2nd MEETING OF THE BALTIC PHYSIOLOGICAL SOCIETIES

New Frontiers in Neurophysiology and Neurology

Programme and Abstracts

March 23-24, 2017
Kaunas, Lithuania

Organizers:

Lithuanian, Latvian and Estonian Physiological societies
Lithuanian University of Health Sciences

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Welcome

Dear colleagues,

We would like to welcome you in the second meeting of Baltic physiologists. Physiology is among the most important branches in modern medicine with a plethora of unresolved issues. Our joint meetings are crucial for information and experience sharing as well as participation in the study process in order to promote young researchers who could ensure physiology research development in the future.

In the context of globalisation, intercontinental contacts are extremely important. However, we sometimes forget our closest neighbours, which makes it difficult to assess what we could achieve through cooperation. One of the core aims of the Baltic physiologists' meetings is to consolidate our capabilities and chances to achieve sustainability in developing research at the regional level with the purpose to become strong partners for global projects.

With an increasing average age of the population and rising numbers of chronic diseases, it is crucial to investigate development mechanisms of these processes in order to manage outcomes of diseases and treat chronic ailments.

The focus of this meeting is on the Alzheimer's disease, neurodegenerative and circulatory disorders. We have been successfully able to combine discussions on innovations in physiology and the study process. The meeting will incorporate two lectures on physiology of vascular and nervous activity, devoted to second and third year students of relevant programmes.

We are pleased to welcome you in Kaunas. We would also like to express our gratitude to all lecturers, researchers and sponsors for their contribution to this event.

Sincerely,

Prof. Edgaras Stankevčius
President of Lithuanian Physiological Society

Programme

Thursday, 23 March
Faculty of Pharmacy, hall 203, Sukilėlių str. 13

15:00 – 16:00 **Lecture** for medical students in connection with teaching module *Circulation*:
Vascular function: from physiology to pathophysiology
Prof. Ulf Simonsen (Aarhus, DK)

Friday, 24 March
Faculty of Pharmacy, hall 203, Sukilėlių str.13

Registration (8:00 – 10:00)

8:30 – 9:30 **Lecture** for medical students in connection with teaching module *Sensation and perception*:
Physiology of astroglia
Prof. Alexei Verkhratsky (Manchester, UK)

Session 1 (10:00 – 12:00)
New frontiers in understanding cellular mechanisms of Alzheimer's disease

Chairs: Edgaras Stankevičius
Alexei Verkhratsky

Alexei Verkhratsky (Manchester, UK, Bilbao, Spain)
Principles of astrogliopathology and astrocytes in Alzheimer's disease (20 min + 2 min)

Wendy Noble, (London, UK)
Astrocytes as mediators of synaptotoxic Abeta-tau interactions in Alzheimer's disease (20 min + 2 min)

Lisa Mohamet (Manchester, UK)
Induced Stem Cell Technology for Alzheimer's disease modelling and drug discovery (20 min + 2 min)

Dmitry Lim (Novara, IT)
Astroglial calcineurin and its role in neuronal dysfunction in Alzheimer's disease (20 min + 2 min)

Ramunė Morkūnienė (Kaunas, LT)
Size dependent toxicity mechanisms of Aβ1-42 aggregates in neuronal-glial cell cultures (20 min + 2 min)

Lunch (12:00:13:00)

Session 2 (13:00 – 15:30)
New frontiers in neurophysiology and treatment strategies of Alzheimer's disease

Chairs: Kęstutis Petrikonis
Ulf Simonsen

Wojciech Maksymowicz (Olsztyn, PL)
Looking for the way of restoration of impaired nervous system function (20 min)

Baiba Jansone (Riga, LV)
New drugs and targets: focus on neuroinflammation and GABAergic systems in Alheimer's disease animal model (20 min)

Vladimir Matckov (Aarhus, DK)
Disturbances in pathology of familial hemiplegic migraine type 2 (20 min)

Ulf Simonsen (Aarhus, DK)
Vascular disturbances in Alzheimer disease (20 min)

Aistė Jekabsone (Kaunas, LT)
Evaluation of peptide-functionalized polyethylene glycol hydrogels as designed ECM-like substrates for neuronal-glial cell culture based in vitro models (20 min)

Greta Pšemeneckienė (Kaunas, LT)
Parallels between Alzheimer's CSF biomarkers and TNF alpha genetic polymorphism (20 min)

Anton Terasmaa (Tartu, EE)
Comparison of phenotype of Wfs1 deficient animals with pathology of Wolfram syndrome (20 min)

Lectures

VASCULAR FUNCTION: FROM PHYSIOLOGY TO PATHOPHYSIOLOGY

Ulf Simonsen
Department of Biomedicine, Aarhus University, Aarhus, Denmark

Aim: The aim is to overview the physiology and pathophysiological aspect of small arteries.

Methods: Overview based on physiology books and scientific papers focused on small artery investigation.

