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Abstracts

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Oral Presentations

Down-regulated expression of the post-synaptic scaffolding protein SHANK3 by aluminum and miRNA-34a associates with multiple neuropsychiatric disorders

Peter N. Alexandrov[1], Lin Cong[2], Yuhai Zhao[2,3], Vivian Jaber[2], Aileen I. Pogue Aileen[4], Walter J. Lukiw[1,2,4,5,6]

[1]Russian Academy of Medical Sciences, Moscow, Russian Federation
[2]LSU Neuroscience Center, Louisiana State University Health Sciences Center New Orleans, New Orleans LA, USA
[3]Department of Anatomy and Cell Biology, Louisiana State University Health Sciences Center New Orleans, New Orleans LA, USA
[4]Alchem Biotek Research, Toronto ON, Canada
[5]Department of Ophthalmology, Louisiana State University Health Sciences Center New Orleans, New Orleans LA, USA
[6]Department of Neurology, Louisiana State University Health Sciences Center New Orleans, New Orleans LA, USA
wlukiw@lsuhsc.edu

Extensive use of advanced LED-Northern assays, micro-RNA- and messenger RNA (mRNA)-based-expression arrays, RNA sequencing, reverse transcription polymerase chain reaction (RT-PCR), highly controlled short post-mortem interval human brain tissues, luciferase-reporter vector-transfection assays, Western-, ELISA- and bioinformatics-analysis has enabled us to identify a small group of up-regulated miRNAs and their mRNA targets that are significantly down-regulated in autism spectrum disorder (ASD). Two of these up-regulated, inducible and pro-inflammatory miRNAs, miRNA-34a and miRNA-146a appear to target and down-regulate SHANK3 (SH3 and multiple ankyrin repeat domain 3), a 185 kDa multi-domain post-synaptic scaffolding protein whose deficits are strongly linked to the acquisition of the ASD phenotype. The abundance of SHANK3 protein has been found to be significantly down-regulated in autism spectrum disorder (ASD), Phelan-McDermid syndrome (22q13.3 deletion-syndrome; PMS), bipolar-disorder (BD), schizophrenia (SZ), Alzheimer's disease (AD) and other related neurological diseases that exhibit progressive cognitive impairment. Interestingly, in contrast to several other known neurotoxins, two environmentally-abundant neurotoxic metals, aluminum (Al) and mercury (Hg) were found to drive miRNA-34a- and miRNA-146a-up-regulation and down-regulation of SHANK3 gene-expression in primary human brain cell cultures and/or C57BL/6J murine models. Our hypothesis is that aluminum and mercury are two environmental-factors capable of driving the altered miRNA-regulated expression of pro-inflammatory, immune- and synaptic-genes such as SHANK3 resulting in the acquisition of the ASD-phenotype. These studies not only expand our mechanistic understanding of the ASD process but also advance and develop improved models to further elucidate the molecular-genetics of ASD, an incapacitating human neuropsychiatric disorder.

The effect of amyloid beta-peptide on ER-mitochondria contact in Alzheimer's disease

Maria Ankarcrona[1], Nuno Santos Leal[1], Giacomo Dentoni[1], Birgitta Wiehager[1], Per Nilsson[1], Tomas Rauramaa[2], Ville Leinonen[2]

[1]Karolinska Institutet, NVS, Division of Neurogeriatrics, Novum 5th floor, 141 57, Huddinge, Sweden
[2]Kuopio University Hospital, Neurosurgery, PL 1627, 70211, Kuopio, Finland
maria.ankarcrona@ki.se

Cellular organelles are in close proximity at specialized contact sites where important cellular processes take place. For example, calcium homeostasis, lipid synthesis, apoptosis and autophagy are regulated at the endoplasmic reticulum (ER)-mitochondria axis and interestingly all these processes are altered in Alzheimer's disease (AD). These contacts are formed by the close (10-30 nm) apposition between the mitochondrial outer membrane and a specialized region of the ER, referred to as mitochondria associated membranes (MAM). Here we have investigated the effect of Abeta on the structure and dynamics of ER-mitochondria contacts in human tissue and AD mouse models.

Human brain biopsies were collected patients undergoing neurosurgery due to normal pressure hydrocephalus. Mouse brain tissue was obtained from wt and tgAPPswe/lon mice of different ages. ER-mitochondria contact length and number were analyzed by structural electron microscopy.

In addition, ER-mitochondria contact is currently analyzed by proximity ligation assay (PLA) in primary neurons derived from knock-in APPNL-F mice. Levels of MAM-associated proteins were detected by Western blot.

From a unique material we show the structure of ER-mitochondria contacts in human brain. EM analysis mouse brain material reveals that ER-mitochondria apposition is affected by Abeta accumulation. Increased length of ER-mitochondria contacts were detected in tgAPPswe/lon mice at 6 months of age. This is accompanied by altered expression of MAM-associated proteins before onset of pathology.

In conclusion, we show the close relationship between Abeta accumulation and regulation of ER-mitochondria contact and discuss its role in AD pathogenesis.

Mechanisms of the effects of prenatal stress on neuro-development: genetics and epigenetics, and potential mitigating factors

David Q. Beversdorf[1,2], Patrick Hecht[1], Zohreh Talebizadeh[3], Calvin Sjaarda[4], Xudong Liu[4]

[1]University of Missouri Interdisciplinary Neuroscience Program, Columbia MO, USA
[2]Departments of Radiology, Neurology, and Psychological Sciences, University of Missouri, Columbia, MO, USA
[3]Children's Mercy Hospital, Kansas City, MO, USA
[4]Queens University, Kingston, ONT, Canada

beversdorfd@health.missouri.edu

Autism spectrum disorder (ASD) is a highly heterogeneous disorder affecting development of social communication, and repetitive behaviors. While genetic etiologies are well established, there is increasing understanding of the role of environmental factors. One of these factors is exposure to prenatal stress. Our lab has revealed that susceptibility to prenatal stress is mediated by maternal genetic susceptibility to stress for the development of ASD-associated behaviors in animal models. This relationship has also been observed in patient populations. Our subsequent work has revealed alterations in the GABA-ergic system and dopaminergic system associated with this gene x environment interaction in animal models. In order to begin to understand the mechanism, have now examined the epigenetic factors associated with this interaction. Finally, we are exploring the possibility of mitigation of this effect by administration of docosahexaenoic acid (DHA).

Relationship of metformin and sphingolipids in brain of rats fed with high fat diet

Halina Car[1], Kamil Bienias[1], Anna Fiedorowicz[1,2], Sławomir Prokopiuk[1], Anna Sadowska[1], Małgorzata Żendzian-Piotrowska[3], Jan J. Braszko[4], Adrian Chabowski[5]

[1]Department of Experimental Pharmacology, Medical University of Bialystok, Szpitalna 37, 15-295, Bialystok, Poland
[2]Laboratory of Tumor Molecular Immunobiology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114, Wrocław, Poland
[3]Department of Hygiene, Epidemiology and Ergonomy, Medical University of Bialystok, Mickiewicza 2c, 15-022 Bialystok, Poland
[4]Department of Clinical Pharmacology, Medical University of Bialystok, Waszyngtona 15A, 15-274, Bialystok, Poland
[5]Department of Physiology Medical University of Bialystok, Mickiewicza 2c, 15-222, Bialystok, Poland

halina.car17@gmail.com

Development of type 2 diabetes is strongly related with high fat diet (HFD) intake. It is well recognized that short term HFD can induce systemic changes leading to peripheral insulin resistance (IR). We showed in this study that short term HFD evoked alterations in ceramide (Cer) and sphingomyelin (SM) levels in prefrontal cortex (PFC) and hippocampus of rats. 3-week HFD increased concentration of total Cers and several Cer and SM species (Cer: -C14:0, -C16:0, -C18:0, -C20:0, -C22:0, -C16:1, -C18:1, and -C24:1 SM: -C18:1) in PFC, which were subsequently rising with HFD prolongation to 6 weeks. Changes in SF concentrations reported in hippocampus were less profound than in PFC. Thus, we found rise of several Cer and SM species (Cers: -C18:0, -C20:0; SMs: -C18:0, -C20:0, -C16:1 and -C18:1) and reduction of Cer C16:1 in rats fed with HFD for 3 weeks. Importantly, administration of metformin to rats fed with HFD reduced concentrations of both Cers and SMs in studied brain structures. To our knowledge this is the first report showing a protective activity of metformin in the CNS. The most important observation of this study is that even short term HFD induces changes in lipid metabolism in CNS, and metformin effectively abrogates this changes.

Maternal immune activation alters immune response and induces synaptic protein changes in rat offspring

Magdalena Cieślik[1], Magdalena Gąssowska-Dobrowolska[1], Henryk Jęśko[1], Paweł M. Boguszewski[3], Robert K. Filipkowski[2], Agata Adamczyk[1]

[1]Mossakowski Medical Research Centre Polish Academy of Sciences, Department of Cellular Signalling, Warsaw, Poland
[2]Mossakowski Medical Research Centre Polish Academy of Sciences, Behavior and Metabolism Research Laboratory, Warsaw, Poland
[3]Nencki Institute of Experimental Biology Polish Academy of Sciences, Laboratory of Animal Models, Neurobiology Center, Warsaw, Poland

mcieslik@imdik.pan.pl

Autism spectrum disorders (ASDs) are life-long neurodevelopmental diseases characterized by two groups of core symptoms: social communication/interaction deficit and repetitive behavior/interests. While ASDs have

a strong genetic component, environmental factors including toxins, drugs, and infections are also known to contribute to their pathogenesis. Epidemiological studies have revealed a clear association between maternal immune activation (MIA) during early pregnancy, and increased risk of ASDs. However, the molecular link between infection-induced fetal development alterations and autism occurrence are still unclear. To gain insights into mechanisms involved in neurodevelopmental pathology including synaptic protein changes and Akt/mTOR pathway alteration, we used a rodent MIA model obtained by exposing pregnant rats to lipopolysaccharide (LPS; 0.1 mg/kg, intraperitoneally) on gestational day 9.5. Our data have shown significant impairment of social interaction (play behavior – tickling test on post-natal day PND 47). We have observed induction of oxidative stress (glutathione oxidation) and pro-inflammatory cytokine expression in adolescent rat offspring. MIA has also induced presynaptic protein alterations. These alterations included decreased synaptobrevin and syntaxin-1, the key components of SNARE complex, as well as synapsin expression changes. In addition, we observed down-regulation of scaffold proteins, including SHANK family proteins and PSD-95, which are crucial regulators of many key signaling pathways. These changes were accompanied by altered phospho-Akt and mTOR kinase substrate 4E-BP1 protein. We hypothesize that disturbed Akt/mTOR signaling could be responsible for the observed aberrant synthesis of selected synaptic proteins, in turn leading to ASD-like changes in synaptic structure and function.

