control. The role that Miro-mediated control of mitochondrial trafficking and turnover plays

in regulating neuronal development, function and pathology will also be explored.
Keywords Mitochondria, mitophagy, parkinson's

L34

Principles of mitochondrial fusion and fission cycle in neurons

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SpringerPlus 2015, **4(Suppl 1):**L34

Mitochondrial fusion-fission dynamics play a crucial role in many important cell processes. These dynamics control mitochondrial morphology, which in turn influences several important mitochondrial properties including mitochondrial bioenergetics and quality control, and they appear to be affected in several neurodegenerative diseases. The molecular machineries behind mitochondrial fusion and fission events are relatively well known. The regulation of fusion and fission events beyond the molecular machinery involved is less clear, fusion and fission are not random occurrences but form a cycle whereby fission typically follows fusion. Mitochondrial fission machinery may somehow sense mitochondrial length and become active when the mitochondrion is oversized and cease when mitochondria are smaller. In contrast, mitochondrial fusion events depend heavily on mitochondrial trafficking. Fusion only takes place when two mitochondria meet and motile mitochondria will be more likely to encounter one another. In cultured cortical neurons, for example, only one in every 14th contact between mitochondria results in fusion. The purpose of this presentation is to provide insight into the complex crosstalk between different processes involved in mitochondrial fusion-fission dynamics and to discuss the potential physiological purpose of mitochondrial fusion and fission.
Keywords Mitochondrial dynamics, neurons, mitophagy

L35

Bioenergetic failure, mitochondrial dysfunction and mitophagy

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Mitophagy specifically describes autophagy of damaged or dysfunctional mitochondria and occurs in programmed cell death when mitochondria fragment and remodel their cristae. Cellular bioenergetics is entwined with mitochondrial dynamics, and mitochondrial insults, including depolarization and inhibition of electron transport chain, trigger mitochondrial fragmentation. Here we investigated mitophagy in neurons during manipulation of mitochondrial bioenergetics. Dysfunction of mitochondria was induced by pharmacological inhibition of respiratory chain complexes I-V (rotenone, 3-nitropropionic acid, antimycin A, KCN & oligomycin, respectively) in primary cultures of cerebellar granule cells. The extent of bioenergetic failure was determined by measuring [ATP], depolarization of mitochondrial membrane potential and decrease in oxygen consumption rate in the Seahorse XF24. All stressors produced mitochondrial dysfunction as shown by concentration- and time-dependent decline in [ATP] over 4–24 h. Complexes I, III or IV showed rapid loss of mitochondrial membrane potential and decreases in oxygen consumption rate over 4–24 h. Autophagolysosomal flux was increased as shown by increased LC3-II and labelling of acidic vacuoles with monodansylcadaverine. Immunocytochemistry for PINK1 showed translocation of PINK1 from cytoplasm to mitochondria after injury, indicating likely involvement of mitophagy during bioenergetic dysfunction. Transfection of fluorescent pH-biosensor Rosella (Rosado CJ, et al. Autophagy. 2008;4:205) targeting mitochondria indicated the pH of the mitochondrial location dropped with the inhibition of complex I and II, implying acidification of mitochondria, presumably in acidic vesicles undergoing mitophagy. Dieback of neuronal arbor observed here paralleled that seen with a GFP-plasmid. Together these data indicate that bioenergetic dysfunction produces mitophagy in primary neurons and is likely to be involved in neuronal dynamics.
Keywords Mitophagy, PINK1, autophagolysosome

L36

Mechanisms of mitochondrial quality control in autosomal recessive Parkinson's disease

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The genes encoding the E3 ubiquitin protein ligase Parkin (PARK2) and the mitochondrial serine/threonine kinase PINK1 (PARK6) are mutated in clinically similar, autosomal recessive early onset Parkinson's disease (PD) forms. Over the past ten years, a number of studies in different model systems have demonstrated that PINK1 and Parkin regulate jointly several processes relevant to maintenance of mitochondrial quality, including mitochondrial trafficking and dynamics, mitophagy and biogenesis. By using a combination of approaches of cell biology, confocal imaging and biochemistry in different cell models, we recently showed that loss of protein import efficiency triggers recruitment of Parkin by PINK1 in proximity of the translocase of outer mitochondrial membrane (TOM). We provided evidence that the degradation of specific TOM subunits plays a key role in initiating the autophagic degradation of damaged mitochondria. We also showed that PINK1 and Parkin interact with the TOM machinery on polarized mitochondria. Our results suggests that this interaction modulates the import of the multifunctional mitochondrion-protective matrix enzyme 17beta-hydroxysteroid dehydrogenase 10, which is depleted in Parkin-deficient mice and Parkinson's disease patients. Electron and confocal microscopy, and calcium imaging approaches used to characterize the endoplasmic reticulum (ER)-mitochondria interface, a compartment previously linked to neurodegenerative processes, revealed enhanced juxtaposition between these organelles, associated with increased ER-to-mitochondria calcium transfer in cells from Parkin-deficient mice and patients with PARK2 mutations. Our current work aims at investigating the relevance of mitochondrial quality control mechanisms regulated by PINK1 and Parkin in different cell types of the central nervous system, to evaluate the contribution of each of them to the physiopathology of autosomal recessive Parkinson's disease.
Keywords Parkinson's disease, mitophagy, ER/mitochondria interface

L37

Activity-dependent neuroprotective protein (ADNP): from autism to Alzheimer's disease

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We have originally discovered activity-dependent neuroprotective protein (ADNP) as a major regulatory gene, a component of the SWI/SNF chromatin remodeling complex, essential for brain formation. Others found ADNP as a most frequent autism spectrum disorder (ASD)-associated gene. Furthermore, ADNP is the only protein significantly decreasing in the serum of Alzheimer's disease (AD) patients. Our most recent results revealed sex-related learning/memory differences in mice, reflecting hippocampal expression changes in ADNP and ADNP-controlled AD/ASD risk genes1. Hippocampal ADNP transcript content was doubled in male vs. female mice, with females showing equal expression to ADNP+/– males and no significant genotype-associated reduction. Increased male ADNP expression was replicated in human postmortem hippocampal samples. The hippocampal transcript for ApoE (the major risk gene for AD) was doubled in female mice compared with males, and further doubled in the ADNP+/– females, contrasting a decrease in young ADNP+/– males. ADNP regulates >400 genes during embryonic development, with ApoE being a major target. Other AD related proteins regulated by ADNP include tau (with pathological tau constituting the neurofibrillary tangles and with AD being the major tauopathy). Furthermore, ADNP associates with microtubule end binding proteins, controlling dendritic spine density, which is compromised in AD and ASD. Previously, overexpression

of the eukaryotic translation initiation factor 4E (eIF4E) led to ASD-like phenotype in mice and we have shown that hippocampal eIF4E expression was specifically increased in young ADNP+/– male mice. Understanding ADNP expression, positioned as a master regulator of key ASD and AD risk genes, introduces a novel concept of hippocampal gene-regulated sexual dimorphism toward gender-based biology and therapeutics.

Acknowledgements Funded by AMN, ISF, and the Israeli Ministry of Science.

Keywords ADNP, Autism, Alzheimer's disease

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L38

Neuronal-activity regulated gene expression: emphasis on BDNF

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Neurotrophin brain-derived neurotrophic factor (BDNF) is a growth factor that has important roles in the development and functioning of nervous system by promoting the survival, differentiation and synaptic plasticity of specific neuronal populations. Modifiability of neuronal connectivity by formation of new synapses, and alteration of the strength and stability of existing synapses, is regarded as the main cellular basis for memory and long-term behavioral adaptations. The gene encoding BDNF is considered to be one of the master genes of synaptic plasticity. BDNF has also received particular interest for its deregulation in nervous system disorders. Decreases of BDNF and its receptor TrkB levels and activity are accompanied by and are believed to lead to several pathologies, particularly nervous system diseases like neurodegenerative, psychiatric and cognitive diseases. Therefore knowledge about the regulatory mechanisms of BDNF gene is important both for understanding of nervous system function and for finding new drug targets. Results of our studies on the molecular mechanisms of neuronal activity-regulated expression of BDNF gene in the nervous system will be presented and discussed.

Acknowledgements Supported by by Estonian Research Council (institutional research funding IUT19-18), National R&D program „Biotechnology" (grant AR12030), Norwegian Financial Mechanism (grant EMP128) and Estonian Academy of Sciences.

Keywords BDNF, synaptic plasticity, gene regulation

L39

Epigenetic mechanisms of hypoxic preconditioning

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Hypoxic preconditioning is a pre-exposure to the repetitive mild hypoxia which results in development of brain hypoxic/ischemic tolerance and cross-tolerance to injurious factors of another nature. The endogenous defense processes mobilized by hypoxic preconditioning and resulting in formation of brain tolerance are based on evolutionary acquired gene-determined mechanisms of neuroprotection and adaptation. Key event is an activation of pro-adaptive transcriptional factors HIF-1, CREB, NF-kB, c-Fos, NGFI-A and down-stream expression of their target genes in vulnerable brain neurons. An important role can thus be suggested for the epigenetic regulation of gene expression, in particular, acetylation of core nucleosome histones leading to changes in chromatin structure which ensure access of the transcriptional factors activated by the preconditioning to the promoters of target genes. It has been shown that hypoxic preconditioning considerably increases an acetylation status of all histones and, particularly, H3 in the neurons of rat hippocampus and neocortex, whereas injurious severe hypoxia causes global repression of histone acetylation. Diverse effects of the severe hypoxia and mild hypoxic preconditioning on the methylation of DNA and histones have also been observed. The complex of the epigenetic modifications induced by the hypoxic preconditioning is attributed to the relaxed DNA which becomes available for activation of gene expression by pro-adaptive transcriptional factors up-regulated by the preconditioning.

Acknowledgements The work was supported by Russian Foundation for Basic Research (grant No. 14-04-00516).

Keywords Hypoxic preconditioning, brain hypoxic/ischemic tolerance, epigenetic mechanisms

L40

Role of amyloid precursor protein (APP) in regulation of neuronal genes

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Understanding the mechanisms regulating gene expression in the course of development and adaptation of the organism to the permanently changing environment and in the case of pathology is fundamentally important. Special attention in recent years has been paid to the processes of epigenetic regulation of neuronal genes involving chromatin modifications at the level of DNA methylation and histone acetylation. In these processes an important role belongs to the histone deacetylases (HDAC) which control gene silencing. Recently it was shown that the amyloid precursor protein (APP) and its intracellular domain (AICD) participate in regulation of expression of a number of neuronal genes, including those involved in amyloid metabolism and clearance. The list of APP-regulated genes includes the major amyloid-degrading enzyme neprilysin (NEP), a transport protein transthyretin (TTR), aquaporin and others. Our studies strongly indicate that AICD regulation of NEP and TTR is APP isoform-dependent and cell-type specific. In this process AICD competes for gene regulation with HDACs and treatment of cells or animals with HDAC inhibitors such as valproic acid results in up-regulation of NEP and TTR mRNA and protein levels and increased NEP activity leading to a reduction in total cellular amyloid (Aβ) peptide levels. Regulation of other proteins, e.g. acetylcholinesterase, does not involve AICD but requires full length APP molecules. APP overexpression in neuronal cells was also shown to affect the levels of HDAC gene products which might explain its role in gene regulation. Further studies of the APP interactome are important for better understanding of its role in brain development and functioning and for designing the drugs protecting the brain against neurodegeneration, in particular Alzheimer's disease.

Acknowledgements Supported by RFBR (13-04-00388), Program of RAS "Fundamental Sciences for Medicine", ARUK.

Keywords Amyloid precursor protein, neprilysin, histone decetylases

L41

Anti-inflammatory activities of carbon monoxide-releasing molecules (CO-RMs) in the brain

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The transcription factor Nrf2 and its downstream target heme oxygenase-1 (HO-1) are ubiquitous protective systems against oxidative stress, inflammation and tissue injury. The products of HO-1 enzymatic activity, biliverdin and carbon monoxide (CO), possess interesting anti-oxidant and anti-inflammatory properties suggesting that exploitation of this pathway as a target for drug discovery may offer therapeutic avenues in a variety of disorders [1]. In this context we have developed carbon monoxide-releasing molecules (CO-RMs), a class of compounds that deliver CO in a controlled fashion and exert a variety of pharmacological effects [2]. Data will be presented showing that CO-RMs modulate neuroinflammation and neuroprotection in vitro and in vivo. In BV2 microglia cells challenged with thrombin and interferon gamma, CO-RMs reduced the production of inflammatory mediators both in normoxic and hypoxic conditions. Similarly, in a rat model of collagenase-induced intracerebral hemorrhage, CO-RMs

modulated microglia activation and the production of TNF-α while partially protecting against brain damage [3]. The mechanism(s) by which CO liberated from CO-RMs exert protection remains elusive. However, our laboratory has recently produced findings in BV2 microglia pointing to mitochondria as a plausible target for the anti-inflammatory action of CO. Studies are also currently ongoing on the synthesis and characterization of novel hybrid molecules that potently activate Nrf2 and simultaneously release CO in order to maximize the anti-inflammatory effects of the HO-1 pathway [4].

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Keywords HO-1, carbon monoxide, microglia

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L42

Carbon monoxide targeting mitochondria in astrocytes: modulation of cell metabolism, redox response and cell death

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The endogenously produced gasotransmitter carbon monoxide (CO) has been studied as a factor involved in cytoprotection, homeostasis and anti-inflammation. Small amounts of reactive oxygen species (ROS) are described as signaling factors in CO's biological mode of action. Mitochondria are the main source of ROS and are also key organelles in orchestrating cell function: metabolism, cell death control and redox signaling. Astrocytes are most abundant glial cells and essential for neuronal function, namely metabolic and physical support, expression of neurotransmitters and promotion of neuroprotection. In this work it is shown that CO prevents astrocytic cell death and improves cell metabolism by targeting mitochondria, and some of the underlying molecular mechanism are disclosed. CO directly targets non-synaptic mitochondria and inhibits their mitochondrial membrane permeabilization, by preventing mitochondrial swelling, depolarization and inner membrane permeabilization. Thus, CO limits the release of cytochrome c into the cytosol and the activation of apoptotic cascade in astrocytes. All these events are ROS-dependent and involve glutathionylation of adenine nucleotide translocator (ANT), whose activity is ATP/ADP transport through mitochondrial inner membrane. In addition, low amounts of exogenous CO increase ATP production by improving oxidative metabolism. Mitochondrial population and specific cytochrome c oxidase activity are higher upon CO treatment. The CO-induced metabolic improvement is dependent on Bcl-2 expression. Dysfunctional mitochondrial can be eliminated by mitophagy, which is a crucial process for maintaining their function and quality control. In astrocytes, CO promotes mitophagy at 1 h of treatment, while following 24 h mitochondrial population is back to basal levels, indicating that CO contributes to mitochondrial turnover. Furthermore, CO limits astrocytic cell death in an autophagic dependent manner.

Keywords Carbon monoxide, astrocytes, mitochondria

L43

Transcription Factor Nrf2: a novel target to modulate inflammatory and neuroprotective responses in Parkinson's disease

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Stopping the succession of events that lead to development of Parkinson's disease (PD) is a principal challenge that requires a brain protective approach in early diagnosed patients. Although PD ethiopathology may not have a single causative factor, information from sporadic and familial cases together with chemical and genetic animal models strongly suggests that low-grade chronic inflammation and oxidative stress play a critical role. Our team is studying the relevance of the transcription factor NRF2 (Nuclear factor (erythroid-derived 2)-like 2), a master regulator of oxidant and inflammatory defense, as a new therapeutic target in PD. The pro-inflammatory activation of microglia and astroglia in response to LPS, MPTP and human α-synuclein over-expression is exacerbated in Nrf2-deficient mice, thus demonstrating an immunomodulatory role of this protein. In PD patients, some evidence gathered from epidemiological, genetic and anatomopathologic studies also support a protective role of NRF2. Several compounds activate NRF2 and provide an immunomodulator and cytoprotective response in preclinical animal models of PD. At this time a crucial point to translate these promising results to the clinic is the discovery of a drug with good pharmacokinetics and pharmacodynamics that fulfill criteria of safety, tolerability and efficacy. The use of repurposing drugs, such as dimethyl fumarate used nowadays for treatment of remitting relapsing multiple sclerosis, may provide excellent candidates.

Keywords Neuroinflammation, Nrf2, Parkinson's disease

L44

Neuroprotective effert of carbon monoxide and Nrf2 in cerebral ischemia

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Carbon monoxide (CO) is a gaseous second messenger produced when heme oxygenase (HO) enzymes catabolize heme. We have demonstrated that CO can be therapeutic in ischemia-reperfusion brain injury; however, it is unclear whether CO can also offer protection in permanent ischemic stroke or what mechanism underlies the effect. HO1 neuroprotection is shown to be regulated by Nrf2; therefore, we investigated whether CO might partially exert neuroprotection by modulating the Nrf2 pathway. To evaluate potential protective effects of CO, we exposed wildtype and Nrf2–/– mice to 250 ppm CO or control air for 18 h immediately after permanent middle cerebral artery occlusion. Infarct volume and neurological deficits were assessed on day 7. Molecular mechanisms of Nrf2 pathway activation by CO were also investigated. Mice exposed to CO after permanent ischemia had 29.6 ± 12.6% less brain damage than did controls at 7 days. Additionally, 18 h CO treatment led to Nrf2 dissociation from Keap1, nuclear translocation, increased binding activity of Nrf2 to HO1 antioxidant response elements, and elevated HO1 expression 6–48 h after CO exposure. The CO neuroprotection was essentially completely abolished in Nrf2–/– mice. Low-concentration of exogenous CO represents a neuroprotective agent for stroke combination treatment and its beneficial effect would be at least partially mediated by activation of the endogenous Nrf2 pathway.