Results: Small arteries play an important role for regulation of vascular resistance, and hence blood pressure and organ perfusion. Neurotransmitters including ATP, noradrenaline, and neuropeptide Y are released from sympathetic nerves causing contraction in systemic small arteries through activation of purinoceptors, α-adrenoceptors, and neuropeptide Y receptors followed by membrane depolarization, calcium influx, and activation of the contractile elements in the smooth muscle cells. In addition, a series of kinases including Rho kinase, protein kinase C, and tyrosine kinases are involved in the intracellular events leading to contraction. Circulating angiotensin and endothelium-derived contractile factors also contribute to the contractility. Vasodilator nerves including parasympathetic nerves and sensory afferent innervation also play a role in certain vascular beds. Endothelium-derived relaxing factors including nitric oxide and many others released by agonists or by shear stress play an important role in induction of vasodilatation. They induce vasodilatation mainly by increasing smooth muscle cyclic nucleotides and by activation of potassium channels. High blood pressure, hypertension, is characterized by increased sympathetic activation in young subjects followed by remodeling and narrowing the small arteries and by endothelial cell dysfunction which is worsen by the presence of other risk factors for cardiovascular disease. Attempts to restore/counteract endothelial cell dysfunction have been numerous in animal studies, but reliable and repeatable methods to detect endothelial dysfunction in man are lacking making it a challenge to develop drugs with this aim.

Conclusion: There has been a rapidly growing knowledge concerning the physiology and pathophysiology of small arteries, but further investigation allowing evaluation of these small arteries in vivo is required.

PHYSIOLOGY OF ASTROGLIA

Alexei Verkhratsky
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The nervous system in mammals represents complex network formed by several distinct cell types of neural and non-neural origin. In the course of evolution from most primitive diffuse nervous system to the human brain, these cellular types underwent remarkable degree of specialisation. Neurones become perfect elements for signalling and information processing, whereas housekeeping functions went to the neuroglia, which have themselves specialised into many types of cells to provide for specific aspects of nervous system homeostasis. Homeostatic function of neuroglia is executed at many levels, and includes:

- Whole body and organ homeostasis (for example astrocytes control the emergence and maintenance of the CNS, peripheral glia are essential for communication between the CNS and the body, and enteric glia are essential for every aspect of gastrointestinal function);
- Cellular homeostasis (e.g. astroglia and NG2-glia are both stem elements);
- Morphological homeostasis (glia define the migratory pathways for neural cells during development, shape the nervous system cytoarchitecture, and control synaptogenesis/synaptic pruning, whereas myelinating glia maintain the structural integrity of nerves);
- Molecular homeostasis (which is represented by neuroglial regulation of ion, neurotransmitter and neurohormone concentration in the extracellular spaces around neurons);
- Metabolic homeostasis (e.g. neuroglial cells store energy substrates in a form of glycogen and supply neurones with lactate);
- Long-range signalling homeostasis (by myelination provided by oligodendroglia and Schwann cells);
- Defensive homeostasis (represented by astrogliosis and activation of microglia in the CNS, Wallerian degeneration in CNS and PNS, and immune reactions of enteric glia, all these reactions providing fundamental defence for neural tissue).

Moreover, some astrocytes act as chemosensitive elements of the brain that perceive systemic fluctuations in CO_2, pH and Na^+ and thus regulate behavioural and systemic homeostatic physiological responses. Since any brain disease results from failure in brain homeostasis, neuroglia are involved in many, if not all, aspects of neurological disorders and hence neuroglia may represent a novel target for medical intervention in treatment of neurological diseases.

Oral presentations, session 1
New frontiers in understanding cellular mechanisms of Alzheimer's disease

PRINCIPLES OF ASTROGLIOPATHOLOGY AND ASTROCYTES IN ALZHEIMER'S DISEASE.

Alexei Verkhratsky
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The common and prevailing set of neurological thoughts considers neurones as the primary substrate of pathological progression. This "neurone-centric" concept, however, undergoes a dramatic change. It has become universally acknowledged that integration and information processing in the brain occurs though close interactions of synaptically connected neuronal networks and complex fabric of neuroglial cells. There is compelling evidence demonstrating that astrocytes create the compartmentalisation in the CNS, and integrate neurones, synapses, and brain capillaries into individual and relatively independent units. Astroglial syncytia allow intercellular communication, accomplished through translocation of ions, metabolic factors and second messengers. Many levels of integration, both morphological and functional, presented by neuronal-glial circuitry ensure the spatial and temporal multiplication of brain cognitive power. Neuroglial cells contribute to all forms of neurological diseases and glial reactions, determine, to a very large extent, the progression and outcome of neuropathology. Astrocytes are specifically involved in various neurodegenerative diseases including Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease and various forms of dementia. Astrocytes undergo both atrophy and reactivity, which are specific for different stages of the disease evolution. Astroglial reactivity represents the generic defensive mechanism, and inhibition of astrogliotic response often exacerbates neuropathology.