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Ceramide and sphingosine-1-phosphate crucial modulators of neuronal death. Relevance to neurodegenerative disorders

Kinga Czubowicz, Przemysław L. Wencel, Robert P. Strosznajder

Laboratory of Preclinical Research and Environmental Agents, Department of Neurosurgery, Mossakowski Medical Research Centre, Polish Academy of Sciences, Pawińskiego 5 St, 02-106 Warsaw, Poland

kinga.czubowicz@interia.pl

The metabolic turnover of sphingolipids produces several signaling molecules including bioactive sphingolipids such as: ceramides and sphingosine-1-phosphate (S1P) which are crucial regulators of cell survival and death. Ceramides affect various aspects of neuronal physiology determining cell differentiation, growth, survival, senescence and apoptosis. Ceramides can be hydrolyzed by ceramidases to sphingosine, which can be phosphorylated by sphingosine kinases to sphingosine-1-phosphate. Several studies indicate that S1P promotes cell proliferation and viability acting as a primary messenger and exerts its effect by binding to five specific G protein coupled-receptors (S1PR1-5). Alterations of opposed acting bioactive sphingolipids ceramide/S1P ratio are observed in: brain ischemia, inflammation, cancer, Alzheimer's disease (AD) and other neurodegenerative disorders. Numerous evidences demonstrate that ceramides are associated with several pathological aspects of AD, including for example: amyloid beta peptides accumulation, reactive oxygen species generation, mitochondrial dysfunction and initiation of apoptosis.

The aim of our study was to investigate the role of S1P in molecular mechanisms of neuronal cells survival/death in the model of oxidative stress evoked by ceramide. The experiments were carried out on human neuroblastoma SH-SY5Y cells and PC12 cells transfected with β-amyloid precursor protein (APP – wild type and APP – Swedish mutation) and with empty vector. Our results indicate that "sphingolipid rheostat" between pro-survival S1P and pro-apoptotic ceramide plays a crucial role in regulation of neuronal cells' fate. Stimulation of S1P receptors signaling may offer novel therapeutic strategy in treatment of neurodegenerative diseases characterized by accumulation of ceramide.

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Pharmacological interference with PARP-1 activity in treatment of human diseases: one strategy but different cellular outcomes

Józefa Gądek-Węsierski

Cell Cycle Regulation Group, Department of Medicine I, Div.: Institute of Cancer Research, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria

Jozefa.Gadek-Wesierski@meduniwien.ac.at

Poly(ADP-ribose)polymerase-1 (PARP-1), a highly expressed nuclear enzyme, is involved in cellular responses to various stress stimuli. Activated PARP-1 is significantly involved in the signaling of DNA breakage, repair of single-strand DNA breaks and/or apoptosis in stressed cells. The induction of adequate response(s) to stress strongly depends on the cellular context. It has been found that transient inactivation of PARP-1 may result in different outcomes. Shutting down PARP-1 activity after ionizing radiation

or massive oxidative stress (e.g. after myocardial infarction or stroke) has tissue protective effects. In contrast, inhibition of PARP-1 in some type of cancers enhances the action of anti-cancer drugs, increasing the rate of apoptosis.

In this context it should be mentioned that most conventional anti-cancer drugs such as alkylating or crosslinking agents and inhibitors of topoisomerases I/II, strongly affect DNA integrity, resulting in DNA breakage. If these DNA lesions are not repaired, the continuing DNA damage can generate mutations and genomic instability, subsequently giving rise to secondary cancers or cell death, with the precise outcome depending on the collective extent of DNA damage. Interestingly, DNA-repair deficient cancer cells (e.g. BRCA1/2) are generally extremely sensitive to PARP-1 inhibitors. Based on these observations a novel therapeutic approach termed "synthetic lethality" has been developed that relies on the conditional blockage of base excision repair in DNA-repair deficient cancer cells. Treatment of patients with DNA-repair deficient cancer by administration of selective PARP-1 inhibitors in conjunction with anti-cancer drugs selectively kills cancer cells.

Convergence of non-genetic factors in Autism Spectrum Disorders

Andreas M. Grabrucker

University of Limerick, Dept. of Biological Sciences, Cellular Neurobiology and Neuro-Nanotechnology lab, Limerick, Ireland
University of Limerick, Bernal Institute, Limerick, Ireland
andreas.grabrucker@ul.ie

One in every one hundred Europeans born today is diagnosed with autism, with prevalence rates on the rise. The steep rise in the incidence rate of Autism Spectrum Disorders (ASD) makes it unlikely that purely genetic causes underlie ASD. Thus, genetic factors might be responsible or facilitate the occurrence of ASD, but in addition to a combination of autism-related genes, specific environmental factors may act as risk factors triggering the development of the disorders. Zn deficiency is frequently reported in autistic patients shortly after birth and we have recently shown that prenatal Zn deficiency leads to autism-like behavior in mice. Prenatal Zn deficiency affects the microbiome leading to an increase in inflammation, and in addition or through this, alters neuronal function, ultimately affecting brain connectivity and lateralization. Thus, our data reveals links between prenatal stress, immune system abnormalities, impaired gastro-intestinal functions, and zinc deficiency, all factors associated with autism. Building on this knowledge, several new strategies for the treatment and prevention of autism emerge, such as increasing the bioavailability of zinc during pregnancy or altering local zinc levels in the brain.

SK channels are involved in mitochondrial metabolic shifts: implication for longevity?

Inge E. Krabbendam[1], Birgit Honrath[1,2], Carsten Culmsee[2], Martina Schmidt[1], Amalia M. Dolga[1]

[1]Department of Molecular Pharmacology, University of Groningen, Groningen, The Netherlands
[2]Department of Pharmacology and Clinical Pharmacy, University of Marburg, Marburg, Germany
a.m.dolga@rug.nl

Attenuation of mitochondrial respiration, cell metabolic shifts and preservation of calcium homeostasis have been proposed as therapeutic strategies against neurodegeneration and to increase longevity. Recent data showed that small-conductance Ca^{2+}-activated K^+ (SK) channels modulate calcium homeostasis, mitochondrial respiration and cell survival. We investigated the effects of SK channel activation on mitochondrial complex activity and a possible metabolic shift that might underlie the molecular mechanisms of increased neuronal resilience against oxidative stress. Mitochondrial respiration was measured using Extracellular Flux Analyzer and high-resolution respirometry. To distinguish between effects of SK channel activation on either glycolytic activities and OXPHOS, we applied different concentrations of CyPPA, a positive modulator of SK channels to cells grown in glucose-based (OXPHOS and glycolysis) or galactose-based medium (no glycolysis). High-resolution respiratory revealed that CyPPA reduced mitochondrial complex I and II activity and reduced mitochondrial superoxide formation. CyPPA treatment prevented cell death in conditions of oxidative stress mainly in glucose-grown cells and to a lesser extent in galactose-grown cells, indicating that CyPPA has a reduced neuroprotective potential in the absence of glycolysis. Interestingly, extracellular flux analysis revealed a CyPPA-mediated fast initial increase in glycolysis and a slight reduction of OXPHOS activity. These experiments were validated by cell viability assays where activation of SK channels in galactose-based medium promoted cell death in the absence of glycolysis and when mitochondrial complex I and V were inhibited. These findings shed light on new molecular mechanisms mediated by SK channels that could explain the increased neuroprotective capacity in conditions of increased oxidative stress.

Cytoprotective role of Parkin against alpha-Synuclein evoked mitochondrial failure in PC12 cells. Relevance to Parkinson's disease and other neurodegenerative disorders

Anna M. Lenkiewicz, Anna Wilkaniec, Agata Adamczyk

Department of Cellular Signalling, Mossakowski Medical Research Centre Polish Academy of Sciences, Pawinskiego 5, 02-106 Warsaw, Poland

amlenkiewicz@gmail.com

Parkin and alpha-Synuclein (ASN) are two proteins that are associated with the pathophysiology of Parkinson's disease (PD). Excessive release of ASN, its oligomerization, aggregation and deposition in the cytoplasm contribute to the neuronal injury and cell death through oxidative-nitrosative stress induction, mitochondrial impairment and synaptic dysfunction. In recent years, several studies have demonstrated the neuroprotective function of Parkin and its ability to protect neurons against a wide variety of insults, including those mediated by neurotoxins. However, the role of Parkin in ASN toxicity is still unknown. To investigate the cytoprotective function of Parkin against ASN-induced mitochondria failure and cytotoxicity, PC12 dopaminergic cells with Parkin overexpression and Parkin knock-down were treated with exogenous ASN oligomers. Then, the mitochondria condition, the expression of proteins involved in maintaining mitochondrial function, as well as cell viability were analysed.We found that exogenous ASN oligomers affect Parkin function leading to its S-nitrosylation, autoubiquitination and degradation. Furthermore, ASN-evoked oxidative-nitrosative stress as well as Parkin knock-down triggered mitochondria dysfunction including stimulation of mitochondrial reactive oxygen species production, decreased mitochondrial membrane potential and depletion of cellular ATP level. Finally, we showed that Parkin overexpression prevents mitochondrial failure and attenuates ASN-dependent dopaminergic cell death. In summary, our data showed for the first time that the direct interaction between ASN and Parkin, results in mitochondria dysfunction in dopaminergic cells. Thus, maintaining an optimal function of Parkin appears to be an important issue for ensuring homeostasis of neuronal cells in PD and other neurodegenerative disorders.