Keywords Medical gas, neuroprotection, Nrf2-regulated protein, stroke

L45

The lipogenic regulator Sterol Regulatory Element Binding Factor-1c is required to maintain peripheral nerve structure and function

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Myelin is a membrane characterized by high lipid content to facilitate impulse propagation. Changes in myelin fatty acid (FA) composition

have been associated with peripheral neuropathy [1], but the specific role of peripheral nerve FA synthesis in myelin formation and function is poorly understood. We explored the extent to which lack of the key regulator of FA synthesis as Sterol Regulatory Element Binding Factor-1c (Srebf-1c) could result in the development of peripheral neuropathy. We found that Srebf-1c null mice display a neuropathic phenotype consisting in hypermyelinated small caliber fibers, the result of changes in myelin periodicity. Unexpectedly, transcriptomics and metabolomics revealed activation of peroxisome proliferator activated receptor α (Pparα) signaling in Srebf-1c null peripheral nerve as a result of increased levels of two distinct phosphatidylcholine-based Pparα ligands, PC-C16:0/C18:1 and PC-C18:0/C18:1 [2,3]. Pparα is a nuclear receptor that directs uptake, utilization and catabolism of FAs [4]. As a consequence of abnormal local Pparα activation, Srebf-1c null peripheral nerve exhibit increased fatty acid utilization, a detrimental condition leading to peripheral neuropathy. Treatment with a Pparα antagonist rescues the neuropathy of Srebf-1c null mice. These findings reveal the importance of FA synthesis to sustain peripheral nerve structure and function.

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Keywords Peripheral neuropathy, Schwann cells, metabolism

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L46

The molecular control of GnRH neuron development

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Fertility critically depends by a small number of hypothalamic neurons secreting the neurohormone GnRH. During development GnRH neurons migrate from the nasal placode to the hypothalamus by following the migratory path formed by the vomeronasal axons. Developmental defects of this process can cause congenital GnRH deficiency (GD), characterized by absent/delayed puberty and consequent infertility. The underlying mutated loci are for the majority of GD cases unknown, partially because of a poor understanding of the molecular mechanisms that control the development of these crucial neuroendocrine cells. Here we provide evidence of the importance of class 3 semaphorins and their receptors in this process. Specifically, I will explain how two different semaphorins play distinct roles during the migration of GnRH neuron.s Thus, semaphorin3A affects the migration of GnRH neurons in the nasal compartment, via the co-receptors Neuropilin-1 and 2, whereas semaphorin3E via its receptor plexind1 controls the survival of GnRH neurons once positioned in the hypothalamus. Accordingly, disrupted semaphorin signalling may be involved in the aethiopathogenesis of genetic diseases characterized by GnRH deficiency.

Keywords GnRH neuron, semaphorin, migration

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L47

The role of microvascular endothelial WNT signaling the formation of the blood brain barrier

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We analyzed the pathological consequences of abnormal Wnt/β-catenin signaling in endothelial cells of brain vessels using a murine model of Cerebral Cavernous Malformation (CCM) disease that develops after endothelial-cell-selective ablation of the CCM3 gene. We report increased transcription activity of β-catenin in CCM3-knockout endothelial cells in in-vitro and in-vivo models. Such activation is cell-autonomous, independent of Wnt-receptor stimulation, does not induce canonical Wnt/β-catenin signaling and represents an early response to CCM3 ablation that initiates the expression of EndMT makers before the onset of Tgf-β/BMP signaling which is required for the progression of the pathology, as we have previously described. We also show that the NSAIDs sulindac sulfide and sulindac sulfone, which attenuate β-catenin transcription activity, significantly reduce the number and dimension of vascular lesions in the central nervous system of mice with endothelial cell CCM3 knockout. These NSAIDs thus represent pharmacological tools for inhibition of the formation of vascular lesions, particularly with a view to patients affected by the genetic variant of CCM, who continue to develop new malformations over time.

Keywords Beta-catenin, cerebral cavernous malformation, endothelium

L48

WNT signaling mechanisms in nociception and sensitization of afferent neurons

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Wnt signalling represents an ancient and highly versatile signalling system, which plays diverse critical roles in embryonic development. Sensory neurons of the dorsal root ganglia require Wnt signalling for initial cell fate determination as well as patterning and synapse formation over later development. Recent studies have functionally linked mis-regulation of Wnt signalling to cancer, bone disorders and abnormal synaptic function in adult life. We will discuss a novel, functional role of Wnt signalling pathways in sensitization of peripheral adult sensory neurons in a pathophysiological context, and the underlying molecular mechanisms. Using an interdisciplinary approach, through different technique spanning molecular, genetic, and behavioural experiment in vitro as well as in vivo pain models in mice, we show that Wnt3a is able to recruit different Wnt-pathways, to alter pain sensitivity in a modality-specific manner, acting via intracellular kinases in peripheral nerves. We found evidence for an intriguing dichotomy of non-canonical signalling pathways in mediating mechanical and thermal hypersensitivity. Indeed, while the calcium pathway is mainly involved in thermal hypersensitivity, mechanical hypersensitivity is driven by the planar cell polarity pathway. Interestingly we did not find clear functional evidence for the canonical Wnt signalling pathway in Wnt3a-induced sensitization of nociceptors. Finally, we will provides evidence for a translational potential for targeting peripheral Wnt signalling in tumour-nerve interactions and pathological pain hypersensitivity, paving the way for therapeutic interventions.

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Keywords Wnt signalling, DRG, nociception

L49

WNT signaling in midbrain dopaminergic neuron development and cell replacement therapies for Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disease characterized by the loss of midbrain dopamine (mDA) neurons. Clinical trials using human embryonic midbrain tissue for transplantation have provided proof of concept that cell replacement therapy (CRT) can lead to not only symptomatic relief, but also changes in the course of disease and

withdrawal of medication. Human pluripotent stem cells are currently regarded as the main candidate cell type for CRT because they are readily available, expandable, and can be standardized and differentiated into mDA neurons capable of inducing functional recovery in animal models of PD. However, protocols for mDA differentiation are still far from optimal and require further improvement. We previously found that members of the Wnt family of morphogens regulate multiple aspects of mDA neuron development [1]. Different branches of the Wnt signaling pathway, such as Wnt/β-catenin, activated by Wnt1, and Wnt/PCP, activated by Wnt5a, have been thought to regulate separate or opposing functions. However, we found that Wnt5a cooperates with Wnt1 to promote mDA neurogenesis and that Wnt1 cooperates with Wnt5a to promote the differentiation of postmitotic mDA neuroblasts [2]. We are currently applying this knowledge to improve protocols for the differentiation of human stem cells into mDA neurons suitable for transplantation and functional recovery in animal models for PD [3].

Keywords WNT, dopaminergic neurons, Parkinson's disease

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L50

WNT signaling in microglia and the glioma microenvironment

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WNT signaling in microglia and the glioma microenvironment Gunnar Schulte WNT signaling is important during embryonic development and organogenesis having specific roles in the development of the CNS such as regulation of neural tube formation, axon guidance and CNS stem cell regulation. Our work has recently established a role of WNT signaling in the regulation of the brain's macrophages, the microglia and thus WNTs emerge as novel regulators of CNS inflammatory responses. First of all, it appeared that b-catenin levels are elevated in microglia in Alzheimer disease (AD) brains as well as microglia cells in AD mouse models. Employing in vitro studies of primary mouse microglia isolated from newborn mouse pups indicated that both WNT-3A and WNT-5A induce diverse signaling routes in microglia leading to differential proinflammatory modulation of the cells. Interestingly, the net effect of WNT stimulation on the inflammatory potential of mouse microglia is context dependent. While WNTs increase inflammatory markers when giving to microglia alone, they are able to act in an anti-inflammatory manner when microglia are activated by prestimulation with lipopolysaccharides. Our findings thereby indicate that WNTs act on microglia as a homeostatic regulator, further underlined by yet unpublished data that suggest that WNT-5A is elevated in human glioma associated with a distinct inflammatory signature of the tumor as well as a substantial invasion of microglia.

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Keywords WNT, CNS inflammation, brain tumor

L51

Translational repression in the pathogenesis of FUS- and C9orf72-dependent ALS

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The major focus of ALS research has recently moved to RNA control of motor neuron functions, as most of the newly identified genes, that alone account for more than half of ALS familial cases, are clearly associated to RNA regulation. These include FUS and TDP43, two DNA/RNA binding proteins with a role in the regulation of RNA transcription, splicing, transport and translation, and C9orf72, a gene that is marked by the presence, in carriers, of an highly expanded GGGGCC repeat that is believed to provide the mutant gene of an acquired, toxic feature by an RNA-dependent gain of function mechanism. Thus, RNA dysmetabolism is likely to represent a central issue in ALS pathogenesis. Yet, whether a specific step of RNA processing is particularly affected in ALS motor neurons is unclear. We have recently obtained evidence that a prominent effect of FUS and C9orf72 expression is the induction of stress granules-associated translational repression. In this presentation I will discuss our recent work on the molecular mechanisms underlying these effects and their potential relevance in ALS pathogenesis.

Keywords ALS, RNA trafficking, stress granules

L52

Post-transcriptional modifications caused by TDP-43 mutations in mouse and man

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SpringerPlus 2015, **4(Suppl 1):**L52

TDP43 is a ubiquitously expressed prevalently nuclear protein involved in RNA splicing, RNA stability and miRNA processing. Amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) and inclusion body myopathy (IBM) are characterized by TDP43 being depleted from the nucleus and accumulating in cytoplasmic inclusions, which are the pathological hallmark of the disease – these diseases are also defined as "TDP43 proteinopathies". Mutations in TDP43 have been found to be causative of a proportion of ALS cases reinforcing the primary importance of this molecule in disease pathogenesis. The pathogenic mechanism by which TDP43 acts is unclear, and both loss of nuclear function (LOF) and gain of function (GOF) mechanisms have been proposed. We here discuss how we used muscle tissue, which provides high quality RNA of patient disease tissue, to investigate the consequences of TDP43 mislocalization. Further, we characterize two novel mouse TDP43 mutant lines, carrying ENU-induced mutations in order to study the effects of TDP43 mutations expressed at physiological levels in the mammalian central nervous system. One mutation (deltaRNA) strongly reduces the RNA-binding capacity of the protein; the second mutation (C-TERM) is in the glycine-rich C-terminal domain where the majority of human pathogenic mutations are found. Our results underline the importance of studying models with physiological expression levels of TDP43 mutations and shed light on the different effects on RNA metabolism caused by the TDP43 loss and gain of function.

L53

Sm-like proteins in the pathogenesis of Spinal Muscular Atrophy

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Proximal Spinal Muscular Atrophy (SMA) is caused by an insufficient supply of the Survival of Motor Neurons (SMN) protein. It is characterized by the selective degradation of the alpha motor neurons in the spinal cord. Analysis of the mouse model, which faithfully mimics the SMN insufficiency and the motor neuron phenotype, shows that motor neuron degeneration starts in the axons, specifically at the neuromuscular junctions. SMN is a house-keeping factor that is necessary for the assembly of the seven-membered ring of Sm proteins around the spliceosomal snRNAs and that is therefore required for pre-mRNA splicing. Such a function cannot easily explain the selective motor neuron phenotype. It has been proposed that insufficient supply of the SMN protein affects alternative splicing, especially of U11/U12-dependent introns, in mRNAs that are crucially required in

motor neurons. Details, however, remain elusive. In addition to the Sm proteins, there are the like-Sm (LSm) proteins that also form heptameric complexes and participate in various steps of mRNA metabolism. We have previously shown that one such LSm complex is involved in the transport of mRNAs to the neurites, especially the axons of motor neurons. mRNA transport and local protein synthesis is an important mechanism to bring about sudden changes in the proteome at distal regions of the cell. Here, we further explore the role of the LSm proteins in neuronal mRNA regulation and how this could be relevant for the SMA pathology. Interestingly, we find that one such LSm protein is significantly depleted in the SMA mouse model before the onset of the disease, possibly indicating a causal involvement.
Keywords SMA, mRNA regulation, Sm-like proteins

L54

Diverse roles of FUS in Amyotrophic Lateral Sclerosis

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A number of different genes have been found mutated in patients with Amyotrophic Lateral Sclerosis (ALS). Several of these genes encode for proteins involved in multiple steps of RNA processing, suggesting that mRNA dys-metabolism has a role in the degeneration of motor neurons. This is the case also for FUS-linked ALS. FUS (Fused in Sarcoma) is a DNA/RNA binding proteins with an established, yet not completely clear, role in the regulation of RNA transcription, splicing, transport and translation. However, recent evidence indicates that (similarly to mutant ALS-linked SOD1) the toxic function of this protein may lie also in its propensity to aggregate and sequester other proteins, and/or in its ability to induce mitochondrial damage and oxidative stress. In this presentation I will discuss our recent work on the molecular mechanisms underlying these effects and their potential relevance in the pathogenesis of ALS.
Acknowledgements This work is supported by ARiSLA (Project OligoALS to M.T.C.)
Keywords ALS, protein aggregation, mitochondrial damage

L55

The protein quality control system in motoneuron diseases

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Spinobulbar muscular atrophy (SBMA) and amyotrophic lateral sclerosis (ALS) are two related motorneuron diseases (MNDs), both characterized by the presence of inclusions o aggregates of proteinaceous materials. In SBMA, aggregates contain mutant androgen receptors (AR) with an elongated polyglutamine tract (ARpolyQ), while in ALS aggregates contain TDP43, ubiquilin, optineurin, etc. Exceptions are familial ALS (fALS) forms linked to superoxide dismutase 1 (SOD1) mutations, in which aggregates are composed of mutant SOD1. Protein aggregation occurs when a large excess of proteins with aberrant conformations (misfolding) in produced and poorly cleared from the cells. Neurons contains an efficient protein quality control (PQC) system, but this may be insufficient to correctly remove misfolded proteins, especially during aging. The PQC system requires the activities of efficient chaperones and of the two major intracellular degradative systems: the ubiquitin-proteasome (UPS) and the autophagic systems. When misfolded protein are recognized by chaperones, they can be removed via autophagy by their engulfment into autophagosomes which then fuseto lysosomes. We found that motoneurons may responds to misfolded species by activating the expression of a small HSP, HSPB8, which facilitate the clearance of misfolded species via autophagy, usually acting by restoring the proper autophagic flux, found altered in MNDs. HSPB8 requires its co-chaperone BAG3. BAG3 binds the protein 14-3-3 and with this it interacts with dynein in a complex which also includes HSC70-CHIP. Dynein moves this large complex on the microtubules organization center where autophagosomes are assembled. Here, CHIP ubiquitinated misfolded protein substrates allowing their recognition by p62 and clearance from the motoneurons. Thus, together, the PQC and the HSPB8 proteins help to protect motoneurons from damages associated to the presence of aberrant protein species accumulating in affected cells.
Keywords Motoneuron diseases, protein misfolding, autophagy

L56

Neurochemical changes in different brain regions induced by PACAP – relations to neuroprotection

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide with diverse occurrence and functions. One of the most well-known effects of PACAP is its strong neuroprotective effect. In this presentation we give an insight into recently described neurochemical changes induced by PACAP or altered by PACAP the lack of it. In an invertebrate model for Parkinson's disease we found that PACAP effectively counteracts the dopamine-decreasing effect of rotenone, a mitochondrial neurotoxin. Similarly, in a 6-hydroxydopamine-induced rat model of Parkinson's disease, we found that PACAP effectively increases dopamine levels. Furthermore, our proteomics analysis shows that PACAP treatment also counteracts the 6-OHDA-induced decrease in PARK-7 protein, effective against oxidative stress. Studying the role of endogenous PACAP, we found that PACAP-deficient mice show higher susceptibility to toxic agents causing degeneration of the substantia nigra dopaminergic neurons. Using proteomic analysis we revealed that the expression of numerous proteins is altered in the mesencephalon and striatum of knockout mice. Among the altered proteins, several are involved in metabolic processes, energy homeostasis, and structural integrity. ATP-synthase and tubulin beta-2A were expressed more strongly in PACAP-knockout mice. In contrast, the expression of more peptides/proteins markedly decreased in knockout mice, like pyruvate kinase, fructose biphosphate aldolase-A, glutathione S-transferase, peptidyl propyl cis-trans isomerase-A, gamma enolase, beta-synuclein and aspartate amino transferase. The altered expression of these proteins might partially account for the decreased antioxidant, cytoprotective and detoxifying capacity of PACAP-deficient mice. The described changes may provide further explanation for the neuroprotective potency of PACAP.
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Keywords PACAP, neuroprotection, proteomics

L57

Contribution of the galanin system to inflammation

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Neurogenic inflammatory components mediated by peptidergic sensory nerves have a crucial impact on the symptoms of inflammatory diseases. Galanin is a regulatory sensory neuropeptide, which was shown to attenuate neurogenic inflammation, but our current understanding about its endogenous targets, and physiologic importance is incomplete. Among the endogenous receptors of

galanin (GAL1, GAL2, GAL3) we found GAL3 to be the most abundantly expressed on the vasculature and GAL2 on different types of immune cells including polymorphonuclear neutrophils and natural killer cells. Therefore, we evaluated if galanin exerts direct or indirect effects on these immune cells. Our data revealed that galanin can be regarded as an immunomodulatory peptide as it can sensitize polymorphonuclear neutrophils and natural killer cells towards proinflammatory cytokines. Since there are only scarce in vivo data concerning the role of GAL3 in inflammatory disease conditions, we analysed its involvement in the K/BxN serum transfer model of autoimmune arthritis and the oxazolone-model of allergic contact dermatitis, employing GAL3 gene-deficient mice. After arthritis induction, GAL3-knockout mice demonstrated increased clinical disease severity and earlier hindlimb edema than wildtype mice. Vascular hyperpermeability was also elevated compared to wildytpes, but neutrophil myeloperoxidase activity and arthritic hyperalgesia were not significantly different. In contrast, disease severity, vascular, and immune components were not affected in allergic contact dermatitis in GAL3 knockouts in comparison with wildtypes. Our findings suggest GAL3 activation as a substantial anti-inflammatory pathway in neutrophil-dominated autoimmune arthritis, modulating the early neurogenic vascular hyperpermeability and consequent edema formation. However, the involvement of GAL3 activation in the T-cell dependent allergic contact dermatitis remains unsupported.