ASTROCYTES AS MEDIATORS OF SYNAPTOTOXIC A-BETA-TAU INTERACTIONS IN ALZHEIMER'S DISEASE

Wendy Noble, Martina M. Hughes, Diane P. Hanger, Beatriz Gomez Perez-Nievas
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Alzheimer's disease (AD) brain is characterised by the presence of extracellular deposits of Aβ in amyloid plaques and hyperphosphorylated aggregates of tau in neurofibrillary tangles. The presence of these lesions is closely associated with the progressive synaptic dysfunction that gives rise to the clinical symptoms of disease. There are intensive research efforts worldwide focused on uncovering the mechanisms that lead to synapse loss in AD since this may lead to the identification of tractable therapeutic targets. A recent study demonstrated that the presence of activated glia and mislocalisation of hyperphosphorylated tau to synapses are key biological correlates of synapse loss and dementia in AD. We are building on this work to elucidate the mechanisms linking astrocyte activation with changes in tau processing and synapse loss in AD. We have previously shown that astrocytic inflammatory responses influence neuronal responses to Aβ. In primary neural cell cultures, inflammatory factors secreted from activated astrocytes mediate Aβ-induced tau phosphorylation, cleavage and function. We have now generated data in primary neural cell cultures that directly links astrocytic secretions and neuronal tau mislocalisation to synapse loss in AD. These data are supported by our studies in postmortem AD brain where we observe a period of marked astrocyte activation and accumulation of tau in neuronal soma prior to tau redistribution to synapses were tau regulates Abeta-induced excitotoxicity. We have evidence to suggest that specific astrocytic receptors mediate the damaging responses of astrocytes to Abeta and we are exploring the therapeutic potential of blocking these receptors. In summary, our data indicate that astrocytes are an important mediator of Abeta- and tau- induced synaptotoxicity in AD and our initial findings suggest that blockers of astrocytic regulatory receptors may represent as a novel target for dementia treatment.

References

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Kurbatskaya K, Phillips EC, Croft CL, Dentoni G, Hughes MM et al. (2016). Upregulation of calpain activity precedes tau phosphorylation and loss of synaptic proteins in Alzheimer's disease brain. Acta Neuropathol Commun. 4:34. doi: 10.1186/s40478-016-0299-2.

Perez-Nievas BG, Stein TD, Tai HC, Dols-Icardo O, Scotton TC, et al. (2013). Dissecting phenotypic traits linked to human resilience to Alzheimer's pathology. Brain. 136(Pt 8):2510-26. doi: 10.1093/brain/awt171.

INDUCED PLURIPOTENT STEM CELL TECHNOLOGY FOR ALZHEIMER'S DISEASE MODELLING AND DRUG DISCOVERY

Lisa Mohamet
StrataStem Limited, Manchester and Faculty of Medical, Biological & Human, Sciences, University of Manchester, United Kingdom

Alzheimer's disease (AD) represents one of the largest medical burdens currently facing public health, of which there is no cure or prophylaxis. Progress in understanding AD has been hindered by a lack of suitable disease models, reflected in an inability to translate results from animal studies to successful human therapies. The sporadic form of AD (SAD), which is without significant non-Mendelian genetic bias, dominates human pathology. Nevertheless, most of our knowledge of AD derives from studies that utilise cell- and animal-based models of the rare, early-onset, familial AD (FAD). Therefore, there is a pressing need for new technologies that can model early human pathology and aid in the development of new therapeutic targets. Induced pluripotent stem cells (iPSCs) are an ideal model for human disease as they are genetically identical to the donor and are able to form any cell type of the body. We have developed a platform technology that enables iPSCs to be differentiated at high purity into neurones. We show that neurones differentiated from iPSCs harvested from individuals suffering from AD resemble the pathologically affected cells in vivo and express key disease hallmarks. Furthermore, we show AD patient-derived neurones are amenable to an automated platform for phenotypic high content screening (HCS) for repurposed drug screens. Latterly, we report development of a human iPSC-derived astrocyte model created from healthy individuals and FAD- or SAD-affected patients. Induced astrocytes derived from SAD and FAD patients exhibit a pronounced pathological phenotype. Harnessing this potential in AD research will provide an opportunity to significantly reduce timeframes and costs associated with developing novel therapeutics, ultimately improving patient outcomes in the future.

ASTROGLIAL CALCINEURIN AND ITS ROLE IN NEURONAL DYSFUNCTION IN ALZHEIMER'S DISEASE

Dmitry Lim
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Alzheimer's disease (AD) is the most common age-related neurological disorder with an enormous social and economic impact. AD is characterized by progressive loss of memory, social deficit and dementia. Currently, there is no cure or preventive therapy for AD and therefore novel approaches for understanding the AD pathogenesis are desperately needed. Astrocytes, fundamental housekeeping cells in the CNS, undergo complex biphasic changes during AD development, being atrophic at the very early AD stages, but turning to hypertrophy in advanced, plaque-stage AD. In late AD Ca^{2+}/calmodulin-activated phosphatase calcineurin (CaN) plays a central role participating to the development of reactive astrogliosis and setting up neuroinflammation. Little is known, instead, about the role of CaN in early AD with no information being available regarding the role of CaN in astroglial physiology. Work from our lab suggest that the activation of CaN mediates the beta-amyloid (Aβ)-induced alterations of astroglial Ca^{2+} signalling. Exposure of hippocampal astrocytes to soluble oligomeric Aβ triggers a cascade that involves increase in cytosolic Ca^{2+}, activation of CaN and its downstream targets, transcription factors NFAT and NF-kB, that deregulate expression of key components of the astroglial Ca^{2+} signalling including mGluR5. The main features of this cascade have been confirmed on post-mortem brains of symptomatic AD patients. Analysis of Ca^{2+} signals and transcriptome of hippocampal astrocytes from 3xTg-AD mouse pups, which model early stage of familial AD, revealed striking differences in comparison with astrocytes, exposed to Aβ. In particular, mGluR5 signalling was down-regulated in 3xTg-AD astrocytes, while P2Y receptors-mediated signalling was up-regulated. Furthermore, a set of genes, involved in Ca^{2+}-dependent cell adhesion was downregulated. Taken together, our experiments indicate that, at least in terms of Ca^{2+} signalling and homeostasis and transcriptome, the early AD-related changes in astrocytes differ from those found after the burden of Aβ at the late AD stages. The data are discussed in context of the spatio-temporal progression of AD and of the role of CaN in astroglial physiology.