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Neurochemical dementia diagnostics: the role of the CSF biomarkers in early diagnosis of Alzheimer's disease

Piotr Lewczuk

Department of Psychiatry and Psychotherapy, Universitaetsklinikum Erlangen, and Friedrich-Alexander Universitaet Erlangen-Nuernberg, Erlangen, Germany
Department of Neurodegeneration Diagnostics, Medical University of Bialystok, Bialystok, Poland

Piotr.Lewczuk@uk-erlangen.de

Alzheimer's disease (AD) is a major neurodegeneration disorder affecting a large proportion of elder populations worldwide. In its diagnostics, two groups of biomarkers in the cerebrospinal fluid (CSF) play the crucial role: Amyloid beta (Ab) peptides and Tau proteins, along with the hyperphosphorylated forms of the latter (pTau). Their analysis reveal alterations as early as twenty years before the onset of the first clinical symptoms. In pre-clinical stages, like mild cognitive impairment (MCI), changes in the CSF reliably predict increased risk of progression into AD dementia. Correspondingly, biomarkers of amyloid pathology and neurodegeneration were included in diagnostic and research criteria for AD. Current further research directions include: (a) search for novel CSF biomarkers with improved analytical or diagnostic performance, (b) optimization of the analysis of the biomarkers already available (for example, to improve quality control and inter-laboratory comparison of results), (c) development of novel sensitive laboratory technologies, and (d) search for biomarkers in the blood.

Benefit statins in spinal cord injury

Nadezda Lukacova, Katarina Bimbova, Andrea Stropkovska, Maria Bacova, Jan Galik

Institute of Neurobiology, Slovak Academy of Sciences, Soltesovej 4, 040 01 Košice, Slovak Republic

lukacova@saske.sk

Statins are potent inhibitors of cholesterol biosynthesis. Recently, it has been recognized that statins also protect the blood spinal cord barrier when administered up to 6 h after spinal cord injuries, and have powerful anti-inflammatory and antioxidant effects. The aim of our study was to limit the inflammatory response at the lesion site by acute post-SCI administration of atorvastatin (ATR; 5 mg/kg; i.p), and to decrease the extent of secondary damage after short and long-term survivals. The ani-

mals (Wistar rats) underwent spinal cord injury (SCI) at Th9 level (40 g/15 minutes) and they survived 4h, 24 h, and 6 weeks. We evaluated the effect of ATR on the level of pro-inflammatory cytokines, glial scar formation, axonal sprouting, the sparing of neurofilaments, and on neurological outcome. Strong inflammatory response was noted early after Th9 compression. ATR treatment affected posttraumatic inflammatory reaction within the first few hours, reduced the activation of astrocytes and microglial cells, and increased overgrowth of axons and the expression of neurofilaments. We suggest that neuroprotective effect of ATR seen early after SCI could be crucial for tissue regeneration and functional recovery in the chronic phase of SCI. ATR inhibits synthesis of isoprenoid intermediates in the cholesterol biosynthetic pathway. It seems that this drug indirectly depletes the supply of isoprenoids and inactivates small GTP-binding protein Rho, which plays crucial role in pathophysiology of SCI. The activation of Rho and its downstream effector ROCK triggers growth cone collapse leading to significant barrier to axon regeneration.

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Alteration of bioactive sphingolipids signalling and locomotor function in an animal model of Parkinson's disease. Neuroprotective effect of Fingolimod

Joanna Motyl, Joanna B. Strosznajder

Department of Cellular Signalling, Mossakowski Medical Research Centre Polish Academy of Sciences, 5 Pawinskiego Street, 02-106 Warsaw, Poland

jpyszko@imdik.pan.pl

Our previous study demonstrated that sphingolipid homeostasis was altered in a cellular model of Parkinson's disease (PD) model. We also found that sphingosine kinase 1 (SPHK1, EC 2.7.1.91), the key enzyme in pro-survival mediator sphingosine-1-phosphate (S1P) synthesis, was significantly down-regulated/inhibited.

This study focuses on SPHK1/S1P signalling in a PD mouse model, specifically examining the effect of the S1P receptor modulator Fingolimod/FTY720 on molecular alterations in selected brain regions and PD mouse locomotor activity.

The PD model was induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 40 mg/kg), administered intraperitoneally to adult mice. FTY720 (1mg/kg) treatment continued for 10 days and was followed by open field and rotarod testing. Midbrain and striatum were isolated for immunochemical, spectrofluorometric, and QPCR analyses.

Significant reductions in Sphk1 expression and activity were identified in MPTP-lesioned mouse midbrain, with FTY720 administration partially reversing these effects. FTY720 exerted a neuroprotective effect by enhancing tyrosine hydroxylase levels in the striatum significantly affected in PD mice. Moreover, FTY720 activated Akt kinase and BAD phosphorylation in midbrain and striatum, which may be related to S1P receptor signalling. Additionally, FTY720 improved the acquisition of motor skills, as treated PD mice showed an increased latency to fall from a rotarod. FTY720 also improved rotarod performance during training days in PD and control mice. Lastly, PD mice treated with FTY720 travelled significantly longer distances at several time points in the open field test.

In summary, SPHK1/S1P alterations appear to play an important role in the PD pathomechanism. S1P receptor(s) stimulation may offer novel opportunities for neurodegenerative disease therapy.

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Interplay between bioactive lipids and presynaptic proteins – critical in initiation and progression of neurodegenerative disorders

Joanna B. Strosznajder

Department of Cellular Signalling, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

jstrosznajder@imdik.pan.pl

Our previous studies indicated significant role of arachidonic acid (AA) and other polyunsaturated fatty acids (PUFA) in oxidative stress (OS), alterations of synaptic function and suggested their participation in changes of presynaptic proteins. PUFA can influence alpha synuclein (ASN) conformation, amyloid precursor protein (APP) metabolism, amyloid beta (AB) liberation and oligomerization. We have demonstrated that OS induced release of ASN from synaptosomes and that extracellular ASN (eASN) affected glutamatergic and dopaminergic signaling, enhanced free radicals level, Ca^{2+} influx and AB secretion. Through this mechanism eASN significantly increased AB toxicity leading to irreversible changes and cells death. Our last data indicated that eASN significantly

affected expression of genes for APP-degrading enzymes. eASN downregulated transcription of alpha secretase (ADAM10) and metalloproteinases (Mmp2, Mmp10). In consequence it may decrease the neuroprotective effect of alpha APP and enhance amyloidogenic pathway of APP processing. Concomitantly the expression of Sirtuin-1 (SIRT-1) which is involved in the regulation of alpha secretase and in the antioxidative defense of cells is downregulated by eASN and AB. Moreover, eASN inhibited also other pro-survival pathways regulated by sphingosine kinase/sphingosine-1-phosphate and Akt kinase and suppressed transcription of anti-apoptotic Bcl2 gene with simultaneously higher expression of pro-apoptotic proteins.

On the basis of our data we suggest that changes of presynaptic bioactive lipids evoke alteration of ASN and subsequent APP/AB metabolism leading to several molecular disturbances in mitochondria and synaptic dysfunctions in Parkinson's and Alzheimer's Disease.

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New insights on docosahexaenoic acid (DHA) and its peroxidation products in microglia

Grace Y. Sun, Runting Li, Bo Yang, Jiancun Cui, Zezong Gu, C. Michael Greenlief, Kevin L. Fritsche, David Q. Beversdorf

University of Missouri, Columbia, MO 65211

SunG@missouri.edu

Polyunsaturated fatty acids (PUFA), especially arachidonic acid (AA) and docosahexaenoic acid (DHA) are known to play multi-functional roles in brain health and diseases. While release of AA from membrane phospholipids has been regarded mainly through the cytosolic phospholipase A2 (cPLA2), the release of DHA is thought to be mediated by the Ca^{2+}-independent iPLA2 instead. PUFAs undergo enzymatic and non-enzymatic conversions to form eicosanoids and oxylipins, which are powerful lipid mediators. In many instances, increase in reactive nitrogen/oxygen species (RNS/ROS) causes lipid peroxidation and production of aldehyde products such as 4-hydroxynonenal (4-HNE) from AA and 4-hydroxyhexenal (4-HHE) from DHA. These products not only can form adducts with proteins, lipids and DNA, but also possess electrophilic properties capable of modulating signaling pathways. Studies with BV-2 microglial cells demonstrated ability for DHA as well as the lipid aldehydes (4-HHE and 4-HNE) to inhibit LPS-induced NO and ROS production, and also upregulate the Nrf2 pathway for synthesis of heme oxygenase (HO-1). However, effective concentrations for DHA (10-100 μM) are 10-fold higher than those for 4-HHE and 4-HNE (1-10 μM). We used an UPLC-MS/MS protocol for the analysis of 4-HHE and 4-HNE levels in microglial cells. The results showed that LPS enhanced production of 4-HNE but inhibited 4-HHE levels. On the other hand, with DHA pretreatment, oxidative stress induced by LPS resulted in an increase of 4-HHE. Taken together, studies with microglia demonstrated protective effects of DHA to be due partly to the enhanced production of 4-HHE.