Keywords Galanin, inflammation, immune cells

L58

Characterization of neuropeptides which control cerebellar granule cell survival, migration and differentiation

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During cerebellar development, granule cell precursors are produced from a secondary germinative zone forming the external granule cell layer (EGL). Immature granule neurons from the inner part of the EGL then start a tangential migration followed by a centripetal inward radial migration across the molecular and Purkinje cell layers to reach their final destination at the bottom of the forming internal granule cell layer (IGL). This complex migratory process is highly regulated and takes about 2 days in rodents and it is essential for the proper formation of the cortical layers forming the mature cerebellum. In the IGL, granule cells differentiate to establish functional excitatory synapses with GABAergic neurons including Purkinje, basket, stellate and Golgi cells, or die. Some neurotrophins and neurotransmitters have been shown to be involved in the control of cerebellar granule cell survival, migration and differentiation. Initially, when I started my carrier as a researcher, we used to claim that very few neuropeptides were produced in the cerebellum. Nevertheless, we now know that this was wrong as we have recently identified by mass spectrometry over 70 peptides expressed in the cerebellum during development. Over the years, the involvement of some of these peptides such as somatostatin, PACAP or ODN, has been established in the control of cerebellar granule cell survival, migration and differentiation as will illustrate my presentation.

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Keywords Cerebellar granule neurons, neuropeptides, brain development

L59

A new face of orexins action – neuroprotection

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Orexins A and B (hypocretins 1 and 2) are two closely related peptides produced mainly by hypothalamic neurons that project to numerous brain structures. Orexins exert their biological activity by binding to two subtypes of GPCR receptors, OX1R and OX2R. The orexin system has been shown to orchestrate multifaceted physiological functions, including vigilance and the sleep/wake cycle, energy homeostasis, endocrine, visceral functions and pathological states, such as narcolepsy and drug abuse [1]. In addition, a neuroprotective potential of orexin A has been recently demonstrated in rats using a model of focal cerebral ischemia [2]. In our studies we investigated effects of orexins on survival of cultured neurons from the rat cerebral cortex. Quantitative real-time PCR revealed the presence of OX1R and OX2R in cortical neurons. Orexins and [Ala11-D-Leu15]orexin B (a selective agonist of OX2R), used at 0.001-1 microM concentrations, markedly increased neuronal cells viability, an effect associated with an attenuation of caspase-3 activity. Comparable potency of the three peptides suggests a predominant role of OX2R in the studied phenomenon. Under conditions of chemical hypoxia, orexins potently increased neuronal viability and protected cortical neurons from oxidative stress. The pro-survival effect of orexins was blocked by U0126 and 10-DEBC, inhibitors of MEKK and Akt, respectively. In addition, orexins A and B stimulated Akt and ERK1/2 in cortical neurons in a time- and concentration-dependent manner. It is suggested that both Akt and ERK1/2 play an important role in the pro-survival effects of orexins in neurons.

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Keywords Orexins, neuroprotection, oxidate stress

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POSTER PRESENTATIONS

P1

Characterization of ligand binding to dopamine receptors with fluorescence anisotropy based assay

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Dopamine receptors are G-protein-coupled receptors (GPCRs), which are involved in a wide variety of physiological processes. Abnormalities in dopaminergic signal transduction are associated with many different diseases. Therefore, dopamine receptors are targets for variety of drugs involved in disorders like schizophrenia, Parkinson's disease, depression and many others. In order to develop drugs with less side effects and better efficacy it is necessary to understand and characterize receptor-ligand interactions in further detail. In addition, measuring the on- and off-rates of different ligands provides important information about the kinetic profiles of potential drug candidates. We have applied fluorescence anisotropy (FA) assay to investigate kinetic properties and affinities of different ligands for dopamine D1 receptor. For that we have implemented budded baculoviruses as a source of recombinant protein (Veiksina et al., 2014). As a result, we have seen that fluorescent ligand Bodipy-FL-SKF-83566 is suitable for the pharmacological characterization of non-labelled dopaminergic ligands. The obtained results are in good agreement with the data obtained from the radioligand [3H]SCH 23390 binding experiments with the same baculovirus preparations. In conclusion, by using fluorescence based detection assay, we are now able to perform real-time monitoring of ligand binding. Obtained results demonstrate that fluorescence anisotropy based assay is applicable for the study of dopamine receptors and their ligands.

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Keywords G protein-coupled receptors, Fluorescence anisotropy, dopamine receptors

P2

The effect of P2Y12 receptor inhibition in chronic inflammatory pain

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Previous studies of our group indicate that genetic deletion and pharmacological antagonism of P2Y12 receptors alleviate mechanical allodynia in acute inflammatory pain. In this project we investigated the role of P2Y12 receptors in chronic inflammation. For this study we have introduced a CFA-model in wild type and P2Y12R gene deficient (P2ry12–/–) mice. By the injection of Complete Freund's Adjuvant (CFA) in the plantar surface of the right hind paw, we could induce local inflammation persisting at least 14 days. During this 2 week period, mechanical sensitivity was evaluated at several time points using dynamic plantar von Frey aesthesiometer. Mechanical allodynia could be observed 3 days after CFA injection. In P2Y12 receptor gene deficient mice the decline in the paw withdrawal threshold (PWT) was significantly lower than in wild type mice. Both intrathecal and intraplantar administration of PSB-0739, a selective and potent P2Y12 receptor antagonist had robust antihyperalgesic effect in wild type mice, whereas treatment did not affect the PWT in the P2ry12–/– group. Intraperitoneal injection of A-803467, a potent and selective NaV1.8 sodium channel blocker evoked antihyperalgesic effect similar to the PSB-0739. When these two compounds added together, no additive effect was observed, i.e. A-803467 occluded the effect of PSB-0739. To investigate whether P2Y12 receptors on thrombocytes contribute to this effect, we used anti-mouse CD41 antibodies to induce depletion of platelets. Mice were submitted to intraperitoneal injection of this antibody at the end of the first week and measurement of the mechanical allodynia was performed on the second week. Our findings indicate that P2Y12 receptor inhibition is a potential therapeutic approach in chronic inflammatory pain, although its exact mechanism of action needs further investigation.
Keywords Inflammation, pain, purin

P3

Acute stimulation of glycolytic flux in cultured primary astrocytes by the tyrosine kinase inhibitor tyrphostin 23

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Tyrphostin 23 (T23) is a well-known inhibitor of protein tyrosine kinases. To investigate potential effects of T23 on the viability and the glucose metabolism of brain cells, we exposed cultured primary rat astrocytes to T23. While the viability and the morphology of the cells were not acutely affected during an incubation of the cultures for up to 4 hours with T23 in concentrations of up to 200 µM, the presence of T23 rapidly stimulated glycolytic flux as demonstrated by a time- and concentration-dependent increase in glucose consumption and lactate release. Maximal effects were observed for incubations with 100 µM T23 which caused a doubling of glucose consumption and lactate production. The stimulation of glycolytic flux by T23 was fully reversible upon removal of the compound. In contrast to T23, the structurally related tyrosin kinase inhibitor tyrphostin 25 did not affect glycolytic flux, nor was the stimulation by T23 substantially affected by the trichloracetate-induced activation of pyruvate dehydrogenase. Further experiments are now required to elucidate the mechanism of T23-induced stimulation of astrocytic glycolysis.
Keywords Tyrphostin 23, astrocytes, glycolysis

P4

Increase of the FGFR1 signaling in the FGFR1-5-HT1A heteroreceptor complex in midbrain raphe 5-HT neuron systems via allosteric receptor-receptor interaction

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The ascending midbrain 5-HT neurons known to contain 5-HT1A autoreceptors may be dysregulated in depression due to a reduced trophic support. New findings show existence of FGFR1-5-HT1A heteroreceptor complexes in the rat hippocampus with a partial characterization of their interface and in midbrain raphe 5-HT nerve cells. With *in situ* Proximity Ligation Assay (PLA) and supported by co-location of the FGFR1 and 5-HT1A immunoreactivities in midbrain raphe 5-HT cells, evidence for the existence of FGFR1-5-HT1A heteroreceptor complexes were obtained in the dorsal and median raphe nuclei of the Sprague–Dawley rat. Their existence in the rat medullary raphe RN33B cell cultures was also established. After combined FGF-2 and 8-OH-DPAT treatment, a marked and significant increase in PLA positive clusters was found in the RN33B cells. Synergistic receptor-receptor interactions in these receptor complexes indicated their enhancing role in hippocampal plasticity. The existence of FGFR1-5-HT1A heteroreceptor complexes also in midbrain raphe 5-HT nerve cells open up the possibility that antidepressant drugs by increasing extracellular 5-HT levels, can cause an activation of the FGF-2/FGFR1 mechanism in these nerve cells as well. Therefore, the agonist modulation of the FGFR1-5-HT1A heteroreceptor complexes and their specific role is now determined in rat medullary raphe RN33B cells and in the caudal midline raphe area of the midbrain rich in 5-HT nerve cells. The combined icv treatment with FGF-2 and the 5-HT1A agonist 8-OHDPAT synergistically increased FGFR1 and ERK1/2 phosphorylation in the raphe midline area of the midbrain and in the RN33B cells. Cotreatment with FGF2 and the 5-HT1A agonist induced RN33B cell differentiation as seen from development of the increased number and length of extensions per cell and their increased 5-HT immunoreactivity. These signaling and differentiation events were dependent on the receptor interface since they were blocked by incubation with TMV but not by TMII of the 5-HT1A receptor. Taken together, the 5-HT1A autoreceptors by being part of a FGFR1-5-HT1A heteroreceptor complex in the midbrain raphe 5-HT nerve cells appears to have also a trophic role in the central 5-HT neuron systems besides playing a key role in reducing the firing of these neurons.
Keywords Fibroblast growth factor receptor, serotonin 5-HT1A receptor, heteroreceptor complexes

P5

Copper oxide nanoparticles: Synthesis, toxic potential and modulation of astrocytic metabolism

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To test for potential consequences of an exposure of brain cells to copper oxide nanoparticles (CuO-NPs), we have synthesized dimercaptosuccinate-coated CuO-NPs. These particles had a diameter of around 5 nm as determined by transmission electron microscopy but were dispersed as aggregate as demonstrated by their average hydrodynamic diameter in aqueous dispersion of 136 ± 4 nm. Exposure of cultured primary astrocytes to CuO-NPs increased the cellular copper levels and compromised the cell viability in a time- and concentration-dependent manner. CuO-NPs in concentrations above 100 µM (6.3 µg

copper/mL) severely affected the viability of the cells, as demonstrated by a lowered tetrazolium dye reduction capacity, a lowered cellular lactate dehydrogenase activity, a increased membrane permeability and the generation of reactive oxygen species. In contrast, exposure of astrocytes for 24 h with 100 μM CuO-NPs did hardly affect the viability of astrocytes but stimulated the glycolytic flux, increased the cellular glutathione content, stimulated the release of glutathione and elevated the level of the metal storing proteins metallothioneins. Presence of the intracellular copper chelator tetrathiomolybdate throughout the incubation with CuO-NPs protected the cells against the toxicity of CuO-NPs and prevented the stimulation of the glycolytic flux as well as the increased levels of metallothioneins. These data demonstrate that CuO-NPs can severely damage cultured astrocyes and that copper ions derived from sub-toxic concentrations of CuO-NPs strongly affected the metabolism of astrocytes.

Keywords Copper, nanoparticles, sstrocytes

P6

Alteration of the protein quality control system in motor neuron and muscle expressing mutant proteins causing ALS and SBMA

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Motor neuronal system and muscle tissue are two districts differently affected at onset and during the progression of diseases like amyotrophic lateral sclerosis (ALS) or spinal and bulbar muscular atrophy (SBMA). The bases of these diseases are linked to mutated proteins: fALS is commonly caused by mutations in the SOD1 or the TDP43 genes and SBMA is caused by a of CAG repeat in the AR gene. A fraction of these proteins can not reach a mature conformation and misfold. The Protein Quality Control (PQC) system is responsible for the correct protein homeostasis: the chaperones maintain proteins in their correct conformations. If they fail, mutated proteins are directed to the proteasome or the macroautophagy. When misfolded proteins are not correctly removed, they aggregate in nucleus and cytoplasm. To understand cellular behavior in presence of misfolded toxic proteins we investigate the different activation of the PQC system in the two mayor tissues involved. Initially we investigated the differences in PQC activation between NSC34 motor neuronal and C2C12 muscular cell models. Using RTq PCR, western blot and immunocytochemical analysis for p62 and LC3 expression, localization, and turnover we demonstrated that C2C12 cells have a more active autophagic system than NSC34 cells. Then, we compared the two models in presence of misfolded protein inhibiting degradative systems. With Filter Retardation Assay, we observed that these proteins tend to aggregate when PQC system is impaired. Then, we potentiated the PQC response to reduce the insoluble species. By overexpressing the small heat shock protein B8 in both systems we demonstrated that AR polyQ and SODG93A insoluble species were reduced. Also autophagy activation by trehalose caused a reduction in protein aggregation in both cell models. In conclusion misfolded protein aggregates can be reduced by modulating macroautophagy and this could represent a new therapeutical strategy for disease like SBMA and ALS.

Keywords Neurodegeneration, misfolded proteins, PQC system

P7

Neuroprotection by Nerve Growth Factor (NGF) involves modulation of neuronal autophagy

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Nerve Growth Factor (NGF) plays a key role in development and function of specific neuronal populations of the CNS. Decreased NGF availability is also responsible for neuronal vulnerability in neurodegenerative disorders, such as Alzheimer's disease (AD). We used PC12 cells and primary neurons to investigate the potential role of NGF in modulating neuronal autophagy, a cellular process whose deregulation has been linked to the loss of neuronal. The mammalian target of rapamycin has been recently proposed as a therapeutic target for AD due to the role of the autophagy pathway in improving cognitive function by reducing Aβ and Tau pathology. We here show that NGF treatment induces LC3 conversion (LC3-I to LC3-II), a marker of autophagy, through the activation of AMP-activated protein kinase (AMPK). We show that NGF increases the autophagic flux (the dynamic process of autophagosome synthesis, delivery to the lysosome and degradation) as indicated by western blot and fluorescence microscopy analyses in the presence of autophagy inhibitors. These data were confirmed by RT-PCR array analysis and RNA interference-mediated knockdown of autophagy-related genes: Atg9b, Atg12 and AMBRA1, a positive regulator of the BECLIN 1-dependent program of autophagy. In addition, flow cytometry analysis by DCF-DA showed that treatment with autophagy inhibitors determined a strong increase of reactive oxygen species (ROS) followed by decreased cell survival. These changes were fully reversed by NGF treatment, suggesting its potential role in clearing dysfunctional mitochondria. This hypothesis was further confirmed by fluorescence microscopy studies using LC3 and Cox-IV antibodies, showing co-localization of autophagosomes and mitochondria. Overall these data identify a novel aspect of the neuroprotective function of NGF in promoting the clearance of dysfunctional mitochondria (or mitophagy), thus further supporting its therapeutic potential in neurodegenerative pathologies.

Keywords NGF, Alzheimer disease, autophagy

P8

Brain extracellular levels of dimethylarginines (ADMA, SDMA) and cGMP and their modulation by exogenous S-adenosylmethionine in rats with acute liver failure

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Acute liver failure (ALF) instantly evokes symptoms of hepatic encephalopathy (HE), mainly attributed to hyperammonemia. Ammonia neurotoxicity is related to disturbances in the nitric oxide (NO)/cGMP pathway. The methylated derivative of L-arginine (MDALs) – asymmetric dimethylarginine (ADMA) but not symmetrically methylated arginine (SDMA) is an endogenous inhibitor of nitric oxide synthase. Elevated blood and brain ADMA was reported in HE patients and experimental animals. The question arose whether ALF evokes changes in the incorporation of the methyl donor S-adenosylmethionine (SAM) to MDALs, and how they affect cGMP accumulation. To this end, we investigated the effect of intracortical perfusion of SAM on the brain extracellular levels of ADMA, SDMA and cGMP in rats with ALF in the thioacetamide (TAA) model. Sprague Dawley rats (250–270 g) received three i.p. injections of TAA (300 mg/kg) at 24 h intervals. Bilateral microdialysis of the prefrontal cortex was carried out 24 h after the last TAA administration. SAM at 2 mM concentration in artificial cerebrospinal fluid was infused for 40 min and then the medium was changed back. The extracellular levels of ADMA and SDMA were analyzed using positive mode electrospray LC–DMS–MS/MS and cGMP was determined with cGMP enzyme immunoassay. ALF resulted in the increased extracellular levels of ADMA (by ~800%) and SDMA (by ~250%). Moreover, in ALF rats infusion of SAM decreased the ADMA and SDMA (~30%) as compared to the basal value. It seems reasonable that an excessive amount of substrate inhibited the enzymes synthesizing MDALs. On the other hand, the cGMP level did not differ between control and TAA rats. SAM increased by ~90% cGMP only in the control group, what indicates affected NO/cGMP pathway in TAA model. The study demonstrates, that ALF modulates methylation of arginine to MDALs in a manner affecting cGMP accumulation.