References:

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SIZE DEPENDENT TOXICITY MECHANISMS OF $A\beta_{1-42}$ AGGREGATES IN NEURONAL-GLIAL CELL CULTURES

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Aim: Amyloid beta (Aβ), a molecule implicated in pathogenesis of Alzheimer's disease, is a highly aggregation-prone peptide which can form a variety of aggregates including soluble Aβ oligomers and insoluble amyloid fibrils. The proposed mechanisms of neurotoxicity of Aβ peptides include perturbation of cellular membranes and disruption of Ca^{2+} homeostasis, promotion of inflammatory reactions or mitochondrial dysfunction. Part of the existing controversy may relate to the fact that Aβ toxicity depends on its assembly state.

Methods: We used primary rat neuronal/glial cell cultures to analyze effects of various $A\beta_{1-42}$ assemblies such as monomers, small (1-3 nm) and large (5-10 nm) oligomers and $A\beta_{1-42}$ fibrils on viability of brain cells.

Results: We found that small $A\beta_{1-42}$ oligomers at submicromolar concentration induced rapid neuronal necrosis whereas bigger $A\beta_{1-42}$ aggregates or monomers did not cause neuronal death. Small $A\beta_{1-42}$ oligomers-induced neuronal death was preceded by NMDA/AMPA-R-independent plasma membrane depolarization, mitochondrial superoxide generation, mitochondrial depolarization and NMDA-R-dependent glutamate release into extracellular medium. In microglial cells, small Aβ oligomers caused NMDA-R-dependent depolarization of plasma membrane and mitochondrial superoxide generation. Astrocytes were resistant to small $A\beta_{1-42}$ assemblies-induced cell membrane perturbation, however, they showed increase in mitochondrial superoxide as well as mitochondrial depolarization. These effects were prevented in the presence of selective mitochondria-targeted antioxidant MitoTEMPO which also suppressed glutamate release to culture medium, neuronal death and loss, and microglial proliferation. Hence, pharmacological inhibition of NMDA-R and mitochondrial ROS can protect neurons from small $A\beta_{1-42}$ oligomer-induced damage in Alzheimer's disease. Larger (5-10 nm) $A\beta_{1-42}$ oligomers did not induce neuronal death directly, however, they caused disappearance of neurons in neuronal/glial cultures due to stimulation of phagocytic activity of microglia. In addition, the toxicity of large $A\beta_{1-42}$ oligomers and fibrils was substantially increased in the presence of specific anti-Aβ antibodies due to Fc-dependent microglial activation suggesting that therapies resulting in antibodies to oligomeric Aβ should be used with caution or with suppression of microglial activation.

Conclusions: Our results suggest that various $A\beta_{1-42}$ aggregates may cause neuronal death via multiple mechanisms and that integrated drug targeting systems need to be investigated.

Acknowledgements: This work was supported by the Lithuanian State Science and Studies Foundation (T31/2009 AMILOIDE) and the Research Council of Lithuania (LIG-04/2012 MALPAMA).

Oral presentations, session 2
New frontiers in neurophysiology and treatment strategies of Alzheimer's disease

LOOKING FOR THE WAY OF RESTORATION OF IMPAIRED NERVOUS SYSTEM FUNCTION

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The human dream about the regeneration of impaired nervous system is not too distant future. The development of the methods of collection, separation, culturing and administration of stem cells)SCs) shows new possibilities. Actually the use of humoral activity (growing factors secretion) of SCs is more practical but there are few examples of true cell transplantation with clinical effect. Author presents own experience on this field including new research on intraparenhymal cell injections to the spinal cord in animal and clinical experiments. The other important tool promoting the functional regeneration of nervous system is the use of neuromodulation. One of the best definitions of this new branch of clinical neuroscience is subtitle of the journal „Neuromodulation" – „Technology at the neural interface". Epidural spinal cord stimulation is usefull for the treatment of spasticity and pain syndroms. But there is observed the improvement of peripheral blood supply as result of sympathetic activity supression. The ulcers of the foot fingers are cured, but also cerebral blood flow is increasing thanks the upper cervical epidural spinal cord stimulation. It is used for the treatment of patients in minimally conscious state. Our experience in this kind of treatment is limited to 14 patients. First 7 patients were controlled 6 months after surgery and the clinical as well as neuroimaging (functional MRI and SPECT-CT) examination showed different level of improvement. Next study is necessery for the explanation other than vascular influence of stimulation (eg. propagation of the activity of the reticulo-thalamo-cortical tract). The use of electric Central Nervous System stimulation could be considered together with deep magnetic brain stimulation for the treatment of Alzheimer disease.