Pharmacological inhibition of PARP-1 in experimental model of Alzheimer's disease: the cross-talk between PARPs and Sirtuins in regulation of cell's fate

Przemysław L. Wencel[1], Walter J. Lukiw[2], Joanna B. Strosznajder[3], Robert P. Strosznajder[1]

[1]Laboratory of Preclinical Research and Environmental Agents, Department of Neurosurgery, Mossakowski Medical Research Centre, Polish Academy of Sciences, Pawińskiego 5, 02-106, Warsaw, Poland
[2]LSU Neuroscience Center, Louisiana State University Health Sciences Center, 2020 Gravier Street, Suite 904, New Orleans, LA, 70112, USA
[3]Department of Cellular Signalling, Mossakowski Medical Research Centre, Polish Academy of Sciences, Pawińskiego 5, 02-106, Warsaw, Poland

pwencel@imdik.pan.pl

Sirtuins (SIRTs) and Poly(ADP-ribose) polymerases (PARPs) are NAD^+ dependent enzymes that regulate energy homeostasis, transcription and DNA repair. Alzheimer's disease (AD) is characterized by oxidative stress, release oligomerization and accumulation of amyloid beta (Aβ) peptides which may lead to the mitochondria dysfunction and synaptic loss. SIRTs during oxidative stress may promote cell survival whereas PARPs can act both as survival and death inducing factor. Alterations of these enzymes may play crucial role in pathomechanism of AD.

We examined the influence of PARP1 inhibition (by PJ-34) on gene expression of SIRTs, PARPs and beta-amyloid precursor protein (βAPP) cleaving enzymes in PC12 cells under Aβ42 oligomers (AβO) toxicity. Moreover, the effect of endogenous Aβ in PC12 cells stably transfected with human gene for APP wild-type (APPwt) was analyzed.

Our results demonstrate that AβOs have inhibitory effect on mRNA level of nuclear *Sirt1*. Moreover, AβOs significantly enhance mRNA level of mitochondrial *Sirt4,5*

and crucial subunits of γ-secretase (*Psen1,2*) in PC12 cells. Concomitantly, PJ-34 enhances mRNA level of *Sirt1,4,6, Parp3,* α-secretase (*Adam10*), β-secretase (*Bace1*), and *Psen1,2* in PC12 cells subjected to AβOs toxicity. Moreover, Aβ peptides in APPwt cells activate expression of *Bace1*, *Psen1,2* and *Parp1*.

Our results indicate that PARP1 is important SIRTs transcription regulator. Aβ peptides through modulation of APP secretases transcription may lead to a vicious metabolic circle, which could be responsible for maintaining Aβ at high level. Moreover, PARP1 inhibition modulates βAPP metabolism enzyme(s) transcription and this action can be potentially applied against Aβ peptide toxicity.

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Aberrant purinergic signalling contributes to neurodegeneration and mitochondrial impairment induced by alpha-synuclein

Anna Wilkaniec, Anna M. Lenkiewicz, Agata Adamczyk

Department of Cellular Signalling, Mossakowski Medical Research Centre Polish Academy of Sciences, Pawinskiego 5, 02-106 Warsaw, Poland

aniakazmierczak@gmail.com

Oligomerization and accumulation of alpha-synuclein (ASN) have been implicated in the pathogenesis of Parkinson's disease (PD) and other neurodegenerative disorders. In parallel to the ASN-related hypothesis, alteration in purinergic neurotransmission, particularly the P2 family receptors have been recently shown to participate in the neurodegenerative processes. However, the interplay between extracellular ASN and neuronal purinergic receptors and its involvement in the mechanisms underlying neurodegeneration were not investigated. Therefore, in this study, we have examined the effect of ASN on P2 purinergic receptors and ATP-dependent signalling. We used neuroblastoma SH-SY5Y cell line and rat synaptoneurosomes treated with exogenous soluble ASN. The experiments were performed using spectrofluorometric, radiochemical, and immunochemical methods. We found that extracellular ASN directly activates purinergic P2X7 receptors, leading to pannexin 1 recruitment to form an active complex responsible for ATP release. Moreover, ASN greatly decreased the activity of extracellular ecto-ATPase responsible for ATP degradation. Stimulation of P2X7 receptor by exogenous ASN led to disruption of calcium and redox homeostasis and activation of cellular stress response proteins like Stat3 or SAPK/JNK. We also observed that P2X7–dependent inhibition of PI3K-Akt pathway led to decrease in mTOR activity. Finally, P2X7 antagonists prevented de-regulation of mitochondria function and turnover as well as apoptosis induced by extracellular ASN. Thus, we concluded that purinergic receptors might be putative pharmacological targets in the molecular mechanism of extracellular ASN toxicity. Interference with P2X7 signalling seems to be a promising strategy for the prevention or therapy of PD and other neurodegenerative disorders.

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Clinical genetics of alpha-synucleinopathies and tauopathies

Zbigniew K. Wszolek

Department of Neurology, Mayo Clinic Florida, 4500 San Pablo Road, Jacksonville, FL 32224, USA

wszolek.zbigniew@mayo.edu

Discovery of SNCA nod MAPT gene mutations in late 1990's open the road to understanding of pathophysiological implications of many neurodegenerative conditions. New classifications of these neurodegenerative disorders emerged. Alpha synucleinopathies encompass disorders such as Parkinson disease, Parkinson disease dementia, dementia with Lewy bodies, and multiple system atrophy. The list of tauopathies is growing and includes progressive supranuclear palsy, corticobasal degeneration, Pick disease, chronic traumatic encephalopathy, globular glial tauopathy, argyrophilic grain disease, primary age-related tauopathies, and frontotemporal dementia with parkinsonism linked to chromosome 17. Alzheimer disease, the most common neurodegenerative disorder is considered to be both amyloidopathy and tauopathy. Many of the newer tauopathies can be diagnosed only on pathological grounds. Some of them are clearly the genetic disorders usually transmitted in autosomal dominant fashion. During my lecture I will discuss the clinical presentations of common alpha synucleinopathies and tauopathies and their genetic forms.

Posters

Mitochondrial biogenesis and neural differentiation of human iPSC is modulated by Pyrroloquinoline quinone in a developmental stage-dependent manner

Justyna Augustyniak[1], Jacek Lenart[2], Marzena Zychowicz[1], Gabriela Lipka[1], Piotr P. Stepien[3,4,5], Leonora Buzanska[1]

[1]Stem Cell Bioengineering Unit, Mossakowski Medical Research Centre Polish Academy of Sciences, 5a Pawinskiego Str., 02-106 Warszaw, Poland
[2]Department of Neurochemistry, Mossakowski Medical Research Centre Polish Academy of Sciences, 5a Pawinskiego Str., 02-106 Warsaw, Poland
[3]Department of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, 1 Miecznikowa Str., 02-096 Warsaw, Poland
[4]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 5a Pawinskiego Str., 02-106 Warsaw, Poland
[5]Centre of New Technologies, University of Warsaw, 2C Banacha Str., 02-097 Warsaw, Poland
jaugustyniak@imdik.pan.pl

Pyrroloquinoline quinone (PQQ) belongs to small molecules which modulate mitochondrial biogenesis and may affect the fate decisions. The PQQ efficiency was proofed in many cell types, but not in neural stem cells (NSC). In our studies, we evaluated the impact of PQQ on different stages of hiPSC neural differentiation: neural stem cells (NSC), early neural progenitors (eNP) and neural progenitors (NP). Main tested parameters were the effect of PQQ on viability, proliferation, mitochondrial biogenesis and differentiation potential. Our results indicated that sensitivity to PQQ is dependent on its concentration and the stage of development. Antioxidant properties of PQQ were proved in all tested stages of neural development, however induction of mitochondrial biogenesis was stage specific. PQQ potential to stimulate mitochondrial content was evaluated at DNA, mRNA and protein level. Changes in the expression of important for mitochondrial biogenesis genes (NRF1, TFAM and PPARGC1A) upon PQQ treatment were observed at all developmental stages, but only at the eNP stage the up-regulation was significant and coexist with increase the mtDNA copy number and high levels of expression of SDHA and COX-1 proteins. In addition, a strong induction of GFAP, with down-regulation of MAP2 gene expression upon PQQ treatment in eNP was observed. Thus, the existence of a "developmental window" of eNP cells for PQQ-induced mitochondrial biogenesis is proposed. Our study indicates the possibility of in vitro shifting cell differentiation in the favor of glia, but more research is needed at this point.

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Exogenous electric stimulation has anti-inflammatory effect and promotes healing after spinal cord injury in rats

Maria Bacova, Katarina Bimbova, Jana Fedorova, Jan Galik

Institute of Neurobiology, Slovak Academy of Sciences, Kosice, Slovak Republic
svatova.maria@gmail.com

The aim of this study was to evaluate the effect of oscillating electric field stimulation on inflammatory response and motor function recovery in rats with spinal cord injury (SCI). A rat spinal cord was injured by compression device at the level of Th9 segments with the weight of 40g for 15 minutes. For electric stimulation the miniature oscillating field (OSF) stimulator was used, which was designed in our laboratory. OSF stimulator reverses polarity of electric field every 15 minutes to support axonal outgrowth in both directions. Implanted stimulator delivered current to the injured spinal cord by means of two Ir/Pt electrodes that were inserted into the epidural space, two segments cranial and caudal from the Th9 segment. Experimental animals (Albino Wistar rats) were divided into three groups: control (intact with OSF stimulator), injured (SCI only) and experimental (SCI with OSF stimulator). BBB score and CatWalk test for evaluation of motor function before and after experiments were performed. After month, animals were perfused and the spinal tissue was used for immunohistological and histological analysis. In the control group we observed that OSF implantation did not induce an inflammatory reaction or necrosis of the surrounding tissue, so it doesn't present a risk or any limitation for the animals. Our results also showed that the experimental SCI+OSF group of rats had better motor activity of hind limbs after injury. OSF stimulation decreased the number of activated astrocytes and microglial cells and promoted wound healing after surgery compared with the SCI group of animals.