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Keywords Asymmetric dimethylarginine (ADMA), S-adenosylmethionine (SAM), hepatic encephalopathy

P9

Exogenous carbon monoxide improves neuronal differentiation: a near-death experience

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Cerebral ischemic injuries and neurodegenerative disorders lead to death or impairment of neurons in the central nervous system. Application of stem cell based therapies, namely stimulation of endogenous neurogenesis or cell transplantation, are promising strategies and currently under investigation. Carbon monoxide (CO) is an endogenous product of heme degradation by heme oxygenase. Although there is no published data reporting CO as a factor involved in stem cell differentiation, several evidences support this hypothesis. This gasotransmitter induces mitochondrial biogenesis, which is also broadly described to be involved in metabolic shifts during neuronal differentiation process. Likewise, CO-induced pathways can occur via generation of small amounts of ROS, which are also important signaling molecules in neuronal differentiation. The CO effect on modulation of neuronal differentiation is assessed in three different models with increasing complexity: human neuroblastome SH-S5Y5 cell line, human teratocarcinome NT2 cell line and hippocampal organotypic slice cultures (HOSC). CO does increase the final yield of post-mitotic neurons. During neuronal differentiation, CO promotes an increase on precursor cell proliferation and in parallel CO inhibits cell death. Furthermore, cell mitochondrial population is increased by CORM-A1 supplementation. Further work is needed for assessing the mechanisms underlying CO effects in neuronal differentiation, namely by targeting modulation of cellular metabolic pathways, redox alterations and autophagy related pathways. In conclusion, CO appears as a promising therapeutic molecule to stimulate endogenous neurogenesis or to improve in vitro neuronal production for cell therapy strategies.

Keywords Carbon monoxide, neuronal differentiation, neuroregeneration

P10

sRage plasma level in association with multiple sclerosis risk in Slovak population

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Receptor for advanced glycation end products (RAGE) triggers an intracellular signalling pathways of various proinflammatory ligands. Solubile form of RAGE (sRAGE) in human plasma can hinder the function of RAGE and can have the role in the pathogenesis of inflammatory diseases. In patients with multiple sclerosis (MS), altered level of sRAGE in plasma and cerebrospinal fluid has been found. In our study, we tried to identify possible association of sRAGE serum level with MS. Serum levels of sRAGE were detected by ELISA in 44 MS patients (22 patients with rapid progression of MS and 22 patients with slow progression of MS according to the MSSS score) and 32 healthy control subjects. We found significantly increased level of sRAGE in serum of MS patients when compared to controls. No significant differences in serum level of sRAGE where found between rapidly progressing and slow progressing subgroup of MS patients.Our results suggest for the role of sRAGE in MS ethiopathogenesis, but we did not find any association of sRAGE in serum with the rate of MS disability progression.

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Keywords Multiple sclerosis, sRAGE, ELISA

P11

Characteristics of adenine nucleotides content in acute hypoxia by mathematical analysis methods

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Oxygen starvation is observed in a variety of pathological states and serves as one of the urgent problems in medicine. A decrease in oxygen supply to tissues is accompanied by the inhibition of metabolic processes (primarily of energy metabolism), which impairs functional activity of the brain. The main source of energy for brain is adenosine triphosphate (ATP). It was shown that the components of adenylate pool can be used as early predictors of hypoxia.

Aim of the study The aim of our study was analysis of adenosine triphosphate (ATP) and adenosine monophosphate (AMP) experimental concentrations and integral coefficient K= in intact animals brain tissue and in disturbances of the oxygen regime by methods of mathematical analysis, as well as detection of some regularity in the character of their changes under the impact of hypoxia for the assessment and prediction of direction of production and utilization energy in metabolic pathways.

Methodology In this study empirical dependencies and criteria of statistical significance of mathematical modeling of quantitative relation between specified brain nucleotide stock indicators for the assessment and prognostication of brain energy state in extreme conditions were used.

Results and area of their application The use of empirical dependencies methods allowed to create multiregression models, subtly enough to unite experimental indicators ATP and AMP in hypobaric hypoxia and ischemia with different-term exposure. Obtained models can be used for prognostication of ATP and AMP concentrations in disturbances of the oxygen regime in a short or over a long period of time, as well as to receive information of indicator K= changing depending on brain hypoxia.

Conclusions functional dependencies are presented in this study to analyze shape, closeness and stability of relations between adenine nucleotides characterizing coupling of production and utilization energy processes, and also to predict the direction of these processes under hypoxic condition.

Keywords Adenylate pool, hypoxia, multiregression model

P12

Extracellular acidification leads to mitochondrial depolarization with following free radical formation in rat brain synaptosomes

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Brain ischemia is accompanied by lowering of pHo and pHi. We investigated an influence of of acidosis on free radical formation in synaptosomes. Three models were used: 1) Strong extracellular acidification down to pHo 6.0; 2) Moderate extracellular acidification down to pHo 7.0; 3) Intracellular acidification induced by addition of 1 mM amiloride corresponding to pHi decrease down to 6.65. We have shown that both types of extracellular acidification, but not intracellular acidification, increase DCFDA fluorescence by calcium-independent way that reflects free radical formation. These three treatments induce the rise of the dihydroethidium fluorescence that reports synthesis of superoxide anion. However, the impact of low pHi on superoxide anion synthesis was less than induced by moderate extracellular acidification. Mitochondrial uncoupler CCCP did not inhibit an increase of fluorescence of both dyes at pHo 6.0. In contrast, superoxide anion synthesis at pHo 7.0 was almost completely eliminated by CCCP. Furthermore, using fluorescent dyes JC-1 and rhodamine-123, we confirmed that decrease of pHo leads to mitochondria depolarization. Low pHi was not effective. Iron chelator deferoxamine and antioxidant ionol are inhibits pH-induced increase of DCFDA fluorescence, but does not influenced mitochondria depolarization. We are failed to found sodium influx monitored by fluorescent dye Sodium Green

in case of low pHo. Involving of plasma membrane receptor which is distinct from acid-sensitive ion channels (ASIC) and electron transport chain of mitochondria for moderate acidification can be suggested. Action of strong acidification seems to be mediated by release of iron from proteins. We have shown that low pHo led to oxidative stress in neuronal presynaptic endings that might underlie the long term irreversible changing in synaptic transmission.
Acknowledgements This work was supported by Belorussian Republican Foundation of Basic Investigation (grant B13-066).
Keywords Synaptosomes, ischemia, free radicals

P13

Oxidation of Methionine-35 in Alzheimer's amyloid-beta peptide and the aggregation of the oxidized peptide

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive loss of brain tissue and accumulation of amyloid-β(Aβ) and tau. Aggregation of Aβ peptides into amyloid plaques is considered to be a causative factor in AD, however the precise mechanism behind the AD onset has remained elusive. Oxidative stress (OS) is also characteristic to AD, but it is not known whether OS is a risk factor or a consequence of AD. It is assumed that Aβ aggregates generate free radicals in the presence of copper ions by participation of the Met35 residue, which can increase the OS levels. The aim of our study was to establish the role of Met35 residue in the oxidation of Aβ and peptide aggregation processes. Oxidation of Aβ was studied in the presence of two redox-active compounds: H2O2 and copper ions. In the absence of copper ions the Met35 residue was readily oxidized by H2O2 in a two electron process. The fibrillization of Aβ with Met35 oxidized to sulfoxide was threefold slower compared to that of the native peptide. TEM analysis showed that the fibrils of native and oxidized peptides are similar. The relatively small inhibitory effect of Met35 oxidation on the fibrillization suggests that the possible variation in the Met oxidation state should not affect the in vivo plaque formation. In the presence of copper ions (one-electron process) the oxidation was more complex: addition of the first oxygen was still the fastest process, however, it was accompanied by multiple unspecific modifications of several amino acid residues. Addition of copper ions to the already oxidized Aβ Met35 by H2O2, resulted in a similar pattern of nonspecific modifications, suggesting that the one-electron oxidation processes in Aβ do not depend on the oxidation state of Met35. Thus, it can be concluded that Met35 residue is not a part of the radical generating mechanism of Aβ-Cu(II) complex.
Keywords β-Amyloid, copper(II)ion, methionine oxidation

P14

The effects of optical, electrical and chemical stimulation on serotonin release from median raphe and hippocampus of mice

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The present study has examined several characteristics of the release of [3H]5-HT from the median raphe nucleus (MRN) and hippocampus in terms of its dependence of nerve impulse. We used electrical stimulation and the sodium channel opener veratridine, which excite all of the neuronal processes in the stimulation field, and optogenetics to selectively stimulate those terminals which express channelrhodopsin-2 (ChR2) and compared 5-HT release evoked by electrical and chemical depolarization and by light. We injected an adeno-associated virus containing DNA construct encoding ChR2 into the MRN of mice and investigated [3H]5-HT release from MRN and hippocampal slices. Serotonergic nerve terminals was locally stimulated with 473 nm light (blue laser diode) and electrically by bipolar electrode and by veratridine and transmitter release was monitored by collecting the effluent in a fraction collector. Electrical filed stimulation and veratridine resulted in a significantly increase in the efflux of 5-HT, whereas optical stimulation of ChR2 expressing nerve terminals at various frequencies (10, 20, 50, 100 Hz) elicited only a negligible increase in 5-HT release either from the hippocampus or from the MRN itself. The electrically induced release of radioactive neurotransmitter was completely inhibited by perfusion with tetrodotoxin. We have also applied the 5-HT transporter inhibitor, fluoxetine and the GABAA blocker bicuculline to relieve released 5-HT from re-uptake and any endogenous inhibition. Nevertheless, the effect of optical stimulation remained closed to the detection limit under these condition. In conclusion, whereas our method is suitable to detect [3H]5-HT efflux in response to ongoing neuronal sodium channel activity its sensitivity is too low to detect transmitter efflux evoked by focal optogenetic stimulation. The most likely reason for the failure of detection of 5-HT efflux is that ChR2 is expressed only by a small subpopulation of nerve terminals.
Keywords Channelrhodopsin-2, median raphe nucleus, serotonin

P15

Identification of the antigen recognized by rHIgM22, a remyelination-promoting human monoclonal antibody

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Recombinant human IgM22 (rHIgM22) binds to myelin and to oligodendrocytes, and promotes remyelination in a mouse model of multiple sclerosis [1]. rHIgM22 preferentially reacts with sulfatide-positive (O4-positive) oligodendrocytes [1]. Moreover, binding of rHIgM22 is abolished in CNS tissue slices from Cst(–/–) mice [2], suggesting that its binding to myelin requires the presence of a product of cerebroside sulfotransferase, possibly sulfatide, abundantly expressed in oligodendrocytes and in myelin. However the exact identity of the antigen recognized by this antibody remains to be elucidated. We have tested the binding of rHIgM22 to purified lipids and to lipid extracts prepared from mouse brain, brain myelin, mixed glial cultures, and O4-positive oligodendrocytes using TLC immunostaining and ELISA using liposomes and lipid monolayers with different composition. Our preliminary results show that rHIgM22 binds to sulfatide in vitro, while it does not bind to other myelin sphingolipids, including galactosylceramide and sphingomyelin, suggesting that sulfatide at the oligodendrocyte surface might be important for the binding of rHIgM22 to the surface of these cells and to myelin. However, IgM22 does not bind structures expressing sulfatide outside the nervous system, thus additional factors are likely relevant for the immunoreactivity of IgM22 in CNS. Indeed, we have observed in lipid extracts from different sources another lipid molecule selectively recognized by rHIgM22, whose identity is still under investigation. Remarkably, this lipid is also present in the extracts from mixed glial cultures, which do not contain mature O4-positive oligodendrocytes, suggesting that other glial cells in addition to oligodendrocytes might be important in the response to rHIgM22.
Acknowledgements This work was supported by Acorda Therapeutics.
Keywords Multiple sclerosis, remyelination, sulfatide
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P16

Investigation of P2X7R involvement in maternal poly(i:C) exposure evoked autistic features in mice

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Autism Spectrum Disorder (ASD) is a neurodevelopmental condition with core symptoms of unusual social interaction and communication

and increased repetitive behaviours with limited interests of environment. Recent studies have revealed that purinergic signalling (hyperpurinergia) is one of a key features of autism. Our aim was to establish a reliable model of ASD in our lab utilizing a broad range of behavioural experiments in order to investigate the role of P2X7 in autism. We injected Poly(I:C) (PIC) in two doses to pregnant C57Bl/6 mice: 3 mg/kg on E12.5 and 1.5 mg/kg on E17.5 respectively. Offsprings were weaned 4 weeks of age and behavioural studies started from 8 weeks of age. We performed social preference test, measured the body temperature and sensorymotor coordination (rotarod). We used self grooming and marble burying test in order to investigate manifestation of repetitive behaviour and measured the sensorymotor gating with Prepulse Inhibition (PPI). After behavioural experiments animals were sacrificed. Para-sagittal sections of the cerebellar vermis were cut and Purkinje cells were counted. Synaptosome fractions were made from half brains of animals and examined by electron microscopy. Striatum and Hippocampus monoamine content were measured by HPLC. We compared PIC treated offsprings with naive animals (n = 10–16 animals/group). PIC treated animals showed decreased sociability and sensorymotoric coordination but we did not find change in body temperature of PIC animals. MIA animals showed increased repetitive behaviour in the marble burying and self grooming test. Quantitative Purkinje cell dropout was found in PIC mice and electron microscopy of half brain revealed ultrastructural abnormalities in them. Higher level of monoamins were detected in ASD mice compared to the control group. Based on these results this model seem to be suitable to measure the effect of different compounds or genetic deletion on PIC induced ASD symptoms in rodents.

Keywords Poly(I:C), autism, P2X7R

P17

Effect of extracellular vesicles derived from distinct brain cells on Aβ toxicity and assembly: focus on microglia derived vesicles

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Alzheimer's disease (AD) is a neurodegenerative disorder. The patho-histological features in AD are intracellular accumulation of neuro-fibrillary tangles and extracellular senile plaques. Plaque deposition leads to recruitment and activation of microglial cells, which induces neuroinflammation and drives neurodegeneration.Recent evidence show that soluble pre-fibrillar Aβ species, rather than insoluble fibrils, are highly neurotoxic and correlate with disease severity.Hence, preventing formation of soluble Aβ and its interaction with neurons is a major goal in AD. Despite massive efforts, how extracellular factors regulate assembly of Aβ peptide and neurotoxic activity of Aβ species is still largely undefined. Recent studies indicate that Extracellular Vesicles(EVs),including exosomes and PM-derived microvesicles(MVs), may influence Aβ neurotoxicity. Our findings reveal that production of microglial MVs(m-MVs) is strikingly high in patients with mild cognitive impairment and AD as compared to healthy controls and positively correlates with markers of neurodegeneration and hippocampal atrophy. Furthermore we found that MVs isolated from the CSF of AD patients are toxic to cultured hippocampal neurons. Through in vitro studies we demonstrate that the m-MVs promote generation of neurotoxic soluble species from almost inert Aβ aggregates, which is mediated by lipid components of MVs. Our findings suggest that m-MVs favor formation of neurotoxic Aβspecies throughout the brain, possibly representing the mechanism behind transynaptic spread of Aβ in AD. On the other hand, studies conducted by Yuyama et al., 2012, 2014, and An et al., 2013, suggest that exosomes produced by neurons may exert opposite action by neutralizing neurotoxicity of soluble Aβ. To verify if the overall effect of exosomes and MVs on Aβneurotoxicity may vary depending on parental cell type we are currently studying the influence of EVs (exosomes &MVs) derived from distinct brain cells on Aβ toxicity and assembly.

Keywords Extracellular vesicles (EVs), Alzheimer's disease (AD), neurotoxicity

P18

GnRH level regulation in the hypothalamus of female rats of different age

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In recent years the study of age-related derangements of the gonadotropin releasing hormone (GnRH) synthesis and secretion resulting from both the GnRH gene expression changes and interaction of glia with GnRH-ergic neurons of the hypothalamus is a focus of attention. It was demonstrated earlier that this could result from the decreased activity of monoaminergic and peptidergic systems that control the GnRH preovulatory secretion surge initiation, specifically from the loss of the signal coming from the suprachiasmatic nuclei (SCN) of the hypothalamus. This signal is critical for the emerging of the GnRH regular cyclic secretion, which is mediated prominently by vasoactive intestinal peptide (VIP). We have studied age-related changes in the biogenic amines and VIP content in the hypothalamic structures responsible for the GnRH synthesis and secretion. It has been shown by us that the GnRH level in the median eminence with the arcuate nuclei (ME-Arc) of the hypothalamus of 22-month-old rats is half as high compared to that of 7–8-month-old animals. Beside that, the VIP level in the SCN tended to decrease, with the norepinephrine, dopamine, and 5-hydroxyindoleacetic acid levels decreased significantly in the median preoptic area of the hypothalamus responsible for the GnRH synthesis and in the ME-Arc exercising its secretion into the portal vein of the pituitary. It has been shown that the initial phase of the reproductive failure with 13–14-month-old animals having irregular estrous cycles is characterized by gradual disappearance of the normal biogenic amine diurnal dynamics in the studied hypothalamic structures, which could be due to the loss of regulatory signals coming from the SCN.