NEW DRUGS AND TARGETS: FOCUS ON NEUROINFLAMMATION AND GABAERGIC SYSTEMS IN ALZHEIMER'S DISEASE ANIMAL MODELS

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia. AD is a highly complex disease characterized by the extracellular deposition of beta-amyloid peptide (Ab), intraneuronal tau pathology, mitochondrial dysfunction, altered cellular calcium homeostasis, impaired neural transmission, Ab angiopathy and neuroinflammation, that leads to progressive loss of neurons and synapses, and neurodeficiency (Anand et al., 2014). Currently available treatment options are limited. Although previous research studies were mostly directed on the excitatory (glutamatergic and cholinergic) systems, recent findings indicate a significant role of GABAergic system as a potential tool for the treatment and prevention of AD (Nava-Mesa et al., 2014). Our studies were focused on small molecules in different neurodegeneration models (Klusa et al., 2013). For instances, a carnitine molecule mildronate (meldonium) improved memory in transgenic APPSweDI mice, lowered Ab deposition in the hippocampus and decreased acetylcholinesterase density (Beitnere et al., 2014). The novel 1,4-dihydropyridine (DHP) derivative AP-12 exerted anxiolytic effect and induced learning/memory-stimulating activity in both male C57BL/6J and male and female transgenic APPSweDI mice by enhancing the GABAergic activity and synaptic plasticity processes (Jansone et al., 2015; 2016). Very recently, we have demonstrated the memory-enhancing effects of very low doses of muscimol and baclofen, the GABA-A and GABA-B receptor agonists, respectively, in non-transgenic rat AD model (induced by intracerebroventricular injection of streptozocin). These agonists considerably prevented astrogliosis, decreased acetylcholine degradation (Pilipenko et al., 2017). Our data indicate that neuroprotective effects of studied molecules mostly involve anti-neuroinflammatory action and stabilization of neurotransmission that are essential to prevent decline of cognitive functions in the early AD stages.

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NEUROVASCULAR DISTURBANCES IN PATHOLOGY OF FAMILIAL HEMIPLEGIC MIGRAINE TYPE 2

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Familial hemiplegic migraine type 2 is associated with point-mutations in the α2 isoform of Na,K-ATPase. Heterozygote FHM2 mice bearing G301R mutation were recently characterized for several behavioral and neuronal abnormalities[1]. We have shown that FHM2 mice have abnormal neurovascular coupling in comparison with matched wild types (WT). These abnormalities are shown *in vitro* in isometric myograph, *in situ* in brain slices and *in vivo* in anaesthetized rats.

Pre-constricted middle cerebral arteries from FHM2 mice have exaggerated relaxation to $[K^+]_{out}$ in concentration corresponding to interstitium K^+ surrounding active neuronal tissue (~7-18 mM). This relaxation is inhibited by micromolar $BaCl_2$ suggesting an increased contribution of the inward-rectifying K^+ channels (K_{ir}) and abnormal relaxation of FHM2 cerebral arteries in response to neuronal activation. Accordingly, neuronal electric field stimulation (EFS) in brain slice induced stronger relaxing response of supplying parenchymal arteries in FHM2 mice than in WT. This was, in part, associated with increased contribution of vascular K_{ir} channels and, in part, due with prolonged astrocytic Ca^{2+} waves in brain slices from FHM2 mice. Whiskers stimulation of anaesthetized FHM2 mice also induced stronger increase in perfusion of corresponding sensory cortex in comparison with WT. Interestingly, these K_{ir}-dependent abnormalities of neurovascular coupling in FHM2 mice were not associated with expressional changes in vascular K_{ir} channels.

FHM2-associated mutation of the α2-Na,K-ATPase associated with changes in astrocytic Ca^{2+} signaling and increased relaxation of parenchymal arterioles upon repeated neuronal excitation. This dysfunction in neurovascular coupling might be involved in hyperperfusion associated with the headache stage of migraine attack.

References:

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VASCULAR DISTURBANCES IN ALZHEIMER DISEASE

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Aim: Apart from genetic disposition, aging and cardiovascular disease are risk factors for development of Alzheimer's disease (AD) and vascular dementia. Therefore, the present overview is focused on the association of aging and cardiovascular disease to development of AD.

Methods: Literature overview and focus on the role of transglutaminases in aging, hypertension, and AD.

Results: There is evidence for vascular disturbances including deficits in resting cerebral blood flow, cerebrovascular reactivity, and autoregulation in AD. There is a substantial overlap of mechanistic relationships, as well as genetic and epidemiologic shared risks factors (e.g. hypertension and aging) between cardiovascular/ cerebrovascular disease and AD. Vascular remodeling, arterial stiffness, and endothelial dysfunction may lead to dysfunction of the neurovascular unit contributing to the pathophysiology of AD. The transglutaminase family of enzymes including tissue transglutaminase 2 (TG2), which is a multifunctional and ubiquitously expressed enzyme has been shown to play an essential role in arterial remodeling in hypertension and increment of vessel stiffness related to aging. Furthermore, TG2 is highly expressed in vascular endothelial cells, and there is increasing evidence showing its role in endothelial dysfunction, which leads to endothelial cell apoptosis and alteration of vessel permeability, events that have been shown to occur at early stages of AD in mouse models.