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IL-6 deficiency and hippocampal apoptosis in young adult and aged mice

Izabela Bialuk, Magdalena W. Sokołowska, Tomasz A. Bonda, Magdalena Dziemidowicz, Maria M. Winnicka

Department of General and Experimental Pathology, Medical University of Białystok, Mickiewicza 2c, 15-222 Białystok, Poland

izabela.bialuk@umb.edu.pl

Interleukin 6 (IL-6) is a cytokine with numerous biological functions within the central nervous system. Increasing with age IL-6 expression has been associated with acceleration of senescence and neuronal loss. Because degenerating cells are removed from tissues by apoptosis, the aim of present study was to evaluate whether IL-6 deficiency may influence this process in hippocampus.

Experiments were performed on 4- and 24-month-old IL-6-deficient (IL-6KO) and control (WT) mice. Apoptosis was detected using TUNEL assay, caspase-3 activity was evaluated using immunohistochemistry and expression of apoptosis-related proteins: p53, MDM-2, Bax and Bcl-2 by means of Western Blotting.

TUNEL showed only single apoptotic cells in the hippocampus of young adult and aged animals of both genotypes, what was supported by lack of caspase-3 activity. While the expression of p53 protein in young adult mice was comparable, it was enhanced in the aged animals of both genotypes. Significant increase of p53 expression was observed in 24-month-old WT animals, being 20 times higher in comparison to 4-month-old WT mice. Although the level of p53 in aged IL-6KO mice also increased, its expression was significantly lower in comparison to aged WT animals. MDM-2 expression was significantly lower only in 24-month-old IL-6KO animals in comparison to the young adult IL-6KO mice, while there were no significant differences in the expression of Bax and Bcl-2 proteins.

Significant attenuation of p53 protein expression in aged IL-6KO mice in comparison to aged WT ones may suggest that IL-6 deficiency protects against age-related accumulation of damages in the hippocampal cells.

The release of growth factors and their receptors in spinal cord after endurance training

Katarina Bimbova[1], Maria Bacova[1], Andrea Stropkovska[1], Jana Fedorova[1], Małgorzata Chalimoniuk[2], Józef Langfort[2], Nadezda Lukacova[1]

[1]Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic
[2]Mossakowski Medical Research Center, Polish Academy of Sciences, Warsaw, Poland

bimbova@saske.sk

The goal of our study was to find out whether running on treadmill increases the expression of BDNF (brain-derived neurotrophic factor) and GDNF (glial cell-derived neurotrophic factor), as well as their receptors – TrkB (BDNF receptor) and GFRα1 (GDNF receptor) at thoracic and upper lumbar level. Animals (Wistar rats) were divided into 2 groups. The first group was trained on treadmill 5 days/week for 6 weeks and the second was a control. The intensity of training was gradually increasing (from 27 cm/s to 46.6 cm/s). The spinal cord (SC) tissue, taken from the Th7-L2 region, were divided into three 1.5 cm sections. SC samples were used for RT-PCR to detect relative quantity of BDNF, GDNF, TrkB, GFRα1. BDNF and GDNF were minimally released in the control group throughout the whole area studied (BDNF – 0.254 ± 0.21; GDNF – 0.044 ± 0.037). Similarly, the expression of growth factors receptors in spinal cord was very low (TrkB – 0.039 ± 0.034; GFRα1 – 0.072 ± 0.056). Strong segmental modifiability was seen six weeks after endurance training. The highest BDNF and GDNF levels (5 to 6-times increase) were detected at low thoracic and upper lumbar area. The TrkB values were significantly elevated by the exercise in thoracolumbar segments (3-fold increase) and the GFRα1 levels were mostly affected in the middle of the spinal cord region (4-times increase). The results suggest that BDNF, GDNF and their receptors are strongly modulated by exercise, and that their levels, except for GFRα1 increased with ascending order.

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Neuroprotective effects of metabotropic glutamate receptors group II (mGluR2/3) agonists in an animal model of birth asphyxia

Ewelina Bratek, Apolonia Ziembowicz, Elżbieta Salińska

Department of Neurochem. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

ebratek@imdik.pan.pl

Release of glutamate plays an important role in hypoxia-ischemia (H-I) induced neurodegeneration. It was shown that group II metabotropic glutamate receptors (mGluR2/3) activation before or after ischemic insult results in neuroprotection but the exact mechanism of this effect is not clear.

The aim of present study was to investigate the effect of mGluR2/3 activation on apoptotic factors expression in experimental model of birth asphyxia.

We used hypoxia-ischemia (H-I) on 7-day old rat pups, where the left common carotid artery is permanently occluded and then the pups are subjected to hypoxia (7.4% oxygen in nitrogen for 75 min). Animals were injected intraperitoneal with mGluR2 (LY 379268) and mGluR3 (NAAG) agonists 1h or 6h after H-I (5 mg/kg of body weight). The weight deficit of the ischemic hemisphere and the expression of Bax, Bcl-2, HTR/OMI and BDNF, GDNF, TGF–beta were measured (ELISA). Application of both agonists resulted in decrease of brain weight loss independently on the time of application; both mGluR2/3 agonists decreased the damage and CA1 region disorganization. Both mGluR2/3 agonists decreased expression of Bax and HTR/OMI and increased expression of Bcl-2 compared to untreated H-I. mGluR2/3 agonists application decreased TGF-beta and increased BDNF and GDNF expression in the ischemic hemisphere compared to H-I.

Our results show that activation of mGluR2 or mGluR3 in a short time after H-I insult reduce brain damage and decrease apoptotic processes initiated by H-I in developing brain. Activation of mGluR2/3 receptors increases also expression of neurotrophic factors which promotes neuronal survival.

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Alteration of Akt/mTOR signalling in rats prenatally exposed to valproic acid: relevance to the pathology of autism spectrum disorder

Magdalena Cieślik[1], Magdalena Gąssowska-Dobrowolska[1], Henryk Jęśko[1], Robert K. Filipkowski[2], Paweł M. Boguszewski[3], Agata Adamczyk[1]

[1]Mossakowski Medical Research Centre Polish Academy of Sciences, Department of Cellular Signalling, Warsaw, Poland
[2]Mossakowski Medical Research Centre Polish Academy of Sciences, Behavior and Metabolism Research Laboratory, Warsaw, Poland
[3]Nencki Institute of Experimental Biology Polish Academy of Sciences, Laboratory of Animal Models, Neurobiology Center, Warsaw, Poland

mcieslik@imdik.pan.pl

Mammalian target of rapamycin (mTOR), a serine/threonine kinase, functions as a signal amplifier in the phosphoinositide 3-kinase (PI3K)/Akt/mTOR pathway, and a defect in any of the proteins involved can lead to aberrant signalling. Impairment of Akt/mTOR activity has been suggested in the pathophysiology of autism and other neuropsychiatric disorders. Therefore, the present study aimed to examine the phosphorylation activity of several proteins in Akt/mTOR pathway in the brain of rats prenatally exposed to valproic acid (VPA), which is one of the most used animal models of autism. Pregnant Wistar rats were injected *i.p.* with a single dose of 400 mg/kg VPA on embryonic day 12.5. Autism-like behaviours were verified by measuring ultrasonic vocalizations and elevated plus maze test. Here, we found impaired communication and increased anxiety in VPA animals. Deregulation of Akt/mTOR pathway was observed in the cerebral cortex, hippocampus and cerebellum of autistic model rats. Enhancement of phospho-mTOR protein level was the most pronounced in the hippocampus, where the phosphorylation of mTOR targets was observed: increased p-4E-BP1, and reduced phospho-p70S6K. These changes were accompanied by an increase in p-Akt protein level. In addition, synaptosomes isolated from VPA subjects revealed significant abnormalities in their ultrastructure including unidentified electron-dense matrix material as well as fragile and malformed post-synaptic densities. Altered Akt/mTOR signalling may result in disturbed spine protein synthesis and thereby lead to synaptic dysfunction. These data might help to point possible etiological mechanisms in autism and other neuropsychiatric disorders.

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Amyloid beta oligomers alter transcription of genes involved in stress response and mitochondrial function differently in neuronal and microglial cells: implication in Alzheimer's disease

Magdalena Cieślik, Grzegorz A. Czapski, Sylwia Wójtowicz, Joanna B. Strosznajder

Department of Cellular Signalling, Mossakowski Medical Research Centre, Polish Academy of Sciences, Pawinskiego 5, 02-106 Warsaw, Poland

swojtowicz@imdik.pan.pl

The recent studies underline the important role of microglial cells in maintaining of neuronal circuits and neuronal viability during brain development, aging and neurodegenerative disorders. The cross-talk between neuronal and glial cells is crucial also for the progression of neurodegeneration. Microglial cells are very sensitive to stress evoked by amyloid beta oligomers (AβO). In Alzheimer's disease, activation of microglial cells may play a dual role: it may protect neurons or may promote neurodegeneration/inflammation and neuronal death.

In this study, we compared the impact of AβO on transcription of genes related to antioxidative defense and mitochondrial function in neuronal (SH-SY5Y) and microglial (BV2) cells. The expression of sirtuin-1, mitochondrial sirtuins and genes engaged in mitochondrial dynamics and electron transport chain (ETC) complexes was investigated.