Keywords GnRH, biogenic amines, hypothalamus

P19

Toxicity of amyloid beta 1-40 and 1-42 on SH-SY5Y cell line

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Objectives Amyloid beta plaques are primary hallmark of Alzheimer's disease, which is characterized by specific neurodegeneration. Amyloid beta peptide – the main plaque component- was shown to be neurotoxic in animal models, primary neuronal cultures and immortalized cell lines. However, the results are often controversial and there is no good human cell line model for evaluation of the toxicity of amyloid peptides. Here we studied the effect of amyloid beta 1-40 and 1-42 on undifferentiated and differentiated human neuroblastoma cell line SH-SY5Y.

Results Undifferentiated cell culture was too diverse and unstable to reveal a toxic effect of amyloid beta peptides quantitatively. Differentiated cells established more neuron-like phenotype and were more identical and stable in culture suggesting potential susceptibility to amyloid beta as a neurotoxic agent. Amyloid peptides are prone to form different aggregates with diverse toxic properties, in current study, monomeric amyloid beta 1-40 and 1-42 were applied to the cells. Viability test WST-1 and propidium iodide (PI) uptake tests showed that undifferentiated cells are not susceptible to amyloid beta, however, differentiated cells showed reduced viability and increased PI uptake in case of amyloid beta 1-42, but not in case of amyloid beta 1-40.

Conclusions Current study revealed that amyloid beta has no remarkably toxic effect on undifferentiated SH-SY5Y cell line whereas viability of the neuron-like differentiated cell culture is significantly decreased by the amyloid beta 1-42 peptide that is known to form spontaneously toxic aggregates.
Keywords Amyloid beta, toxicity, SH-SY5Y

P20

Arsenic induced oxidative stress and mitochondrial dysfunction in rat brain

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The present study was undertaken to reveal the effects of chronic arsenic exposure (25 ppm intragastrically for 12 weeks) on mitochondrial functions and oxidative stress in male Wister rats. Chronic arsenic exposure resulted in decrease in the activities of the mitochondrial complexes. There was increased generation of ROS followed by decrease in MnSOD activity. The generation of oxidative stress was associated with increased protein oxidation and lipid peroxidation in rat brain as evident by FTIR spectra. The RT-PCR analysis of NRF 1, NRF 2 and PGC 1α revealed decrease in gene expression suggesting decreased biogenesis following chronic exposure in rat brain. Thus, the findings of the present study reveal that arsenic induced decrease in mitochondrial biogenesis may be responsible for the decreased metabolic response that may be further involved in the generation of oxidative stress and neurodegeneration in rat brain.
Keywords Arsenic, oxidative stress, mitochondria

P21

Neuroprotection exerted by ischemic preconditioning in rat hippocampus involves extracellular signal receptor changes

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Ischemic-reperfusion injury induced by four vessel occlusion affects vulnerable hippocampal CA1 (cornus ammonis 1) pyramidal neurons. Ischemic tolerance evoked by preconditioning (IPC) represents a phenomenon of CNS adaptation to any subsequent ischemia. We refer here for the changes in the external signal receptor protein kinase pathways of the hippocampal area following by IPC. Ischemia was induced by a 4-vessels occlusion (4VO) and the rats were preconditioned by a non-injurious ischemia. Apoptotic markers were used to follow the degeneration process. Western blot and immunohistochemistry identified phosphorylated extracellular signal-regulated protein kinase and p38 proteins in injured hippocampal areas. P-ERK quantification increased after IPC and reached the highest level at 24 h after ischemia. Interestingly, neuroprotection induced by IPC lead to the opposite effect on MAPK/p38, where the level was lowest at 24 h after ischemia. The study clearly shows that phosphorylated form takes part in complex cascades triggered by IPC in the selectively vulnerable hippocampal region. In addition, study reveals an interplay between p-ERK and p-p38 which participates in the tolerence mechanisminduced by preconditioning.
Acknowledgements Study was supported by VEGA 213/12, MZ SR 2012/30-UKMA-7
Keywords Ischemic/reperfusion injury, preconditioning, ERK, rat

P22

Wolfram syndrome 1: from ER stress to impaired mitochondrial dynamics and neuronal development

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Wolfram syndrome 1 (WFS1) is a genetic disorder which has been associated both with impaired early brain development and neurodegeneration. Recent studies have suggested regulation of Ca2+ homeostasis by wolframin (Wfs1) and have demonstrated the involvement of endoplasmatic reticulum (ER) stress in Wfs1 deficiency. Despite the ER dysfunction WFS1 shows several characteristics of pathologies related to mitochondrial dynamics. Therefore our aim was to examine the hypothesis that Wfs1 deficiency could disturb mitochondrial dynamics contributing to impaired neuronal functioning. First we show that Wfs1 deficiency induces mild ER stress leading to Inositol 1,4,5-Trisphosphate Receptor (IP3R) dysfunction and disturbed cytosolic Ca2+ homeostasis, which, in turn alters mitochondrial trafficking, inhibits mitochondrial fusion and augments mitophagy. The overexpression of the active IP3R fragment restores IP3R-mediated Ca2+ release and corrects all perturbations in mitochondrial dynamics suggesting that these events are causally linked. We further demonstrate that suppressing the expression of two Parkinson disease-related proteins, Pink1 and Parkin, leads to reduced Wfs1 deficiency-induced mitophagy and also to the correction of the fusion-fission dynamics and mitochondrial motility. These data suggest that Wfs1 deficiency may over-activate Pink1 and Parkin pathways. Our most important discovery is that Wfs1 deficiency delays neuronal development and axonal growth in primary rat cortical neurons. According to our data, the link between Wfs1 deficiency and delayed neuronal development appears to be mediated by impaired mitochondrial dynamics because suppression of the Pink1-Parkin pathway corrected also the developmental delay. Our data shed light on the mechanisms of neuronal abnormalities in WFS1 and point out potential therapeutic targets. This work may have broader implications for understanding the role of mitochondrial dynamics in neuropsychiatric diseases.
Keywords Mitochondrial dynamics, ER stress, neuronal development

P23

Novel peptides for kinetic studies of ligand binding to melanocortin-4 receptors using fluorescence anisotropy

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Melanocortin receptors (MCRs) are seven transmembrane G protein-coupled receptors that are known for their broad physiological relevance. The subtype 4 melanocortin receptor (MC4R) has emerged as a central element in the regulation of energy homeostasis, eating behavior and regulation of sexual functions. MCRs are governed by a complex dynamic homotropic regulation [1]. There is an increasing trend towards using fluorescence anisotropy (FA) for studying the aforementioned complex receptor-ligand interactions. FA allows the characterization of ligand binding dynamics [2]. The quality of the FA assay can be greatly increased with budded baculovirus particles that display the receptors of interest on their surfaces [3]. The use of a fluorescent ligand Cy3B-NDP-α-MSH has made it possible to study MC4Rs with higher precision and sampling rate [3]. However, this ligand has relatively slow kinetics. Modification of the structure of a MC4R antagonist revealed two new reporter ligands (UTBC101 and UTBC102) for fluorescence labeling. These new reporter ligands selectively bind to MC4Rs and exhibit improved kinetic properties. The association and dissociation rate constants of UTBC101 and UTBC102 are kon = $((2.0 \pm 0.6) \times 10^7\ M^{-1}\ min^{-1})$, koff = $((4.6 \pm 0.3) \times 10^{-3}\ min^{-1})$ and kon = $((1.9 \pm 0.5) \times 10^7\ M^{-1}\ min^{-1})$, koff = $((1.0 \pm 0.2) \times 10^{-1}\ min^{-1})$, accordingly. UTBC101 and UTBC102 enable the characterization of both labelled and non-labelled ligand binding dynamics in regard to the MC4R. UTBC102 could be especially valuable for ligand screening, because of its very high dissociation rate, which makes it possible to achieve equilibrium conditions.
Acknowledgements This work was financed by the Estonian Research Council (IUT20-17) and by the European Union (TK114, 30020).
Keywords Melanocortin-4 receptor, fluorescence anisotropy, kinetic studies

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P24

Survival of retinal ganglion cells in transgenic mice with deficiencies in sialyltransferases or neural cell adhesion molecule (NCAM) or after the administration of neuraminidase

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Neural cell adhesion molecule (NCAM) plays important roles in the regulation of the brain plasticity during its development and in adulthood. NCAM functions may be regulated by the addition of long linear homopolymers of alpha 2-8-linked sialic acid (PSA). PSA is attached to NCAM via either of two specific sialyltransferases: ST8SiaII and ST8SiaIV. PSA-NCAM is expressed abundantly in the retina and optic nerve during development and adulthood. In the retina PSA-NCAM is expressed in the glial cells in close proximity to retinal ganglion cell (RGC). The functions of the PSA-NCAM in the retina remain unknown. The aim of this study was to investigate the roles of PSA-NCAM in the survival of RGCs after administration of the exitotoxin kainic acid (KA). Intraocular administration of KA induced reduction in the density of RGCs approximately by 60%. Administration of endoneuraminidase (Endo-N) an enzyme, which removes PSA residues from the surface of NCAM, enhanced the toxic effect of KA on RGC. In knockout mice with constitutive deficiency of either ST8SiaII or ST8SiaIV genes, the levels of PSA-NCAM did not differ from those in wild type mice. The toxicity of KA on RGC in these animals also did not differ from control. It should be noted, however, that in knockout ST8SiaII–/– adult mice a reduced number of RGCs was found despite the presence of high levels of PSA-NCAM. These data suggest that during development ST8SiaII ensures high levels of PSA-NCAM, which necessary for the developmental survival of RGCs. The PSA-NCAM in the adult retina ensures the resistance of RGCs to injury.

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Keywords PSA-NCAM, Sialyltransferase, Retinal ganglion cell

P25

Mitochondrial biogenesis is rate limiting-factor for axonal growth

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During early development neurons undergo complex morphological rearrangements. This active axo-dendritic growth must be supported by sufficient cellular energy, thus, cellular energy status could be a key factor in initiating this process. However, the precise mechanisms of how cells adapt their energetic status to physiological demand and upcoming energy needs and how they are coupled to adaptive energy generation, e.g., increased ATP production by mitochondria, remain unclear. Previous studies have shown that peroxisome-proliferator-activated receptor gamma co-activator 1 (PGC-1α) is a master regulator of mitochondrial biogenesis and cellular energy metabolism. Our aim was to examine whether neuronal growth depends on mitochondrial biogenesis and whether activation of cell growth pathways could promote mitochondrial biogenesis to support the energetic need of neuronal development. Over-expression of PGC-1α in cortical neurons increased mitochondrial density in the periphery of axonal tree and was two-fold higher in axonal tips compared to control group. Moreover, induction of mitochondrial biogenesis by PGC-1α facilitated the axonal growth and neuronal development. Activation of PGC-1α upstream kinases such as Ca2+/calmodulin-dependent protein kinase kinase 2 (CaMKK2), transforming growth factor-β-activated kinase (TAK1) and STE-related adaptor (STRAD) increased PGC-1α transcriptional activity and mitochondrial density in axons. Most importantly, they promoted neuronal development through mitochondrial biogenesis. This study shows that mitochondrial biogenesis itself is limiting factor for axonal growth and AMPK-PGC-1α upstream pathways sensing cellular energy status could signal not only the energy deficit but also upcoming energy need to generate new mitochondria.

Keywords Mitochondrial biogenesis, PGC-1α, neuronal development

P26

N-terminal proteolytic processing of G proteiin-coupled receptor 37 (GPR37)

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GPR37 is a G protein-coupled receptor that is abundantly expressed in the brain and has been implicated in dopaminergic signaling [1]. The receptor has been identified as a substrate of the ubiquitin ligase parkin and it has been linked to the autosomal recessive juvenile parkinsonism (AR-JP), an early onset familial Parkinson's disease. The loss of parkin function and deficits in the ubiquitin proteasome pathway were proposed to cause intracellular accumulation of unfolded GPR37 leading to the AR-JP pathogenesis [2]. Here, we found that while GPR37 appears to mature normally in a heterologous expression system, the receptor is subject to proteolytic cleavage at its large N-terminal extracellular region. To study this proteolytic processing, we used stably and transiently transfected human embryonic kidney (HEK) 293 and SH-SY5Y neuroblastoma cells that express N- and C-terminally epitope-tagged human GPR37. N-terminal sequencing of the cleaved C-terminal receptor fragment revealed that GPR37 is cleaved between Glu187and Gln188 and the metabolic pulse-chase data suggests that receptor cleavage is a rapid and efficient process. Moreover, our results indicate that the receptor N-terminus is released from the cells by shedding, a phenomenon rarely described for GPCRs. Immunofluorescence microscopy with subcellular markers indicates that GPR37 is still in the full-length form in the trans-Golgi network but is predominantly expressed in the cleaved form at the cell surface. Additionally, experiments with various proteinase inhibitors imply that the receptor is cleaved by a metalloproteinase. As proteolytic processing is involved in the regulation of many cell surface receptors, our findings provide valuable information about GPR37 that help to understand its function and role in AR-JP at the molecular level.

Keywords GPR37, AR-JP, shedding
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P27

The S100B-RAGE pathway is dysregulated in the ALS-linked neuroinflammatory process

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The comprehension of the mechanisms at the basis of astrocytic dysfunction in ALS is crucial to limit neuronal injury. Most of the toxic astrocytic effects highlight the role of intracellular calcium. S100B is a Ca2+-binding protein particularly present in astrocytes, behaving as a neuroinflammatory mediator as it is secreted by astrocytes under pathological conditions and can display paracrine toxicity by binding to RAGE. During ALS progression S100B increases in patient astrocytes and, in a rat model of the disease, S100B is augmented in "aberrant astrocytes", characterized by their neurotoxic potential. The induction of S100B in astrocytes, its release and its interaction with RAGE in motoneurons could represent a hazardous mechanism that takes

place during ALS. Main objectives of this work were to investigate 1) if the expression of S100B protein and RAGE change during the course of the disease in rodent models of ALS, 2) if the expression of mutant SOD1 protein per se is sufficient to modify S100B levels in astrocytic cultures. We observed that S100B levels and localization are modulated in the spinal cord and in the brain cortex of rat and mouse models of ALS. We also demonstrated a differential expression of RAGE subunits in SOD1-G93A-derived CNS tissues. Moreover, we showed that the overexpression of mutant SOD1 in astrocytic cell line is sufficient to increase the intracellular levels and release of S100B, while it is not enough to induce a differential expression of RAGE. Thus, the expression of mutant SOD1 interferes with the physiological expression of S100B and RAGE and reveals that in astrocytes S100B modulation is an early event related to the mere expression of mutant SOD1, while the dysregulation of RAGE might be a phenomenon possibly requiring a more complex interplay between different cell types and pathways. Overall, these data suggest that S100B might be a toxic mediator released by astrocytes in the ALS-linked neuroinflammatory process.
Keywords S100B, RAGE, ALS

P28

Ammonia reduces intracellular ADMA level in cultured astrocytes and endothelial cells: possible involvement of increased y+LAT2-mediated efflux

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Toxic effects of ammonia in the brain are partly related to impaired NO production which depending on the dose/time of ammonia exposure, may be either increased or decrease. Asymmetric dimethylarginine (ADMA), is endogenous NOSs inhibitor and symmetric dimethylarginine (SDMA) is arginine (Arg) transport inhibitor (Teerlink et al., 2009). Previously we reported an increase of ADMA and SDMA concentration in brain of rats with acute liver failure (Milewski et al., 2014), but distribution of the ADMA/SDMA surplus between the particular intra and extracellular compartments has not been studied. Here, we measured the intracellular concentration of ADMA, SDMA and ADMA/SDMA/NO precursor Arg, in cultured cortical astrocytes and rat brain endothelium cells (RBE-4) treated or not with ammonia. In RBE-4 cells not treated with ammonia the ADMA concentration was twice higher and the Arg/ADMA ratio was much lower than in astrocytes, confirming the well documented role of ADMA in endothelial NOS inhibition (Pope et al., 2009). Treatment for 48 h with 5 mM ammonia led to an almost 50% reduction of ADMA and SDMA concentration in both cell type. Since ammonia-dependent Arg transport in astrocytes is specifically mediated by the heteromeric Arg/Gln transporter y+LAT2 (Zielińska et al., 2012), we speculated that this may also hold for both Arg derivatives. Indeed, silencing of the y+LAT2 gene diminished the reduction of intracellular ADMA concentration caused by ammonia treatment in astrocytes. Moreover, the y+LAT2-dependent component of ammonia-evoked Arg uptake was reduced in the presence of ADMA in the medium. The results suggest that increased ADMA (and possibly SDMA) efflux mediated by upregulated y+LAT2 may be one of the ways in which ammonia interferes with intra-astrocytic ADMA content and, subsequently, NO synthesis. Studies are underway to establish if the same sequence of changes holds for ammonia-treated cerebral endothelial cells.
Acknowledgements Supported by NCN grant 2013/09/B/NZ4/00536
Keywords Hepatic encephalopathy, hyperammonemia, methylated arginine
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P29