Conclusions: Vascular alterations may have an important role in the pathophysiology of AD playing a role in the early stages of the disease and in the severity of the symptoms. TGs offers a link between aging, cardiovascular disease and AD. Further study of the role of these enzymes may provide new insights into the mechanistic relationship between these clinical entities, and hence provide a novel pharmacological approach to delay the onset of AD and improve the treatment.

PEPTIDE-FUNCTIONALIZED POLYETHYLENE GLYCOL HYDROGELS AS SUBSTRATES FOR NEURONAL-GLIAL CELLS

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Aim: To test ECM-mimicking substrate from collagen mimetic peptide-polyethylene glycol hydrogels with primary neuronal-glial cells.

Methods: Peptides made by liquid phase synthesis were conjugated to 8-arm polyethylene glycol-maleimide and crosslinked to form shape retaining hydrogels. Microstructured hydrogels were made by PDMS replica molding. Mechanical properties were evaluated by oscillatory rheology and AFM nanoindentation. Cell cultures were prepared from 5-7 day old rat cerebella. Cells were visualized by immuno- and affinity chemistry followed by confocal microscopy. Microglial motility was assessed under brightfield microscope. Ca^{2+} oscillations were measured by registration of Fluo-3 AM fluorescence.

Results: The hydrogels promoted development of cerebellar explants to spheroidal bodies containing neurons, astrocytes and microglia and connected with parallel fibers made of neurites with adherent astrocytes. Neurons in the spheroids formed functional networks observed as Ca^{2+} oscillation with frequency similar to the in vivo activity in cerebellum. Cell attachment, organization, neuritogenesis and size of spheroidal bodies were modulated by altering polymer concentration in hydrogel, introducing IKVAV and RGD peptides, and by microformation.

Conclusions: The matrices developed are easy to operate and compatible with most types of microscopy and common plate readers. The functionalization by bioactive peptides and microformation suggests these matrices as a tool for ECM research, disease modelling, tissue engineering and regenerative medicine.

PARALLELS BETWEEN ALZHEIMER DISEASE CSF BIOMARKERS AND TNF ALPHA GENETIC POLYMORPHISM

Greta Pšemeneckienė, Kęstutis Petrikonis, Daiva Rastenytė
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Aim: CSF biomarkers are known to have good sensitivity and specificity differentiating Alzheimer's dementia (AD) from healthy controls (HC) and reflect main pathogenic pathways of Alzheimer disease. It was reported, that polymorphism in proinflammatory factors may be associated with neurodegeneration and increased AD risk. The study was designed to assess polymorphism SNP's rs1799724(-850C>T) of tumor necrosis factor and estimate association with levels of CSF Aβ1-42, p-tau, t-tau and possession of APOEε4 allele in AD *vs* HC.

Methods: Patients with diagnosed AD were selected for the research. Controls were matched by age and sex. Frozen CSF samples were used for Enzyme-Linked Immunosorbent Assay based measurement of biomarkers levels. DNA was extracted from peripheral blood. APOEε allele revealed via hybridization method. TNF-850C>T polymorphism was detected by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis.

Results: Overall 110 persons were selected (AD, n=84, female 66.66%, mean age 73.22 yrs.; HC, n=26, female 65.38%, 70.38 yrs.). Five demented patients were excluded after follow-up period. TNF-850C>T T allele had all controls, with 10% heterozygotes among them. In AD group 86.36% were TT homozygotes, 12.12% CT heterozygotes, and one with CC genotype. CSF biomarkers were measured for 25 AD patients and 20 HC. Medians of all three biomarkers differed significantly compared with controls ($p<0.05$). In AD group APOEε4 carriers had lower Aβ1-42 concentration ($p=0.033$) compared with ε4- patients. P-tau seemed to be higher in APOEε4 carriers, but did not reached significance ($p=0.070$). These data revealed no significant relations between TNF(-850C>T) and CSF biomarkers or APOEε4+ patients.

Conclusions: Further investigation should be done to make more valuable insights according TNF polymorphism and Alzheimer disease risk or pathogenic links.

COMPARISON OF PHENOTYPE OF Wfs1 DEFICIENT ANIMALS WITH PATHOLOGY OF WOLFRAM SYNDROME

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Aim: Wolfram syndrome (WS) is autosomal recessive disorder that is caused by invalidating mutations in WFS1 gene. Characteristic symptoms of WS include diabetes mellitus, optic atrophy and neurological complications. Here we describe and compare the phenotype of Wfs1 deficient animals, and how well these animal models mirror human pathology of WS.

Methods: Several murine models of WS were created, including mutant mice, where exon8 of Wfs1 gene is invalidated. Phenotype of animals was analyzed using several techniques including biochemical methods, immunohistochemistry, electron microscopy and magnetic resonance imaging.