Our results demonstrated significant inhibitory effect of AβO (24 h) on mitochondrial membrane potential in neuronal SH-SY5Y cells. The AβO also affected transcription of several genes involved in antioxidative defense (Sod2, Gpx4, Sirt1, Sirt4 and Sirt5) in these cells. Moreover, transcription of genes engaged in mitochondria fission/fusion was decreased (Fis1, Ppargc1a) and the level of mRNA for ETC, Sdha was significantly suppressed in SH-SY5Y cells. AβO enhanced expression of genes related to antioxidative defense and mitochondrial biogenesis (Sod2 and Ppargc1a) in microglia cells during the first 24 h, concomitantly not exerted effect on mitochondrial membrane potential. Enhanced expression of genes for antioxidative proteins and Pgc1α in microglia cells at early stage of AβO toxicity may suggest their role in mechanism of cytoprotection.

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The effect of poly(ADP-ribose) polymerase-1 inhibition on expression of antioxidative and mitochondria-related genes: relevance to pathomechanism of Alzheimer's disease

Grzegorz A. Czapski[1], Magdalena Cieślik[1], Przemysław L. Wencel[2], Sylwia Wójtowicz[1], Robert P. Strosznajder[2], Joanna B. Strosznajder[1]

[1]Department of Cellular Signalling, Mossakowski Medical Research Centre Polish Academy of Sciences, Pawińskiego 5, PL 02106 Warsaw, Poland
[2]Laboratory of Preclinical Research and Environmental Agents, Department of Neurosurgery, Mossakowski Medical Research Centre Polish Academy of Sciences, Pawińskiego 5, PL 02106 Warsaw, Poland

gczapski@imdik.pan.pl

Critically important component of the pathogenesis of Alzheimer's disease (AD) is impairment of mitochondrial function. Amyloid beta oligomers (ABO) trigger this phenomenon but the molecular mechanism in not fully understood. The growing body of evidence indicates that mitochondrial function is under tight control of poly(ADP-ribose) polymerase-1 (PARP-1), and involvement of this enzyme in mitochondrial dysfunction was confirmed in many conditions.

Our aim was to evaluate the role of PARP-1 in controlling expression of genes involved in antioxidative defense and in regulation of mitochondrial function. We studied the effect of PARP-1 inhibitor PJ34 on PC12 cells in the absence or presence of ABO.

The data demonstrated that inhibition of PARP-1 enhanced transcription of genes for antioxidative enzymes (Sod1, Gpx1, Gpx4), activated genes regulating mitochondrial fission/fusion (Mfn1, Mfn2, Dnm1l, Opa1, Fis1), subunits of electron transport chain complexes (mt-Nd1, Sdha, mt-Cytb) and modulated expression of several transcription factors (TFs), enhanced Foxo1 and decreased Nrf1, Stat6, Nfkb1. ABO significantly decreased mitochondrial membrane potential and cell viability, and in time-dependent manner affected expression of investigated genes. Under ABO-evoked stress conditions, PJ34 raised transcription of several genes (Gpx1, Gpx4, Opa1, Mfn2, Fis1 and Sdha), but downregulated expression of numerous TFs (Nrf1, Tfam, Stat3, Stat6, Trp53, Nfkb1). Our results indicate the significant role of PARP-1 in regulation of mitochondrial transcriptome which in consequence may influence cellular function.

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Acute liver failure resulted in accumulation of S-adenosylhomocysteine and depletion of glutathione in the brain

Anna Czarnecka, Wojciech Hilgier, Magdalena Zielińska

Mossakowski Medical Research Centre, PAS, Department of Neurotoxicology, Pawińskiego Street 5, 02-106 Warsaw, Poland

aczarnecka@imdik.pan.pl

Methionine adenosyltransferase (MAT) catalyzes the formation of S-adenosylmethionine (AdoMet). It is used in transmethylation reactions and converted to S-adenosylhomocysteine (AdoHcy) which is a potent competitive inhibitor of transmethylation. Abnormalities in hepatic MAT have been found in patients with cirrhosis and in several models of liver injury. Depletion of AdoMet leads to decrease in glutathione synthesis.

The purpose of this study was to assess whether acute liver failure (ALF) induced by thioacetamide (TAA) affects AdoMet and AdoHcy synthesis and as a consequence glutathione metabolism.

Rats were subjected to three injections of TAA (300 mg/kg) which led to ALF with metabolic changes and symptoms typical of acute hepatic encephalopathy. Animals were decapitated 24 h after last injection. HPLC analysis revealed decreased tissue level of AdoMet accompanied by the increase in AdoHcy in the cerebral cortex of TAA rats. In the liver both AdoMet and AdoHcy were decreased. Immunoblot analysis revealed decreased MAT1A liver expression. Cystathionine-β-synthase protein content was decreased both in liver and cerebral cortex. The total glutathione level and GSH/GSSG ratio were decreased.

Summarizing ALF leads to decreased AdoMet affecting brain redox status expressed as ratio of reduced to oxidized glutathione. Moreover, increased brain AdoHcy indicates that its clearance is impaired which may be the cause of the opposing effect on the exogenous AdoMet stimulation in the brain of control and ALF rats (Czarnecka et al., Neurotox Res (2017) 31:99–108).

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Role of CacyBP/SIP protein in beta-catenin dysregulation using YAC128 HD mice and the cacybp zebrafish knockout

Magdalena Czeredys[1], Anna Romaszko[1,2], Jacek Kuznicki[1]

[1]Laboratory of Neurodegeneration, International Institute of Molecular and Cell Biology in Warsaw, 4 Ks. Trojdena Street, 02-109 Warsaw, Poland
[2]Undergraduate student from Biotechnology, Warsaw University of Life Sciences, 166 Nowoursynowska Street, 02-787 Warsaw, Poland

mczeredys@iimcb.gov.pl

Mutated huntingtin has been shown to affect CacyBP/SIP gene encoding calcyclin-binding protein (CacyBP/SIP), that was 2-fold overexpressed in the striatum of YAC128 mice, a model of HD (Czeredys *et al*., 2013). A higher increase in the CacyBP/SIP dimer than in the monomer was found in the striatum of HD mice. Moreover, we detected a decrease in total protein ubiquitination, while the level of beta-catenin was higher in the striatum of HD transgenic mice as compared to wild-type mice.

In these study we determine the effect of increased dimerization of CacyBP/SIP protein in YAC128 model and the effect of decreased level of Cacybp in zebrafish on beta-catenin signaling.

Medium Spiny Neurons (MSNs) with CacyBP/SIP overexpression isolated from the striatum of YAC128 and wild-type mice were used to analyze the level of beta-catenin and protein ubiquitination by western blotting. Proximity Ligation Assay (PLA) was used to study CacyBP/SIP dimerization in these cultures. Zebrafish lines with knockout of cacybp were generated using CRISPR/Cas9 technology.

We observed the presence of CacyBP/SIP dimers using PLA in MSNs. Currently, we are studying if the increased level of CacyBP/SIP dimers affects beta-catenin and its ubiquitination in YAC128 MSNs cultures. Preliminary data from the cacybp zebrafish knockout shows disturbances in beta-catenin protein level in total protein extracts.

Increased dimerization of CacyBP/SIP might disturb degradation of beta-catenin in HD. The cacybp zebrafish knockouts will allow us to find out if Cacybp is involved in beta-catenin signaling.

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The role of bioactive sphingolipid signalling in neuronal cell survival/death

Kinga Czubowicz, Przemysław L. Wencel, Robert P. Strosznajder

Laboratory of Preclinical Research and Environmental Agents, Department of Neurosurgery, Mossakowski Medical Research Centre, Polish Academy of Sciences, Pawińskiego 5 St, 02-106 Warsaw, Poland

kinga.czubowicz@interia.pl

Ceramides are involved in such cell responses as: proliferation, differentiation, growth arrest and apoptotic death. Ceramide may be broken down by ceramidases, thus leading to the formation of sphingosine, which can be phosphorylated by sphingosine kinases (Sphk) to sphingosine-1-phosphate (S1P). Ceramides may be also phosphorylated by ceramide kinase (CERK) to ceramide-1-phosphate (C1P). Several studies indicate that S1P and C1P enhance cell proliferation and antagonize apoptosis. S1P regulates cellular processes by binding to five specific G protein coupled-receptors (S1PR1-5). The aim of this study was to investigate the role of S1P in neuronal cells survival/death evoked by cell-permeable

C2-ceramide. This study was focused on the effect of S1P receptors modulators: phospho-fingolimod (pFTY720) and SEW2871. We also investigated the effect of myriocin (a selective inhibitor of de novo pathway for ceramide synthesis) and NVP-231 (a selective inhibitor of CERK) on neuronal cells survival/death. Our research indicated that C2-ceramide decreased the viability of neuronal cells transfected with β-amyloid precursor protein (APP – wild type and APP – Swedish mutation) and with empty vector. Conversely, exogenously added pFTY720 and SEW2871 significantly increased the viability of neuronal cells through receptors-dependent mechanism. It has been observed that NVP-231 decreased the neuronal cell viability. This study also showed that myriocin had protective effects which could have been caused by the inhibition of ceramide accumulation. Summarizing, our data indicated that modulation of sphingolipid signalling may play a crucial role in regulation of neuronal cells survival/death and may offer therapeutic strategy in treatment of neurodegenerative diseases.