Synapse and dendrite deficits induced by mutations in the X-linked intellectual disability gene Il1rapl1

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Synapse and dendrite deficits induced by mutations in the X-linked intellectual disability gene Il1rapl1 Caterina Montani, Mariana Ramos-Brossier, Pierre Billuart, Carlo Sala Mutations and deletions of Interleukin-1 receptor accessory protein like 1 (IL1RAPL1) gene, localized on X chromosome, are associated to intellectual disability (ID) and autism spectrum disorder (ASD). IL1RAPL1 protein is localized at the postsynaptic compartment of excitatory synapses and plays a role in synapse formation and stabilization. Our project was to characterize IL1RAPL1 mutants identified in patients with ID and ASD and to perform a behavioral and neuronal morphology analysis on IL1RAPL1 KO mice. Specifically, we studied the function of three novel mutations of IL1RAPL1 gene in patients presenting ID. We found that two of the studied mutants lead to a partial loss of function of IL1RAPL1 and we pointed out the important function of the extracellular domain for the trans-synaptic PTPδ/IL1RAPL1 interaction in synaptogenesis. We also characterized the role of IL1RAPL1 wild type and mutants in regulating dendrite morphology using in vitro neuronal cultures and IL1RAPL1 KO mice. We identified, associated to hippocampal cognitive impairment an increased number of dendrite branching points in CA1 and CA2 hippocampal neurons of IL1RAPL1 KO mice. In transfected hippocampal neurons the overexpression of full length IL1RAPL1 and mutants lacking part of C-terminal domains leads to a simplification of neuronal arborisation. This effect is abolished when we overexpressed mutants lacking part of N-terminal domains. Our results indicate the importance of IL1RAPL1 extracellular domains not only in synaptogenesis but also in dendrite development. We also concluded that for this activity PTPδ interaction is not required, suggesting that an unknown IL1RAPL1 binding partner is involved in the effect on dendrite morphology.
Keywords Synapse, intellectual disability, autism spectrum disorder

P30

Organic cation/carnitine transporter (OCTN2) interaction proteome in rat astrocytes: Role of phosphatase PP2A

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L-carnitine is essential for translocation of fatty acids for their mitochondrial oxidation, a process shown in the brain to take place in astrocytes. However, it is not synthesized in the brain and has to be transported to brain cells. Organic cation/carnitine transporter novel family member 2 – OCTN2 (SLC22A5) presence was shown in endothelial cells forming the blood-brain barrier, as well as in neurons and astrocytes. We showed that OCTN2 activity in astrocytes and its presence in plasma membrane are higher upon activation of protein kinase C (PKC), but no phosphorylation of OCTN2 was detected. Therefore, we aimed to identify OCTN2-interacting partners and to define their role in transporter regulation. Mass spectrometry analysis identified several cytoskeletal, ribosomal, mitochondrial, and heat-shock proteins as well as the proteins involved in signaling pathway and trafficking. We focused on protein phosphatase PP2A subunits identified in OCTN2 proteome and we observed co-precipitation of OCTN2 with PP2A structural (A) and catalytic (C) subunits, as well as with two regulatory subunits – striatin and SG2NA. Activation of PKC with phorbol ester (PMA) did not change the amount of co-precipitating subunits A and C but significantly lowered the amount of

co-precipitating SG2NA. Immunocytochemistry analysis of astrocytes showed OCTN2 co-localization with PP2A C subunit and with SG2NA in vesicular structures in the cytoplasm. PMA treatment did not change this co-localization, although an augmented amount of OCTN2 was detected in plasma membrane. We postulate that interaction of OCTN2 with PP2A arrests the transporter in cytoplasm in dephosphorylated state, while PKC activation releases SG2NA subunit from the complex, resulting in transporter trafficking to the cell surface.

Acknowledgements This work has been financed by grant 2012/07/B/NZ3/00225 from National Science Centre in Poland.

Keywords Astrocytes, membrane transporter, protein phosphatase

P31

Evidence for the existence of the A2A-A1 heteroreceptor complex in the rat brain, and comparison of its distribution to that of the A2A-A2A homoreceptor complex

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Adenosine receptors play critical roles in cellular processes and signaling and have been shown to form heteromers with diverse biochemical and/or pharmacological activities that are different from those of the corresponding homomers1. However, despite extensive experimental results supporting the formation of adenosine heteromers in heterologous systems, the existence of such heteroreceptor complexes in the brain remains largely unknown, mainly because of the lack of appropriate methodology. Also no systematic study was carried out on heteromers form by adenosine receptor subtypes alone. In this study, we used several experimental approaches2 to investigate whether adenosine receptor A2A and A1 subtypes can form heteromers among themselves. In situ PLA clearly demonstrated that adenosine receptors (A2A-A2A, A2A-A1) exist as homo/heteroreceptor complexes in rat brain. In the hippocampus, A2A-A1 heteroreceptor complexes are mainly localized to the pyramidal cell layer of the Ammon´s horn and the hilo (PoDG). The complex was also observed throughout the piriformis layer. Several distinct differences were apparent between the distribution of the A2A-A1 heteroreceptor complexes and that of the A2A-A2A homoreceptor complex, which could have important functional consequences. Furthermore, bioluminescence resonance energy transfer analysis of adenosine A2A receptors established that they can physically interact in HEK293T27 cells, as both homomers and heteromers. In addition, static/non-dynamical human GPCR data derived from this and other interaction studies were integrated in a large scale graph, called the GPCR heterodimer network (http://www.iiia.csic.es/~ismel/GPCR-Nets/index.html), which provides global insight into adenosine heteromer connectivity, topology and organization in the context of the adenosine receptor subfamily and the GPCR network as a whole.

Keywords Science, microscope, histology

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P32

Validation of astrocytic reference genes for qRT-PCR in CO treatment studies

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Quantitative real-time reverse transcription-polymerase (qRT-PCR) is a widely used technique to characterize changes in gene expression in complex cellular and tissue processes, such as the cytoprotection or inflammation. The selection of an adequate internal reference gene for accurate and consistent analysis of gene expression is of major importance. Carbon monoxide (CO) affects several metabolic pathways and de novo protein synthesis is crucial in the cellular responses to the gasotransmitter. Herein a selection of commonly used reference genes was analyzed to identify the most suitable internal control genes to evaluate the effect of the CO on gene expression in cultured cortical astrocytes. The cells were exposed to CO by incubation with CORM-A1 (CO releasing molecule A1) and four different algorithms (geNorm, NormFinder, Delta Ct and BestKeeper) were applied to better evaluate the stability of eight putative reference genes. Our results indicate that Gapdh (glyceraldehyde-3-phosphate dehydrogenase) and Ppia (peptidylpropyl isomerase A) is the most suitable gene pair for normalization of qRT-PCR results under the experimental conditions used. Pgk1 (phosphoglycerate kinase 1), Hprt1 (hypoxanthine guanine phosphoribosyl transferase I), Sdha (Succinate Dehydrogenase Complex, Subunit A), Tbp (TATA box binding protein), Actg1 (actin gamma 1) and Rn18s (18S rRNA) genes presented less stable expression profiles in cultured cortical astrocytes exposed to CORM-1 for up to 60 min. Analysis of the effects of CO on the expression of Bdnf and bcl-2 gave different results depending on the reference genes used. A significant increase in the expression of both genes was found when the results were normalized with Gapdh and Ppia, in contrast with the results obtained when the other genes were used as reference. This study highlights the need for proper and accurate usage of reference genes in quantification of qRT-PCR results in studies on the effect of CO in gene expression.

Keywords Carbon monoxide, astrocytes, reference genees

P33

Learned helplessness paradigm in P2xr7 wild type and knock out mice and its effect on synapses in the dentate gyrus

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Although depression is one of the prevailing central nervous system disorders, there is still not enough knowledge about the exact alterations underlying its pathophysiology and the treatment is often inefficient. Our study aims at exploring a potential relationship between major depressive disorder and the purinergic P2X7 receptor (P2X7R), since in previous behavioural tests P2rx7 knock out mice displayed an antidepressant-like phenotype. Among many other known symptoms, the loss of hippocampal spine synapses is a revealing feature of the disorder. Therefore we wanted to measure the density of spine synapses in the molecular layer of the dentate gyrus in the learned helplessness paradigm and later see if genetic inhibition of P2X7R could have influence on this condition. Wild type C57Bl/6 and P2rx7 knock out male mice were exposed to inescapable footshocks (IES) in shuttle boxes during 2 training days and on the 3rd day learned helplessness was tested, where helpless animals usually failed to escape. Control animals were also placed in shuttle boxes but did not receive footshocks until testing. Escape failures and the latency to escape were measured to determine helpless behaviour. Electron microscopy analysis was performed to determine spine synapse density in the different groups. In wild type mice both average escape latency and the number of failed escapes were significantly higher in the IES treated group, however, we could not find such divergency in P2rx7 knock out mice. Electron microscopy analysis confirmed alterations in spine synapse density in the molecular layer of the dentate gyrus subsequent to learned helplessness experiments, as results indicated a significant decrease in spine synapse density in wild type mice, but not in knock out animals. These findings may lead to presume a role of the P2X7 receptor in this disorder and further experiments will hopefully help get a better understanding and thus more effective treatment of major depression.

Keywords p2x7, depression, spine synapse

P34

Trophic support following peripheral axotomy show different behaviour of reactive microglia and astroglia in the ventral horn

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Maintaining a neurotrophic support after peripheral nerve injury can be a key point in reducing the plastic changes which neurons, microglia, and astrocytes in the ventral horn undergo due to the loss of afferent synaptic and neurotrophic stimuli originating from the periphery. Therefore, intrathecal administration of trophic factors or the inhibition of the mechanisms responsible for their degradation could help prevent these changes. The purpose of our study was to analyze the changes in the ventral horn produced by gliopathy determined by the suffering of the motor neurons after peripheral nerve injury following spared nerve injury (SNI) of the sciatic nerve and how the administration of NGF or its synthetic analogue BB14, as well as the increase of endogenous NGF levels by i.t. infusion of GM6001, a MMPs inhibitor modulate these events. Immunohistochemical analysis of spinal cord sections revealed that SNI was associated with increased microglial (Iba1) and astrocytic (GFAP) responses, indicative of reactive gliosis. These changes were paralleled by decreased glial aminoacid transporters GLT1 and GlyT1, and increased levels of neuronal glutamate transporter EAAC1, this maladaptive behavior of neuronal and glial EAATs is paralleled by a net increase of the Glutamate/GABA ratio as measured by HPLC analysis. These molecular changes were found to be linked to an alteration of endogenous NGF metabolism, as demonstrated by decreased levels of mature NGF. The continuous i.t. NGF infusion or of its analogue BB14, or of the generic MMPs inhibitor GM6001 reduced reactive astrogliosis and normalized the expression of neuronal and glial glutamate and glycine transporters, restoring the reduction of the Glutamate/GABA ratio but it showed to be absolutely ineffective in modifying the reactivity of microglia, demonstrating that the two glial populations have different mechanisms of modulation associate to neuronal damage.
Keywords Gliopathy, motoneuron axotomy, NGF

P35

LRRK2 modulates neuronal vesicles cycle through protein interactions

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Mutations in Leucine-rich repeat kinase 2 gene (LRRK2) are associated with familial and sporadic Parkinson's disease (PD). LRRK2 is a complex protein that consists of multiple domains executing several functions, including GTP hydrolysis, kinase activity and a C-terminal WD40 domain devoted to protein binding. Robust evidence suggests that LRRK2 acts at the synaptic site as a molecular hub connecting synaptic vesicles to cytoskeletal elements via a complex panel of protein-protein interactions. We have investigated the impact of pharmacological inhibition of LRRK2 kinase activity on synaptic function. Acute treatment with LRRK2 inhibitors reduced the frequency of spontaneous currents, the rate of synaptic vesicle trafficking and the release of neurotransmitter from isolated synaptosomes. The investigation of complementary models lacking LRRK2 expression allowed us to exclude potential off-side effects of kinase inhibitors on synaptic functions. Next we studied whether kinase inhibition affects LRRK2 heterologous interactions. We found that the binding among LRRK2, presynaptic proteins and synaptic vesicles is affected by kinase inhibition. Interestingly, a sequence variant (G2385R) within the WD40 domain has been implicated as a risk factor in PD, but its physiological and pathological function has not been systematically addressed yet. We analyzed molecular features of the WD40 domain and we addressed the functional implication of the G2385R variant. Our results suggest that LRRK2 WD40 domain serves as a hub for protein interactions setting LRRK2 as part of a protein network involved in synaptic vesicle trafficking. Furthermore we showed that the G2385R mutation influences WD40 domain features in terms of domain folding and binding properties and has an impact on synaptic vesicle dynamic. Our results suggest that different PD mutation might influence synaptic vesicle release via modulation of LRRK2 macro-molecular complex.
Keywords LRRK2, synaptic vesicle, Parkinson's disease

P36

GABAA agonist muscimol ameliorates learning/memory deficits in streptozocin-induced Alzheimer's disease non-transgenic rat model

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Background GABAergic inhibitory action regulates learning/memory processes and contributes to neurotransmission (Gong et al., 2009). Existing evidence suggests GABAergic system is involved in pathophysiology of Alzheimer's disease (AD) via inhibitory interneuron deficits (Verret et al., 2012) and decrease in functional GABAA receptors (Limon et al., 2012). In vitro, GABA and muscimol (GABAA receptor agonist) blocked neuronal death induced by Aβ in rat hippocampal and cortical neurons (Paula-Lima et al., 2005). Our concept: low doses of muscimol may prevent learning/memory deficits in intracerebroventricular (icv) streptozocin (STZ)-induced AD non-transgenic rat model.
Methods Wistar male rats (280 ± 20 g) were pre-treated with saline (control) or muscimol (0.01 and 0.05 mg/kg) for 3 days. On day 4, rats received icv STZ (100 μg/ml) or aCSF. From day 18, rats received muscimol or saline for 4 days; rat spatial learning and memory were assessed in water maze test (4 trials/day) by recording the time to reach the hidden platform (escape latency). A probe trial without platform was carried out 24 h after the training trials, and the number of platform zone crossings has been recorded.
Results STZ statistically increased the escape latency vs. control group ($p < 0.0001$). Muscimol at both doses significantly decreased the escape latency in STZ rats vs. STZ, reversing STZ effect by about 90% on days 3 and 4 ($p < 0.0001$). In probe trial, the number of platform crossings in muscimol+STZ rats' was significantly increased vs. STZ rats. Muscimol at both doses per se showed values comparable to control.
Conclusions Obtained data suggest that icv STZ significantly decreased rat spatial learning and memory and learning ability. Muscimol at both low doses significantly improved rats' learning and memory abilities in both normal and AD-type rats. One may suggest that intensification of GABAergic processes may be a useful pharmacotherapeutic strategy to halt early memory decline in AD.
Keywords Memory, muscimol, streptozocin

P37

Evidence for the existence of dopamine D2R and Sigma 1 allosteric receptor-receptor interaction in the rat brain: role in brain plasticity and cocaine action

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Drug addiction is regarded as one of the most important neuro-psychiatric diseases afflicting our society today. A prototypic drug of abuse is cocaine which directly acts on the brain reward system. In this work we present evidence on the existence of dopamine D2R- Sigma1R heteroreceptor complexes which may play a role in the etiology of cocaine addiction. By means of BRET D2R-Sigma1R heteromers were

demonstrated in HEK293 cells after receptor cotransfection. The existence of D2R-Sigma1R heteroreceptor complexes was demonstrated also in discrete regions of the ventral and dorsal striatum with in situ proximity ligation assay. Through saturation binding assay it was clearly demonstrated that in membrane preparations of HEK293 cells co-expressing D2R-Sigma1R, cocaine (1 nM) significantly increased the D2R Bmax values (998 ± 40 fmol/mg protein) over D2R alone cells (664 ± 37 fmol/mg protein). This effect was counteracted by the Sigma1R selective antagonist PD144418 (Bmax value: 728 ± 39 fmol/mg protein). Furthermore, CREB reporter luc-gene assay indicated that the presence of D2R-Sigma1R significantly reduced the potency of the D2R like agonist quinpirole to inhibit the forskolin induced increase of the CREB signal. In contrast, the presence of a low concentration of cocaine (100nM) was found to markedly increase the quinpirole potency to inhibit the forskolin induced increase of the CREB signal in the D2R-Sigma1R cells. These dynamic changes in D2R-Sigma1R signalling produced by cocaine maybe explained by synergistic allosteric receptor-receptor interactions in the D2R-Sigma1R heteroreceptor complexes at the plasma membrane level. An antagonistic allosteric receptor-receptor interaction between the dopamine D2R and the Sigma1R in absence of cocaine instead of can explain the reduced potency of quinpirole. These dual conformational changes in the D2R-Sigma1R heteroreceptor complexes could be associated with the redistribution of both protomers from the intracellular compartment to the plasma membrane as indicated by means of confocal analysis of agonist induced D2RSigma1R trafficking and internalization. Overall, the dynamic of D2R-Sigma1R heteroreceptor complexes may represent a mechanism that shapes neuronal and addictive responses to cocaine.
Keywords Dopamine D2 receptor, Sigma 1 receptor, heteroreceptor complexes