Results: All Wfs1 mutant animals display glucose intolerance. However, development of insulin dependent diabetes is varied between different models. The metabolic profile of animals shows that heterozygous Wfs1 mutant and wild type mice are similar, while homozygous mutant mice are very different to both, wild type and heterozygous mutant mice. This is consistent with human situation, where heterozygous carriers of WFS1 mutations do not develop WS. Older Wfs1 mutant animals show neurodegeneration, again to a different degree.
Diabetes mellitus is usually diagnosed at the age of 5-6 years, blindness due to degeneration of optic nerve at age of 10-12 years. Comparison of rodent models of WS show that Wfs1 mutant animals show symptoms of WS that correspond to human pathology at approximately 4 to 10 years.

Conclusions: Murine models of WS show symptoms of WS to a different degree Usability of these models in translational research on WS will be discussed.

Poster presentations: general physiology

Antioxidant enzymes activities in brain under selenıum and/or aluminium ions treatment

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Aim. The present study was conducted to determine how Al and Se ions alone and in combination affect superoxide dismutases (SOD) and catalase (CAT) activities in brain of laboratory mice.

Methods. Experiments were done on 4-6 week-old outbred mice. SOD activity was determined after single (24 h) and repeated (14 days) intraperitoneal injections of Al and/or Se solutions. The control mice received injections of the same volume of saline. Enzymes activities in the brain homogenate were determined spectrophotometrically.

Results. The results showed that after single Al dose SOD activity was the same value in control and experimental groups. After single Se dose injection SOD activity decreased by 33.0%. It was observed decrease in SOD activity (by 17.8%) following single Se+Al injections. The results showed that 24 h after injection of Al, Se or their mixed, CAT activity was the same in control and experimental groups. In further experiments, there were evaluated effects of Al and/or Se on antioxidant enzymes activities after 14 days injections. The results showed that injections of these elements alone did not cause changes of SOD activity. The data of the effect of both elements showed that SOD activity decreased by 29.4%. Meanwhile after 14 days injections the obtained results showed that Al caused increase by 22.0% of brain CAT activity and the injections of Se and Se+Al solutions did not affect CAT activity.

Conclusions: Our studies revealed that in the brain Se could counteract the effect of Al on the activity of CAT. Se and Se+Al injection reduce SOD activity in brain after 24 h but after 14 days treatment SOD activity reduce only Se+Al injection.

Aluminium and selenium: effect on lipid peroxidation in mice brain

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Aim: This study aimed to determine concentrations of aluminium (Al) and selenium (Se) in mice brain and evaluate the effect of these elements on the level of malondialdehyde, the final product of lipid peroxidation.

Methods: Experiments were done on 4-6 weeks old Balb C mice intraperitoneally treated with $AlCl_3$ (0.15 LD_{50}) and/or Na_2SeO_3 solution/s (0.025 LD_{50}) for 14 days. Control mice received saline solution injections of the same volume. Lipid peroxides were estimated by measuring malondialdehyde level (MDA). The concentrations of Al and/or Se were measured by inductively coupled plasma mass spectrometry (using NexION 300 D).

Results: After treatment with Al or Se, MDA concentration in mice brain increased by 17% as compared to control. Co-exposure to Se and Al resulted in the increase of MDA concentration by 16% as compared to control. Thus, the pretreatment with Na_2SeO_3 20 min before $AlCl_3$ injections, could not reduce Al-induced brain lipid peroxidation. Moreover, Se itself induced an increase of MDA concentration. After 14-day exposure to $AlCl_3$ or Na_2SeO_3, Al concentration increased statistically non significantly by 12% and 8%, respectively. After treatment with Al and Se, Al concentration in mice brain increased by 38% as compared to control ($P<0.05$). However, after 14-day administration of $AlCl_3$ and/or Na_2SeO_3, Se concentration in mice brain remained almost at the control level.

Conclusions: 14-day exposure to Al induced lipid peroxidation in mice brain, which could not be counteracted by Se co-administration. After co-exposure to Al and Se, the concentration of Al increased by 38%, although Se concentration stayed almost at the control level.

Effect of aluminium and selenium on level of reduced glutathione in mice brain

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Aim: This study aimed to determine sub-acute effects of aluminium (Al) and selenium (Se) on the level of reduced glutathione (GSH), a component of cellular antioxidant system, in mice brain.

Methods: 4-6 weeks old Balb C mice were injected intraperitoneally with $AlCl_3$ (0.15 LD_{50}) and/or Na_2SeO_3 solution/s (0.025 LD_{50}) for 14 days. Control mice were treated with the same volume of saline solution. GSH was measured spectrophotometricaly using reaction with 5,5'-dithiobis (2-nitrobenzoic acid). The concentrations of Al and/or Se were investigated by inductively coupled plasma mass spectrometry.

Results: After 14-day exposure to Al or Se, concentration of GSH in mice brain reached 102% and 108% of control, respectively. Co-administration of Se and Al caused the increase of GSH concentration by 12% as compared to control. Changes in all these groups were statistically insignificant. It should be noted that Se, as a cofactor of glutathione peroxidase, barely induced an increase in GSH concentration. Treatment with $AlCl_3$ or Na_2SeO_3, resulted in the increase of Al concentration by 12% and 8%, respectively ($P>0.05$). After co-exposure of mice to Al and Se, concentration of Al in the brain increased by 38% as compared to control ($P<0.05$). 14-day treatment with Al and/or Se almost did not change concentration of Se in mice brain.