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HPLC studies on glutamate, glutamine and GABA levels in the rat hippocampus in the pharmacological models of autism

Dominik Diamandakis[1], Elzbieta Zieminska[1], Wojciech Hilgier[1], Robert Filipkowski[1], Rafał Polowy[1], Beata Toczylowska[2], Jerzy W. Lazarewicz[1]

[1]Mossakowski Medical Research Centre, Polish Academy of Sciences, Pawińskiego 5, 02-106 Warsaw, Poland
[2]Nalecz Institute of Biocybernetics and Biomedical Engineering Polish Academy of Sciences, Trojdena 4, 02-109 Warsaw, Poland

ddiamandakis@imdik.pan.pl

An imbalance in excitatory/inhibitory neurotransmission has been implicated in the pathogenesis of autism. This study evaluated changes in the content of glutamate (Glu), glutamine (Gln) and GABA in the rat hippocampus in pharmacological models of autism. The rat females at the 11th day of gestation were given orally 800 mg/kg b.w. of valproic acid (VPA) or 500 mg/kg b.w. of thalidomide (THAL). The pups at PND 9 were submitted to ultrasonic vocalization (USV) test, and at PND 30, under anesthesia, to in vivo unilateral microdialysis of the hippocampus. The samples of dialysate representing the basal level followed by 40 min pulse of 100 mM KCl were collected. The contralateral hippocampi were collected and homogenized. After derivatization of the amino acids with o-phtalaldehyde, the samples were submitted to HPLC analysis with a fluorescence detection. In the male, but not female rats a total content of Glu increased in the VPA and THAL groups, to 143% and 158%, respectively; Gln and GABA contents were also significantly elevated. Basal levels of these amino acids in dialysates of the hippocampi in the experimental groups generally did not differ from controls. During application of 100 mM KCl tendency to reduction of Gln concentration in dialysates and increase in GABA level were particularly noticeable in VPA-treated male rats. The results are consistent with a hypothesis on the role of Glu/GABA imbalance in the pathogenesis of autism and revealed differences between sexes in changes in the rat brain content of Glu and GABA in pharmacological autism models.

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Rat compression model of spinal cord injury with low spontaneous motor function recovery

Jana Fedorova, Jan Pavel, Katarina Bimbova, Maria Bacova

Institute of Neurobiology, Slovak Academy of Sciences, Kosice, Slovak Republic

jfedorova@saske.sk

The main aim of our experiments was to specify a standard compression model of traumatic spinal cord injury that offers reproducible results and causes slow spontaneous regeneration of motor hind limb function, which is extremely important for an appropriate possible therapeutic effect determination.

Adult Wistar female rats underwent a spinal cord compression at Th 9-10 level with 30 g, 40 g and 50 g weights lasting 15 minutes. We were testing their motoric hind limb function and monitored a persistence of their bladder function loss. After 4 weeks, we were determined the extent of damage in nervous tissue with the standard histological staining – and quantified by ImageJ program. The quantification of neurofilaments (NF), myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP) was carried out by western blot analysis and confirmed by immunofluorescent labeling.

The largest tissue lost were determined in the impact site and it was reduced to rostral and caudal direction. The rostro-caudal extent of tissue damage was dependent on compression force. The locomotor testing by BBB score demonstrated the slow spontaneous regeneration of hind limb function in all experimental animal group. Neurological function returns, both motoric and sensory also depended on compression force. The lowest amount of MBP, NF and GFAP was found in the epicenter of injury, what reflect a significant damage of axons and astrocytes. On the basis of behavioral testing and histological and biochemical analysis as the most appropriate model was selected 40g compression lasting 15 minutes.

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Analysis of WJ-MSC-secreted factors in response to inflammatory microenvironment *in vitro*

Anna Figiel-Dabrowska, Wioletta Lech, Marzena Zychowicz, Leonora Buzanska, Krystyna Domanska-Janik, Anna Sarnowska

Mossakowski Medical Research Centre, Polish Academy of Sciences, 5 Pawinskiego Street, 02-106 Warsaw, Poland

ania.dabrowska2703@gmail.com

Nowadays clinical application of mesenchymal stem cells (MSC), including Wharton's Jelly derived mesenchymal stem cells, seems to be a promising way of therapeutic intervention in different diseases with strong inflammatory component. Therapeutic effect of transplanted cells could be mainly explained by their innate paracrine regenerative and immunomodulatory properties. However, the extrinsic influence of the defined tissues environment to which cells would be injected may additionally effect these cell responses.

The aim of this study was to assess in vitro the impact of the inflammation-connected factors on secretory properties of WJ- MSC.

Our experiment based on reconstruction of the in vitro environment to which therapeutic cells (WJ-MSC) are usually transplanted. The inflammatory conditions that occur around the transplant were modeled by the increased concentration of TNF-α and IFN-γ. Physioxic, 5% oxygen concentration, 3-dimension transplant structure additionally reconstituted tissue- specific parameters.

Our experiments have shown specific changes in the pallet of WJ-MSC secreted cytokine induced in vitro by the inflammation-like surrounding. We have proved that modelled here environmental modification is able to change expression of IL-6, TGF-β1, VEGF-A, BDNF, GDNF, EGF, CNTF, bFGF genes and proteins in WJ-MSCs. Both extrinsic factors introduced in our experiments, the physioxia and growth of cells in 3–D structures enhanced cytoprotective properties of WJ-MSC.

The results of our experiments have confirmed sensitivity of WJ-MSC secretory profile to surrounding microenvironment in vitro and indicated optimization of 3-D transplant structure can protect and enhance therapeutic properties of MSC.

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The gut microbial peptidoglycan-induced innate immune response in the glia and neuroinflammation

Goutham K Ganjam[1,2], Anika Hunold[1], Wolfgang H. Oertel[2], Carsten Culmsee[1]

[1]Institute of Pharmacology and Clinical Pharmacy, University of Marburg, Germany
[2]Department of Neurology, University of Marburg, Germany
ganjam@staff.uni-marburg.de

A growing body of preclinical investigations discovered bidirectional communication between the brain and the gut microbiome to maintain health. Dysbiosis of gut microbiome may result in a pathophysiological role in human brain disease including neurodegeneration, autism and metabolic syndrome. Increased intestinal permeability as a result of gut dysbiosis allows microbial cell wall products to enter the bloodstream causing endotoxemia and systemic inflammation. Although gut dysbiosis compromises the brain function, the underlying molecular mechanisms of bacterial peptidoglycan-induced inflammation are sparse. Therefore, ''the aim of the study is to investigate the molecular pathways contributing an inflammatory response to bacterial peptidoglycan muramyl dipeptide (MDP) in the brain, especially, primary mouse astrocytes and microglial cells''. Recognition of microorganisms or their products by pattern-recognition receptors (PRR) is considered as the primary component of innate immunity. Nucleotide binding oligomerization domain 1/2 (NOD1/2)-like receptors (NLRs) and toll-like receptors (TLRs) belong to such innate immune PRRs. Glial cells exposed to NOD2 specific ligand MDP augmented the production of pro-inflammatory cytokines TNF alpha, IL-1 beta, and IL-6 by several folds in the presence of TLR homologs. Likewise, the morphology of primary microglial cells demonstrated a shift from resting stage to pro-inflammatory M1 polarization phenotype. Gene expression analysis identified upregulation of NOD2 signaling components and NF-kB luciferase reporter gene by several-fold upon stimulation. Real-time analysis of bioenergetic profiles using Seahorse demonstrates increased glycolytic flux in polarized microglia without affecting mitochondrial oxygen consumption rate. These results establish that glial NOD2 innate immune signaling is regulated by bacterial peptidoglycans promoting CNS inflammation.

Kynurenic acid as a dietary supplement – does it affect memory in adult rats?

Kinga Gaweł[1], Tomasz Kocki[1], Marcin Dziedzic[2], Agnieszka Pławska[1], Magdalena Kozłowska[1], Jolanta H. Kotlińska[3], Jacek Kiś[4], Waldemar A. Turski[1]

[1]Department of Experimental and Clinical Pharmacology, Medical University, Jaczewskiego 8b, 20-090 Lublin, Poland
[2]Department of Laboratory Diagnostic, Medical University, Chodzki 1, 20-093 Lublin, Poland
[3]Department of Pharmacology and Pharmacodynamics, Medical University, Chodzki 4a, 20-093 Lublin, Poland
[4]Department of Department of Human Anatomy, Medical University, Jaczewskiego 4, 20-090 Lublin, Poland
kingagawel@o2.pl

There are many literature data highlighting the role of kynurenic acid (KYNA) an antagonist at the glycine site of N-methyl-D-aspartate (NMDA) receptor and alpha-7 nicotinic acetylcholine (α7nACh) receptor, on learning and memory. KYNA is produced endogenously from tryptophan along kynurenine pathway. It can also be absorbed from the gastrointestinal tract. Noteworthy, high content of KYNA was found in popular foods, e.g. honey, broccoli and potatoes. Therefore, the aim of this project was to determine if chronic alimentary administration of KYNA may affect the memory in adulthood. Experiments were performed on male Wistar rats. KYNA (Sigma) was added to drinking water in concentration of 250 mg/l. Water was provided ad libitum starting immediately after birth and continuing until adulthood. The behavioral tests i.e. novel object recognition test (recognition memory), Barnes maze task (spatial learning, memory retrieval and cognitive flexibility) and passive avoidance test (contextual memory) were conducted beginning on postnatal day 49. Our study revealed that chronic administration of KYNA in drinking water did not affect recognition and contextual memory. Moreover, it did not induce spatial learning, spatial memory retrieval and cognitive flexibility disturbances in adult rats. Thus, obtained results indicate that chronic administration of KYNA in diet during the development may not affect learning and memory processes in adulthood.

SK channels at the ER-mitochondrial interface preserve neuronal survival in conditions of enhanced calcium transfer and impaired mitochondrial bioenergetics

Birgit Honrath[1,2], Inge E. Krabbendam[1], Carmen Ijsebaart[1], Valentina Pegoretti[1], Martina Schmidt[1], Carsten Culmsee[2], Amalia Dolga[1,2]

[1]Faculty of Science and Engineering, Department of Molecular Pharmacology, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands
[2]Institute of Pharmacology and Clinical Pharmacy, University of Marburg, Karl-von-Frisch-Str. 1, 35043 Marburg, Germany
b.honrath@rug.nl

The crosstalk between the endoplasmic reticulum (ER) and mitochondria facilitates calcium transfer between these organelles, thereby maintaining the driving force for calcium into the mitochondrial matrix to facilitate mitochondrial respiration and ATP production. However, a pathological increase in ER-mitochondrial coupling (EMC) induces mitochondrial calcium overload and mitochondrial damage which has been linked to the progression of neurodegenerative diseases. calcium-activated potassium (SK) channels attenuate neuronal cell death by preventing mitochondrial superoxide formation and mitochondrial calcium dysregulation, and are therefore a promising target for the treatment of neurodegenerative diseases.