P38

The role of Negr1 in cortical development via NCAM-FGFR2 signaling

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Autism spectrum disorder (ASD) affects 0.9% of children and it is recognized as the most genetic of all developmental neuropsychiatric syndromes. Mutations in NEGR1 and FGFR2 genes have been recently identified as ASD candidates. Negr1 is a member of IgLON adhesion protein family but its functions are largely unknown. Our original approach has identified Negr1 as a developmentally regulated synaptic protein. Thus we examined the consequences of Negr1 acute downregulation. Strikingly, we found that Negr1 ablation impairs neuronal maturation in vitro1. A combination of biochemical and imaging investigation has demonstrated that Negr1 influences neurites outgrowth via MAPK signaling organizing trans-synaptic heterodimer. In detail, we demonstrated that ectopic Negr1 is sufficient to improve neurite arborization and rescue the morphological phenotype observed in Negr1 KD cells. This function is dependent on the activation of MAPK pathway through tyrosine kinase receptors. In fact, we found that Negr1 physically and functionally interacts with NCAM and FGFR2, modulates FGFR2 response to FGF and consequently influences MAPK pathway. FGFR2/NCAM pathway plays an important role during brain development. Not surprisingly, our investigation of the radial migration of newly generated cortical neurons, revealed that Negr1-FGFR2-NCAM cross-talk controls cortical organization in vivo. Connectivity dysfunctions have been suggested as causative alterations in ASD. Given the functional, physical and genetic correlation among Negr1 and FGFR2 and NCAM, the study of Negr1/FGFR2/NCAM molecular cross talk may offer new therapeutic opportunities.
Keywords Autism, Negr1, MAPK
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P39

Cortical synapses in acute hepatic encephalopathy: morphology and expression of proteins involved in synaptic transmission

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Hepatic encephalopathy (HE) is manifested by impaired glutamatergic transmission. The current view is that HE spares the glutamatergic synapse, its dysfunction being primarily due to impaired astrocytic control. Here we show that in acute HE, the glutamatergic synapse by itself presents discrete changes in the ultrastructure (electron microscope) and expression of functionally critical synaptic proteins (Western blot). We used C57Bl6 mice subjected to a hepatotoxic insult in the azoxymethane (AOM) model. Cerebral cortex of AOM mice was ultrastrcturally characterized by the presence of increased numbers (~by 15%) of enlarged synapses showing abundance of synaptic vesicles in the presynaptic zone. The expression of synaptophysin and synaptotagmin in S2 fraction was increased by ~80% and ~30%, respectively. The expression of the NR1 subunit of NMDA receptor, but not of NR2A, was slightly increased. The amount of PSD-95 in P2 fraction and nNOS in S2 fraction, were elevated by ~40% and ~30% respectively. The expression of PKCζ was increased by ~30% and ~40% at the mRNA and protein level, respectively. PKCζ protein level was reduced by ~20% in P2 membrane fraction and elevated by ~30% in S2 cytosolic fraction suggesting its altered intracellular trafficking. This report is to our knowledge the first to demonstrate distinct changes in the synaptic ultrastructure and composition of synaptic proteins in the acute stage of HE. Except for PKCζ, all the other changes are indicative of a compensatory response of as yet unknown functional implications.
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Keywords Ammonia, synapses, synaptic proteins

P40

Proteasome stress triggers differential cellular responses of neural cells

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Ubiquitin-proteasome system (UPS) represents important intracellular system controlling protein quality and intracellular signalling. Overload or dysfunction of UPS leads to proteasome stress that is implicated in mechanisms of neurodegeneration associated with neurodegenerative diseases, e. g. Parkinson and Alzheimer disease. Proteasome stress is also considered as the main cause of delayed neuronal death observed after transient global brain ischemia. Despite significant progress made to date, the exact mechanism and selectivity of cell death induced by proteasome stress after global brain ischemia is still not completely understood. The aim of our work was to study effect of proteasome stress on cell viability, stress response as well as on mechanism of death of neuroblastoma SH-SY5Y and glioblastoma T98G cells. Proteasome stress was induced by treatment of cells with bortezomib, inhibitor of proteasome 26S complex. Neuroblastoma cells were more sensitive to bortezomib than glioblastoma cells and death of neuroblastoma cells occurred significantly faster than death of glioblastoma cells. With respect to cellular response, treatment of both SH-SY5Y and T98G cells with bortezomib was associated with accumulation of polyubiquitinylated protein aggregates and increased expression of HSP70. With respect to cell death mechanism, we have documented bortezomib-induced release of cytochrome c from mitochondria and activation of caspase 3 in SH-SY5Y cells. In T98G cells, bortezomib induced activation of caspase 4 but not caspase 3 and did not induce release of cytochrome c from mitochondria. Our results indicate that proteasome stress affects neural cells in different way but does not answer the question about selectivity and delay of cell after global brain ischemia.
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Keywords Ischemia, cell death, proteasome

P41

Miro1 overexpression protects against α-synuclein-induced mitochondrial loss in neuronal culture

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Mitophagy is a selective degradation of mitochondria that promotes the turnover of mitochondria and prevents the accumulation of damaged organelles. Pink1 and Parkin proteins are crucial in the removal of damaged mitochondria. Mutations in the corresponding PINK1 and PARK2 genes are associated with Parkinson's disease (PD), and mitochondrial impairments are central to PD pathogenesis. Pink1 has been shown to interact with the atypical Rho GTPases Miro, the outer mitochondrial membrane proteins involved in mitochondrial trafficking. Miro1 is degraded shortly after mitochondrial damage in a Parkin-dependent manner. α-synuclein is a major component of Lewy bodies, the characteristic cellular inclusions in PD. Mutations of α-synuclein, including A53T, are linked to familial PD. α-synuclein has been shown to bind to the mitochondrial membrane, and increased membrane-bound α-synuclein in PD contributes to the functional disturbance of mitochondria. Overexpression of A53T-mutated α-synuclein has been shown to induce mitophagy in vitro and in vivo. We hypothesized that Miro1 function is disturbed in an α-synuclein (A53T)-overexpressing model, and the aim of this study was to elucidate the involvement of Miro1 in α-synuclein-induced mitophagy. We have found that α-synuclein is one component of the Miro1 interactome. Moreover, co-expression of Miro1 restored mitochondrial length and density in primary neuronal culture overexpressing A53T-mutated α-synuclein. Miro1 overexpression did not change the basal mitophagy, but decreased significantly α-synuclein-induced mitochondrial removal. Together, our results suggest that Miro and α-synuclein may interact in the mitophagic pathway.
Keywords Mitochondria, autophagy, Parkinson's disease

P42

Glycohydrolases in the central nervous system: the role of GBA2 in the neuronal differentiation

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Mammalian neurodevelopment is characterized by qualitative and quantitative changes in plasma membrane glycosphingolipids due to a fine regulation of their metabolic pathways. While the biosynthetic pathway is largely studied scant is the information available on the catabolic one. For this reason, I studied the activity of the main glycohydrolases expressed in the central nervous system during neuronal differentiation. To this purpose the activities of the principal glycohydrolases involved in glycosphingolipid catabolism have been evaluated in different experimental models such as brains and cerebella of mouse at different ages and neuronal cell cultures (immortalized mouse neuronal cell lines GN11 and GT1-7, primary cultures of mouse cerebellar granule cells and human neuroblastoma cells SH-SY5Y). The results obtained indicate that the process of neuronal differentiation is associated with a marked increase in the activities of all the glycohydrolases evaluated, in particular, the activity of the non-lysosomal β-glucosylceramidase GBA2 undergoes the most relevant increase representing the prevalent form of β-glucocerebrosidase in mature neurons. In order to evaluate the possible role of GBA2 in the neuronal differentiation, SH-SY5Y cells have been stably trasfected for GBA2 overexpression. Cells overexpressing GBA2 acquired a neuronal phenotype and showed a significant increase in ceramide levels. These results are in line with literature data that demonstrate the involvement of ceramide in the neuronal differentiation. Therefore, it is possible to hypothesize that the hydrolysis of glucosylceramide to ceramide, catalyzed by GBA2 at the plasma membrane level, has a functional role in the neuronal differentiation process. Collectively these findings suggest that GBA2 may represent a possible neuronal marker and demonstrate for the first time its direct involvement in the neuronal differentiation.
Keywords Sphingolipids, neuronal differentiation, GBA2

P43

A new potential mechanism of action of tianeptine – the effect on microglial cell activation

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Tianeptine is an atypical antidepressant drug with proven efficacy, but still not fully understood mechanism of action. Recently it has been suggested that tianeptine may modulate inflammatory processes, however there is a lack of data on its influence on microglia – the main source of pro-inflammatory cytokines in the brain. Therefore this project aimed to investigate whether tianeptine can influence activation of microglial cells. We conducted our study in two experimental models: in vivo – in the hippocampus and frontal cortex of adult rats and in vitro in microglial cultures. Pregnant rats were subjected daily to 3 stress sessions from 14th day of pregnancy until delivery. Control pregnant females were left undisturbed in their homecages. Microglial cells were pre-treated for 30 min with different concentrations of tianeptine and stimulated with LPS (100 ng/ml). Next, expression of microglial activation markers and pro-inflammatory cytokines were evaluated. In the second part of experiments at 3 months of age, after behavioral verification, control and prenatally stressed rats were injected with tianeptine (10 mg/kg i.p.) for 14 days. Next, biochemical studies were carried out on hippocampus and frontal cortex. We observed that in microglial pre-treatment with tianeptine (1–10 μM) reduced the expression of microglial activation markers (CD40 and MHCII) and production of pro-inflammatory cytokines. Moreover, in adult animals subjected to prenatal stress (an animal model of depression) chronic tianeptine treatment inhibited microglial activation (decreased CD40 and CD68 expression) in both examined structures. In conclusion, our results show that tianeptine exerts anti-inflammatory properties suppressing microglial activation in both in vitro and in vivo experimental models.
Acknowledgements This research was supported by grant no. 2013/09/B/NZ7/04096, NCN, Poland. Joanna Slusarczyk and Ewa Trojan are holders of scholarships from the KNOW.
Keywords Tianeptine, microglia, inflammation

P44

The effects of STZ-induced diabetes on cognition and brain amyloid in 5XFAD mouse model of Alzheimer's disease

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Introduction Alzheimer's disease (AD) is an incurable neuro-degenerative disease characterized by progressive dementia. Main neuropathological features of AD include extracellular β-amyloid (Aβ)-containing plaques, intraneuronal aggregates of hyperphosphorylated τ-protein and neurofilaments, microglial activation and clustering around Aβ plaques and synaptic loss. 5XFAD transgenic mice are a model of AD, exhibiting rapid brain accumulation of Aβ and microgliosis. The aim of the study was to characterize the effects of streptozocin (STZ)-indced diabetes on learning and memory of 5XFAD and wild-type (WT) mice in Morris water maze (MWM) at ages 2 and 6 months and on brain amyloid load.
Methods and results Mice were injected with STZ 90 mg/kg or vehicle i.p., once daily for 2 consecutive days. MWM was performed on week 9 and histological analysis of brains of mice injected with STZ or vehicle at 2 months of age was performed on week 16. STZ treatment did not affect locomotion or vision of mice in MWM. At both 2 and 6 months of age, STZ treatment impaired memory of both 5XFAD and WT. Learning

was significantly impaired in STZ-treatedc 5XFAD mice at 2 months. Surprisingly, Congo Red-positive area fraction (%) of hippocampus and amygdala was decreased 5XFAD mice treated with STZ at 2 months. Plaque diameter was not different between STZ treated and vehicle treated 5XFAD mice.
Conclusions Insulin deficiency could affect cognition through mechanisms unrelated to Aβ metabolism. Also different mechanisms may underlie effects of STZ treatment on learning and memory in different age groups, possibly including enhancement of brain amyloid deposition and inhibition of neural cell precursor proliferation. We also hypothesize that STZ treatment might increase the soluble brain amyloid fraction in this model, since it is currently acknowledged that oligomeric (soluble) rather than fibrillar Aβ species disrupt cognitive function in AD.
Keywords Amyloid, diabetes, Alzheimer's

P45

The effect of hypoxic postconditioning after severe hypobaric hypoxia on the expression of Cu, Zn-SOD in hippocampus and neocortex of rats

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Oxidative stress is a key mechanism of cellular damage during and after severe hypoxia. Accordingly, up-regulation of expression and activity of endogenous antioxidants is an important mechanism of cellular adaptation to hypoxia. Do endogenous antioxidants take part in postconditioning-induced neuroprotective mechanisms similarly to their participation in preconditioning-induced ones? In the present work the effect of postconditioning by 3-trial mild hypobaric hypoxia (360 Torr, 2 h, once a day) after 1 session of severe acute hypobaric hypoxia (180 Torr, 3 h) on the expression of Cu, Zn-superoxide dismutase (Cu, Zn-SOD) was studied by immunocytochemical analysis in areas CA1, CA2, CA3, CA4 and DG of hippocampus and in frontoparietal neocortex (NC) of male Wistar rats. Two time points were examined: 3 h after the last session of postconditioning that was 3 days after severe hypoxia and 24 h after the last session of postconditioning that was 4 days after severe hypoxia. It has been shown that postconditioning significantly increases the total number of Cu, Zn-SOD-immunoreactive cells (Nt) at least in two areas of hippocampus studied (CA2 and DG) compared to non-postconditioned rats at 3 days but not at 4 days after severe hypoxia. In contrast, in NC of postconditioned rats, Nt tends to increase compared to non-postconditioned animals at 4 days but not at 3 days after severe hypoxia. The effect of postconditioning on the number of intensely expressing Cu, Zn-SOD neurons differs in various areas and at various time points. The modification of Cu, Zn-SOD expression in some areas of hippocampus and NC, induced by 3-trial hypoxic postconditioning, correlates with the prevention of massive delayed apoptotic neuronal death and amelioration of functional disorders caused by severe hypoxia. Thus, Cu, Zn-SOD and other endogenous antioxidants may play, apparently, an important role in the treatment of severe hypoxia/ischemia stroke by postconditioning in brain neurons.
Keywords Postconditioning, hypoxia, Cu, Zn-superoxide dismutase

P46

The brain acylcarnitine profile depends on nutritional state

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In addition to glucose, also fatty acids are important as an energy source in the brain where about 20% of the energy is gained from mitochondrial oxidation of fatty acids. Fatty acids are transported into mitochondria in the form of acylcarnitine. It is known that acylcarnitine concentration in the heart and blood plasma varies depending on the nutritional state, fed or fasted. The aim of the present study was to compare the concentration of short-chain (C2-C5), medium-chain (C6-C12) and long-chain (C14-C18) acylcarnitines in fed and fasted states in rat brain structures: cerebellum, cortex and hypothalamus. The total concentration of acylcarnitines was not affected by nutrient state in none of brain structures studied, but we found that cortex contained less acylcarnitines than cerebellum and hypothalamus. The nutritional state did not affect the overall concentration of short-chain acylcarnitines in the brain structures.In contrary, the nutritional state significantly affected the concentration of total medium chain acylcarnitines in the cortex: 0.33 and 0.46 nmol/g tissues in fed and fasted state, respectively. The highest concentration of medium-chain acylcarnitines was found in hypothalamus in fed and fasted state compared to cortex and cerebellum. The nutritional state significantly affected the concentration of total long-chain acylcarnitines in the cortex: 4.92 and 5.88 nmol/g tissues in fed and fasted state, respectively. In fed state the highest concentrations of long-chain acylcarnitines was found in hypothalamus compared to cortex and cerebellum. The results demonstrate that the nutrition state affects brain acylcarnitine concentration in different brain structures, and cortex is the most affected brain structure. Further studies are needed to investigate the role of changes in acylcarnitine profile in the brain signalling pathways.
Acknowledgements The study was supported by European Social Fund project No. 1DP/1.1.1.2.0/13/APIA/VIAA/009.ISN travel award
Keywords Acylcarnitine, brain structures, nutritional state

P47

Janus-faced taurine: protection or toxicity?