Conclusions: 14-day exposure to Al and/or Se did not influence the level of reduced glutathione. Co-administration of Al and Se, led to the increase of Al concentration in mice brain by 38%, although Se concentration remained almost the same as in control.

The quantum chemistry and molecular mechanics in the study of acute toxicity of potential local anesthetics

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Aim: The one of the modern approach to the search for promising chemical structures is computer chemistry, particularly quantum chemistry. Quantum chemical studies give a reliable description of chemical reactivity using a quantitative calculation of the characteristics of the reacting system in a stable state or in the process of their chemical transformations. The present study was designed to comparative study the "acute" toxicity using LD 50 (lethal dose) method after single administration and using rational computer design.

Methods: New derivatives of piperidine were synthesized at the Institute of Chemical Sciences with the laboratorial codes MAV140, MAV141, MAV142, MAV143, MAV144, MAV145, MAV146, MAV147. Acute toxicity was studied by traditional method: by subcutaneous administration of 4% aqueous solutions into white mice of both sexes with weight from 17.0 to 22.0 g. The toxicity was assessed by LD50 method. Calculations of the quantum chemical parameters were carried out by the semi-empirical Hartree-Fock method using the MOPAC 8 calculation program.

Results: According to the results of LD50 calculations, all compounds proved to be low-toxic. The correlation between "toxicity-quantum-chemical characteristics" was analyzed. The low-toxic compounds are characterized by a high level of stability in the form of a neutral molecule, due to negative values of energy of formation and total energy, combined with a relatively low reactivity, as approved by a positive dipole moment.

Conclusions: The obtained results reveal the possibility of using the parameters of quantum chemistry and molecular mechanics to predict the toxicity of piperidine derivatives and optimize primary screening.

Effect of *Acanthopanax senticosus* extract on accumulation of cadmium and amount of immune response cells in mice spleen

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Aim: This study aimed to investigate the moderating effect of liquid extract of *Acanthopanax senticosus* (AS) on immunotoxicity of cadmium.

Methods: The mice were subjected to chronic treatment with cadmium chloride (0.05 LD50) i.p. injections. After, the effects of AS extract (0.1 LD50 and 0.05 LD50) i.p. injections on accumulation of Cd, as well as on level of macrophages, T lymphocytes, and B lymphocytes, were investigated in mouse spleen.

Results: Periodical injections of cadmium chloride for 6 weeks caused the increase of Cd concentration in spleen. Injections of AS extract or AS extract and cadmium chloride did not change the number of macrophages comparing to control group, while the number of macrophages increased significantly in mice injected with cadmium chloride only. AS extract or cadmium chloride did not change the number of T-lymphocytes, but in mice injected with Cd +AS, the number of T-lymphocytes was smaller than in mice injected with cadmium chloride only. On the other hand, AS extract and cadmium chloride increased the number of B-lymphocytes, but the number of B-lymphocytes in spleen of mice injected with Cd +AS 0.05 LD50 was smaller and in mice injected with Cd +AS 0.1 LD50 it was statistically significantly higher than in cadmium chloride treated mice.

Conclusions: Injections of AS extract combined with cadmium chloride leads to the increase of cadmium concentration in spleen of experimental mice. AS decreases the activity of macrophages and T lymphocytes induced by cadmium and increases the activity of B lymphocytes.

CGRP induced relaxation in human sigmoid colon musculature of asymptomatic diverticular disease patients

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Aim: It is known that smooth muscle relaxation is a subject to alteration in diverticular disease. Calcitonin gene-related peptide (CGRP) is a potent smooth muscle relaxant that is involved in a multitude of physiological processes within the digestive system. The aim of the study was to investigate CGRP signalling pathway in asymptomatic diverticular disease (ADD) patients.

Methods: Full-thickness sigmoid specimens were obtained from patients undergoing surgery for non-obstructing colorectal carcinoma, that served as control (n = 8, age: 50–75 years) and as ADD (n = 4, age: 57–76 years) samples in presence of colonic diverticula. Longitudinal and circular smooth muscle strips were used to record isometric muscular activity *in vitro* in response to CGRP. The magnitude was expressed as the percentage of a maximal reference relaxation induced by 10^{-3}M sodium nitroprusside (SNP) and reference contraction obtained by addition of 10^{-4}M bethanechol.

Results: In ADD patients both circular and longitudinal muscles displayed reduced relaxation responses, however, we have observed no significant differences between the two muscle layers. CGRP induced relaxation differed between ADD and control patients in both muscular layers ($p<0.05$). ADD tissue displayed an amplified response to CGRP, as the difference between vehicle and CGRP induced smooth muscle relaxation differed in ADD (41%-60%, $p<0.01$), but not in the control group (67%-72%, n.s.). More so, the difference between ADD and control for vehicle induced relaxation (Δ30%) was greater than for CGRP (Δ11%) ($p<0.01$), suggesting that CGRP may have a compensatory role in altered smooth muscle contractility of DD patients.

Conclusions: Our results suggest that smooth muscle response to CGRP is altered in ADD patients. Peptidergic innervation remodeling of the large intestine may be a contributing factor to the etiology of diverticula formation and DD symptoms.

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Author Index

Table of Contents