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Taurine is a simple sulfur-containing amino acid ubiquitously distributed in the tissues of most animals. It is involved in a wide range of physiological processes including osmoregulation, lipid metabolism, intracellular calcium regulation, neuronal development, neuromodulation and cell protection. Using in vivo model of acute ethanol intoxication (5 g/kg) in 7-day-old mice we have found that taurine treatment at total dose 2 g/kg has saved about 50% of dying neurons from ethanol-induced apoptosis in the internal (Taranukhin et al, 2009, 2010) and the external (Taranukhin et al, 2012) granular layers of developing cerebellum. However, any further increase in taurine doses (4–6 g/kg) aiming to protect more neurons against alcohol-induced apoptosis poses to threat to the whole organism and kills 7-day-old mice thus treated. Since the high doses of taurine alone or ethanol alone did not lead to animal death, lethality appears to be due to ethanol and taurine combined toxicity. We reveal the 50% and 100% lethal doses of taurine and ethanol combination for developing (7-day-old), adult (5–6-month-old) and old (12–13-month-old) mice and can conclude that the toxicity of ethanol and taurine combination is age-dependent. A dramatic drop in blood glucose levels observed in 25% of 7-day-old and 40% of elderly mice treated with taurine and ethanol suggests that one of the reason of animal death may be hypoglycemia (Taranukhin et al., 2013, 2015). Based on the results obtained we conclude that taurine may have beneficial effects in protecting brain cells against the apoptosis induced by alcohol. However, our finding on the toxicity of combined taurine and ethanol prompts serious concern particularly for young people mixing taurine-containing energy drinks with alcohol.
Acknowledgements This research was supported by the competitive research funding of Pirkanmaa Hospital District and the Finnish Foundation for Alcohol Studies.
Keywords Taurine, ethanol, hypoglycemia

P48

Peptidomic characterization of peptide processing in the hippocampus of Wfs1 knockout mice

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Mutations in WFS1 gene cause Wolfram syndrome, which is a rare autosomal recessive disorder, characterized by diabetes insipidus,

diabetes mellitus, optic nerve atrophy and deafness (DIDMOAD). WFS1 gene product wolframin is located in the endoplasmic reticulum. Mice lacking this gene have disturbances in processing and secretion of peptides, such as vasopressin and insulin. In the brain, high levels of wolframin protein are observed in the hippocampus, amygdala and limbic structures. The aim of this study was to investigate the effect of Wfs1 invalidation on the peptide processing in hippocampus of mice. Peptidomic approach was used to characterize individual peptides in the hippocampus of wild type and Wfs1 knock-out mice. We identified 126 peptides in the hippocampal extracts and levels of 10 peptides were different in Wfs1 and wild type mice at ($p < 0.05$). Largest alteration was found in the level of peptide little-LEN, which is processed (cleaved) from pro-SAAS (Pcsk1n) in prohormone convertase 2 (PC2) dependent ways. Results of this study reveal alterations of peptide processing in the hippocampus of Wfs1 deficient mice.

Keywords Peptide processing, Wfs1, pro-SAAS

P49

Increased prolyl endopeptidase induces alterations in the expression of neural cell adhesion molecule and its polysialylation in SH-SY5Y neuroblastoma cells in vitro

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Development of the nervous system and its structural remodelling in the adult relies on molecules mediating the structural plasticity of neurons, e specially those involved in cell adhesion, cytoskeletal dynamics or synapse formation. Neural cell adhesion molecule (NCAM) is a membrane-associated glycoprotein that can be modified by glycosylation with polysialic acid (PSA), attenuating NCAM-mediated cell interactions, thereby promoting structural plasticity [1]. It has been demonstrated that in conditions of neuroinflammation, NCAM can be cleaved extracellularly by metalloproteinases and other proteolytical enzymes. Prolyl endopeptidase (PREP) is a cytosolic serine protease, and alterations in PREP expression and activity have been associated with neuronal death and neuroinflammation [2]. Since the precise mechanisms and possible partners of PREP in neuroinflammation remain unclear, the aim of this study was to determine whether increased secretion and activation of PREP could impair NCAM expression and its polysialylation. SH-SY5H cell line overexpressing (o/e) PREP was used as an in vitro model for increased PREP expression and extracellular release. When measuring expression levels of NCAM and PSA-NCAM we found remarkable loss in PSA-NCAM and disrupted expression patterns of NCAM compared to wild-type cells. As matrix metalloproteinase 9 (MMP-9) has been indicated in processes regulating shedding of PSA-NCAM, MMP-9 expression level was measured. An increase in the level of the active form of MMP-9 was found, which was counteracted by a specific inhibitor of PREP, KYP-2047. As demonstrated, PREP might have an important role in processes involved in NCAM degradation and polysialylation, thereby inducing progression of pathologies associated with altered neuroplasticity.

Keywords Neural cell adhesion molecule, prolyl endopeptidase, cellular plasticity

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P50

Corticosterone induces DNA methyltransferases expression in rat cortical neurons

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Corticosterone (CORT) is the main glucocorticoid hormone involved in stress responses in rodents. It is established that CORT exerts its effects via glucocorticoid receptor (GR) and mineralocorticoid receptor that regulate downstream gene expression during development and adulthood. In our previous study, we have shown that maternal separation on postnatal day 15 increases DNA methyltransferase (DNMT) 1, 3A and 3B expression levels in rat nucleus accumbens lasting into adulthood (Anier et al. 2014). However, the exact mechanism how maternal separation alters DNMT expression is unclear. We hypothesize that stress-induced GR stimulation may increase the expression levels of DNMTs and alter long-term DNA methylation-demethylation balance in infant rat brain. Our aim is to evaluate the effect of CORT and maternal separation on the expression levels of DNMTs in rat cortex. In rat primary cortical neurons, CORT treatment increased mRNA levels of DNMT3A and DNMT3B. GR antagonist mifepristone significantly decreased CORT-induced DNMTs mRNA levels indicating GR stimulation-dependent upregulation of DNMTs expression. Higher mRNA levels of DNMT1, DNMT3A and DNMT3B in rat cortex at postnatal day 15 and increased plasma CORT levels suggest that elevated CORT upregulates DNMTs expression. Our results indicated that DNMTs are downstream targets of GR-dependent CORT stimulation and early life stress may induce aberrant DNA methylation pattern that could facilitate long-term changes in gene expression.

Keywords DNA methyltransferase, maternal separation, corticosterone

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P51

Novel positive allosteric modulators of sigma-1 receptor

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Background The Sigma-1 receptor is a chaperone protein that modulates intracellular calcium signalling of the endoplasmatic reticulum and is involved in learning and memory processes. The aim of the present study was to compare in vitro Ca2+ concentration modulating activity and in vivo behavioural effects of enantiomers of methylphenylpiracetam, a novel positive allosteric modulator of Sigma-1 receptors.

Methods The activity of enantiomers was compared in the model of electrically stimulated rat vas deferens and bradykinin-induced intracellular Ca2+ concentration ([Ca2+]i) increase assay in neuro-blastoma-glioma (NG-108) hybrid cells, PRE-084 was used as a selective Sigma-1 receptor agonist. Effects on contextual memory, locomotor activity and antidepressant activity were evaluated in ICR male mice in passive avoidance (PA) response, open field and Porsolt forced swimming tests, respectively. The rota-rod, traction and chimney tests were used to screen effects of the compounds on muscle function and coordination.

Results Enantiomers with R-configuration at the C-4 chiral centre in the 2-pyrrolidone ring both significantly increased PRE-084 activity in vitro and almost two-fold increased the retention time in the PA test in vivo. The behavioural side effects were slightly more expressed for the enantiomers with S-configuration at the C-5 chiral centre. The enantiomers of methylphenylpiracetam did not induce any significant effects on the locomotor and depressive condition in the mice.

Conclusions Pharmacological stimulation of sigma-1 receptor is an emerging approach for cognition enhancement. The R-configuration enantiomers of methylphenylpiracetam are more active positive allosteric modulators of Sigma-1 receptor than S-configuration enantiomers. The Sigma-1 receptor modulatory activity of compounds correlated with the memory enhancing effects.

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Keywords Sigma-1 receptor, positive allosteric modulation, cognition

P52

Allosteric modulation of peptide ligand binding to Neuropeptide Y receptor Y1 revealed by fluorescence-based assay

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Implementation of fluorescence methods in studies of ligand binding to their receptors opens new possibilities to characterise these processes. One of the potential approaches is the detection of changes of the fluorescence anisotropy (FA) and/or total fluorescence intensity (TFI) signals upon binding reaction. However, to achieve significant changes in the FA/TFI signal, some requirements need to be met – the concentration of receptor binding sites as well as the dissociation constant of the interaction should be in the same order as the fluorescent ligand's concentration. We have used FA assay to investigate ligand binding properties to Melanocortin 4 (MC4) receptor [1]. Implementation of budded baculoviruses (BBV) that display G protein-coupled receptors on their surfaces significantly increased sensitivity and temporal stability of this assay [2]. For the first time we demonstrate the applicability of BBV experimental setup to study Y1 receptor system. Here we used TAMRA-PYY, an Y1 receptor specific fluorescent peptide ligand, as a reporter ligand. Besides real-time monitoring of FA signal changes, up to 5 fold decrease in TFI signal was observed within TAMRA-PYY binding to the Y1 receptor. Pharmacological characterization of Y1 receptors with receptor-specific unlabelled ligands gave the rank order of potencies consistent with previously reported values. Additionally, allosteric heterogeneous interactions were revealed as koff values of TAMRA-PYY differed more than 7 times depending on the nature of dissociation initiated ligand. These observations provide evidence for similar allosteric receptor-ligand binding mechanism as previously shown for MC4 receptors [3].

Acknowledgements This work was financed by Estonian Research Council (IUT20-17) and the European Union (TK114, 30020).
Keywords Allostery, fluorescence assay, neuropeptide Y receptor Y1

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P53

Neuroprotection by MKK7 inhibition in excitotoxicity

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Excitotoxicity following cerebral ischemia elicits a molecular cascade, which leads neurons to death. One key molecule of this pathway is c-Jun-N-terminal kinase (JNK), a MAP kinase, which plays both physiological and pathological roles in neurons. We have previously shown that JNK blockade by specific cell permeable peptide inhibitors significantly reduces infarct size and neuronal death. On the other hand, JNK inhibition may have detrimental side effects due to blockade of its physiological function. Here we have designed a new inhibitor, which blocks MKK7, an upstream activator of JNK, which mediates its pathological activation. This inhibitor was designed taking advantage of the growth arrest and DNA damage inducible 45β (GADD45β) ability to bind MKK7, optimizing the essential domain of GADD45β and linking it with a spacer to TAT peptide sequence to penetrate cells. This inhibitor significantly reduces neuronal death in two in vitro models of excitotoxic cell death, one induced by NMDA exposure and the other by oxygen glucose deprivation. We tested the MKK7 inhibitor in vivo, in two models of permanent ischemia, the one obtained by electrocoagulation, and the other by thromboembolic occlusion of the Middle Cerebral Artery. In both models, it blocked MKK7 activation and provided significant protection, significantly reducing the infarct size when injected 30' before the lesion. In the electrocoagulation model, we also tested the efficacy of the peptide when injected 6h after lesion, obtaining similar protection. Therefore, we showed that it is possible to prevent JNK activation in excitotoxicity by specific inhibition of MKK7, preserving the physiological role of JNK driven by MKK4. Targeting MKK7 could represent a novel therapeutic strategy for several diseases involving JNK activation.

Keywords Stroke, JNK, ischemia

P54

Hypoxic postconditioning is an effective method of protection from severe hypoxia induced lipid peroxidation and neuronal apoptosis in rats

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Postconditioning (PostC) is an exposure of the damaged organism to extreme factors of the mild intensity to mobilize endogenous protective mechanisms. In our laboratory method of PostC using three daily trials of mild hypobaric hypoxia (MHH) was developed. It has been found that such method of the PostC effectively prevents degeneration of the hippocampal and neocortical neurons in rats, subjected to severe hypoxia (SH). Present study has been aimed at examination of the impact of oxidative processes in the development of the neuroprotection acquired in the course of hypoxic PostC during first three days of reoxygenation after SH in rats. The levels of thiobarbituric acid reactive substances (TBARS) and Schiff bases (SB) were used as markers of lipid peroxidation. In addition, the intensity of the apoptotic DNA fragmentation has been studied. During the three days after the SH a sustained increase of SB in the rat hippocampus was observed (700–1000% of the control value). After the first PostC episode the SB levels decreased to 150% of the baseline. Subsequently this parameter did not differ significantly from the control values. TBARS showed accumulation on the first day following the SH but afterwards its levels dropped to 40% of control and did not recover then to normal values. In the PostC animals, the levels of TBARS after each of three PostC episodes did not differ from the control values. These facts indicate that the PostC MHH balances the activity of pro- and antioxidant systems in vulnerable brain regions and promotes the effective utilization of components damaged by peroxidation. Fragments ladder typical for cells undergone apoptosis was obtained by the electrophoretic separation of the total DNA, extracted from a rat brain after one, two and three days after the SH. In the PostC group, the DNA fragmentation was revealed only after the first PostC episode, demonstrating antiapoptotic action PostC MHH.

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Keywords Hypobaric hypoxia, hypoxic postconditioning, neuronal apoptosis, lipid peroxidation

P55

Effect of brevican deficiency on neuroplasticity mediating molecules

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Extracellular matrix (ECM) forms the pericellular part of the tissue. Due to the ECM's constitution it defines the biophysical and biochemical properties of the tissue. In vertebrates' brain the neuronal ECM mainly consists of negatively charged chondroitin sulfate proteoglycans (CSPG), hyaluronan and glycoproteins. CSPG are supposed to be involved in cell adhesion, axonal pathfinding and receptor binding. Therefore they are considered to play a role in neural development and plasticity as well as in several neurological and psychiatric disorders (e.g. schizophrenia and depression). One member of the CSPG family is brevican which was previously demonstrated by Blosa et al. (2013) to

occur adjacent to the active zone of synapses. Accessorily, deficiency in brevican was reported to lead to a reduction of hippocampal LTP and recruitment of local plasticity. In this study we therefore focused on the effect of brevican on the neuronal cell adhesion molecule NCAM and its polysialylated form PSA-NCAM. Both of them are known to be involved in the establishment and modulation of neuroplasticity which are essential for memory formation. Furthermore we analysed the expression of the prolyl endopeptidase PREP, the matrix metalloproteinase 9 (MMP9) and tissue inhibitors of MMPs (TIMP). Immunohistochemical and western blot analyses of hippocampus tissue of brevican knockout mice compared with wild type littermates did not reveal changes in the expression of MMP9, PREP, NCAM nor PSA-NCAM, but showed a reduction of TIMP1 and 3 by trend. Consequently, changes of LTP and local plasticity in brevican deficient mice do not seem be mediated by an alteration of NCAM and PSA-NCAM, but might be supported by different expression levels of TIMP 1 and 3 modulating several MMPs' activity.

Keywords Extracellular matrix, brevican deficiency, tissue inhibitor of matrix metalloproteinase

P56

The protective and toxic role of neuromelanins in brain aging and Parkinson's disease

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Neuromelanins (NM) are a family of compounds occurring in all brain regions of human brain. These pigments are contained in special lysosomes together with lipid bodies, protein matrix, and accumulate in aging (Zucca et al. Neurotox Res. 2014). The synthesis of NM is a protective process because the melanic component is generated through the removal of reactive/toxic quinones that would otherwise cause neurotoxicity (Sulzer et al. *PNAS* 2000). NM serves an additional protective role through its ability to chelate and accumulate metals, including environmentally toxic metals such as mercury and lead. Other metals like Fe, Zn, Al, Cr and Mo are also accumulated by NM (Zecca et al. PNAS. 2008). However NM can play also a toxic role in Parkinson's disease (PD), when it is released by dying neurons of substantia nigra. Extracellular NM particles induce microglial activation with production of superoxide, nitric oxide, hydrogen peroxide, pro-inflammatory factors and causes neurodegeneration (Zhang et al. Neurotox Res. 2011). A high content of major histocompatibility class I complex (MHC-I) was found in NM-containing organelles of the neurons in substantia nigra (SN) and locus coeruleus (LC) which degenerate in PD, while other neurons not targeted by PD has low MHC-I. The latter can bind antigens derived from foreign proteins, presenting them on neuronal membrane. Then CD8+ cytotoxic T-cells, which were observed in proximity of MHC-I presenting neurons of SN and LC in PD subjects, can target these neurons inducing neuronal death. Infiltration of T-cells occurs in SN and LC of PD subjects. The presence of MHC-I in catecholamine neurons containing NM could explain their selective vulnerability in PD, revealing a novel inflammatory T-cell mediated neurodegenerative process of PD (Cebrián et al. Nat Commun. 2014). In conclusion NM can play a protective or toxic role depending on the molecular/cellular context.

Keywords Neuromelanin, aging, Parkinson's disease

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Role of caspase-3 in development of neuronal plasticity and memory

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Caspases are known to play an important role in apoptosis and their increased activity in pre- and postsynaptic terminals leads to proteolysis of synapse-associated proteins, resulting in disruption of synaptic functions. Moreover, caspases degrade the C-terminal fragment of amyloid-precursor protein intracellular domain (AICD) which regulates expression of a variety of genes including an amyloid-degrading enzyme neprilysin (NEP). As such, inhibition of caspases is considered as a tool for prevention and compensation of various synaptic pathologies leading to cognitive deficit and Alzheimer's disease pathogenesis. In this study we have evaluated the role of prenatal hypoxia on the activity of caspases and neuronal network characteristics in rat brain and the effect of caspase inhibitors on these parameters. We have found that the brain of rats subjected to prenatal hypoxia (E14, O2 7%, 3 h) is characterised by an increased number of caspase-3-positive neurones and higher activity of this enzyme in the neocortex and hippocampus in the period of intensive synaptogenesis (P20-30) compared to controls. Subsequently, in later life these animals had a reduced number of synaptopodin-positive dendritic spines and reduced activity of NEP accompanied by disruption of cognitive functions. Single i.v. injection of caspase-3 inhibitors (Ac-DEVD-CHO) to hypoxic rats on P18-23 led to a decrease in caspase-3 activity and increased NEP expression. In these animals we have also observed restoration of synaptopodin levels and distribution of the labile synaptic spines in the neocortex and hippocampus which were accompanied by improved memory. The effects of inhibitors on memory was observed within one month after administration but not detected 2.5 months later. These data testify to the involvement of caspase-3 in normal brain development and indicate an important role of this enzyme in neuronal plasticity and regulation of cognitive functions.

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Keywords Caspase-3, brain development, cognitive functions.