In this study, we expressed genetically-encoded ER-mitochondrial linkers which heterodimerize to link ER and mitochondria upon addition of rapamycin in neuronal HT22 cells. We found that cell death induced by glutamate was potentiated in conditions of enhanced EMC, and both, SK channel activation, and enrichment of a mitochondria-targeted SK2 channel variant protected against glutamate toxicity. Using mitochondrial GFP-aequorin for calcium measurements, we show that EML formation indeed enhanced mitochondrial calcium uptake in response to calcium chloride stimulation. Further, we found that strengthening ER-mitochondrial connectivity impaired basal mitochondrial respiration and attenuated maximal uncoupled respiration as assessed by extracellular flux analysis. Together, our results indicate that mitochondrial SK channel activation was able to prevent toxicity induced by increased EMC which leads to dysfunctional calcium homeostasis and impaired respiration. These results highlight the importance of mitochondrial SK channel isoforms in protection against neuronal cell death through enhancing mitochondrial resilience.

Monitoring of global brain ischemia impact on circulating blood

Jana Jachová, Miroslav Gottlieb, Milina Matiášová, Miroslava Némethova, Petra Bonová

Institute of Neurobiology, Slovak Academy of Science, Kosice, Slovak Republic
jachova@saske.sk

The main mechanisms of cerebral ischemic injury include excitotoxicity, reactive oxygen species and inflammation, all together leading to serious brain tissue damage. In clinics, the rapid diagnostic of ischemia is important. For that reason, identifying of early stage of ischemia markers is extremely attractive. Because simply access to patient blood samples, we decided to monitor brain ischemia impact on the systemic level, changes of glutamate concentration and antioxidant enzymes activity respectively.

The global ischemia was induced by four vessel occlusion model. Impaired neurological functions caused by ischemia was evaluated by test – Morris water maze. The count of degenerating neurons was recorded by FluoroJade B staining in the CA1 area of hippocampus. The concentration of glutamate in the blood was measured by an enzymaticfluorimetric method. For the determination of enzyme SOD and CAT activity colorimetric based assays was used. Induction of the global ischemia caused an impairment of learning and reference memory compared with the control group. We recorded damage of the hippocampus also by FloroJade B staining, where we observed a significant neurodegeneration in the CA1 area.

During early phase of reperfusion we observed the increase glutamate concentration with gradual elevating in the following days. Activity of antioxidant enzymes was reduced shortly after the reperfusion.

Results of our experiments confirm a direct impact of the cerebral ischemia on the circulating blood. We found that the glutamate concentration in the blood could serve as a long term marker of brain ischemia, while reduced antioxidant enzyme activity better reflects early postischemic period.

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MitoQ abrogates BID-mediated mitochondrial damage in RSL3-induced ferroptosis

Anja Jelinek[1], Lukas Heyder[2], Michael Daude[2], Matthias Plessner[3], Sylvia Krippner[3], Robert Grosse[3], Wibke E Diederich[2], Carsten Culmsee[1]

[1]Institute of Pharmacology and Clinical Pharmacy, Biochemical-Pharmacological Center, Philipps-University Marburg, Karl-von-Frisch-Straße, 35043 Marburg, Germany
[2]Center for Tumor Biology and Immunology, ZTI, Philipps-University Marburg, Hans-Meerwein-Straße, 35043 Marburg, Germany
[3]Institute of Pharmacology, Faculty of Medicine, Biochemical-Pharmacological Center, Philipps-University Marburg, Karl-von-Frisch-Straße, 35043 Marburg, Germany

anja.jelinek@staff.uni-marburg.de

Neurodegenerative diseases involve oxidative stress in the underlying cell death mechanisms. Here, we sought to elucidate, whether ferroptosis by inhibition of GPX4 using RSL3 comprises mitochondrial death mechanisms. We investigated the effects of RSL3 in several cell lines (HT-22, MEF, Bid CRISPR/Cas9-KO cells) and in primary mouse neurons with a focus on mitochondrial parameters and analyzed the effects of BID inhibition, inhibitors of lipid peroxidation, mitochondria-targeted radical scavengers and iron chelators in ferroptosis induced by GPX4 inhibition.

1S, 3R-RSL3 mediated mitochondrial damage and cell death at nanomolar ranges whereas an inactive RSL3 variant lacking chlorine did not exert cytotoxicity. In Bid CRISPR/Cas9 KO cells, RSL3 was less toxic, suggesting that BID was involved in ferroptotic death. Further, the BID inhibitor BI-6c9 provided full protection in WT HT-22 and MEF cells against the RSL3 challenge with respect to cell death, lipid peroxide and mitochondrial ROS formation, mitochondrial membrane potential and ATP production. Liproxstatin-1, ferrostatin-1 and deferoxamine, and the mitochondria-targeted antioxidant MitoQ also sustained these parameters at control levels. In line with earlier studies implicating AIF to be involved in oxidative cell death by loss of GPX4, we found protection by AIF gene silencing.

In summary, we identified BID-mediated mitochondrial damage as a key feature of oxidative cell death induced by the GPX4 inhibitor RSL3. Therefore, BID inhibition and mitochondrial protection are promising therapeutic strategies in paradigms of oxidative neuronal cell death.

Deregulation of urea cycle enzymes in amyloid β precursor protein over-expressing PC12 cells and in sporadic Alzheimer's disease brain

Henryk Jęśko[1], Anna Wilkaniec[1], Magdalena Cieślik[1], Magdalena Gąssowska[1], Wojciech Hilgier[2], Walter J. Lukiw[3], Agata Adamczyk[1]

[1]Department of Cellular Signalling, Medical Research Centre, Polish Academy of Sciences, PL-02106 Warsaw, Poland
[2]Department of Neurotoxicology, Medical Research Centre, Polish Academy of Sciences, PL-02106 Warsaw, Poland
[3]Neuroscience Center of Excellence and Departments of Neurology and Ophthalmology, Louisiana State University Health Sciences Center, 2020 Gravier Street, New Orleans, LA 70112, USA

hjesko@imdik.pan.pl

Accumulating evidence suggests the implication of altered arginases and other urea cycle enzymes in the pathogenesis of Alzheimer's disease (AD). L-arginine is metabolized via arginase 1 (Arg1) or Arginase 2 (Arg2) to generate ornithine, whereas nitric oxide synthases (NOS) metabolize L-arginine to generate nitric oxide. Amyloid beta (Aβ), the product of Aβ precursor protein (AβPP) cleavage, plays a central role in AD. However, the impact of Aβ on the balance between arginases and NOS is unknown. Herein, we investigated the changes in arginases and other urea cycle enzymes in PC12 cells overexpressing human Aβ precursor protein – either wild-type (APPwt cells) or bearing double "Swedish" mutation (APPsw, K670M/N671L) as well as in post-mortem sporadic AD brains. We used real-time PCR, miRNA arrays and cluster analysis. Our data demonstrated that *Arg1* and *Arg2* as well as argininosuccinate synthase (*ASS*) that metabolizes citrulline was significantly down-regulated in both APPwt and APPsw cells. Argininosuccinate lyase (*ASL*) remained unchanged in APPwt cells while it was up-regulated in APPsw cells. Up-regulation of neuronal (*NNOS*) and endothelial (*ENOS*) nitric oxide synthase was observed in APPwt cells while only *NNOS* was increased in APPsw cells. The changes were found to follow closely those observed in the human hippocampal CA1 region of sporadic AD brains. Moreover, we have found elevated hsa-miRNA-9 and hsa-miRNA-128a in AD brains; their changes might modulate the expression of *ASS* and *NOS*, respectively. In conclusion, Aβ alters the balance between arginases and NOS that might sensitize cells to nitrosative stress.

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DNA damaging effect of para-methoxyamphetamine (PMA) and para-methoxymethamphetamine (PMMA) in the rat brain

Katarzyna Kamińska[1], Alexandra Jurczak[1], Karolina Noworyta-Sokołowska[1], Grzegorz Kreiner[2], Anna Górska[1], Krystyna Gołembiowska[1]

[1]Department of Pharmacology, Institute of Pharmacology of the Polish Academy of Sciences, Smętna 12, 31-343, Kraków, Poland
[2]Department of Brain Biochemistry, Institute of Pharmacology of the Polish Academy of Sciences, Smętna 12, 31-343, Kraków, Poland

katkam@if-pan.krakow.pl

Designer drugs are synthetic compounds developed to provide similar effects to illicit drugs of abuse, which are not subjected to legal control. Although designer drugs still have the reputation of being safe, several experimental studies in animal models and humans indicated risks including life-threatening serotonin syndrome, hyperthermia, neurotoxicity, and abuse potential. Para-methoxyamphetamine (PMA) and para-methoxymethamphetamine (PMMA) are a stimulant and psychedelic drugs closely related to the amphetamine-class. PMMA is invariably always found in combination with PMA in "Ecstasy" pills, sometimes along with amphetamine, methamphetamine or ephedrine.

The aim of the present study was to investigate the effects of PMA and PMMA on oxidative damage of DNA in the rat brain.

The DNA damaging effect was examined with the alkaline comet assay technique.

It was found that PMA (5-10 mg/kg) and PMMA (5-10 mg/kg) produced single and double-strand DNA breaks in the rat cortex. The effect of chronic administration of PMA and PMMA on DNA damage was stronger than effect of PMA and PMMA given in single doses.

It is concluded that oxidative stress induced by PMA, PMMA results in overflow of DA and 5-HT and leads to neurotoxic changes in the cell bodies innervated by DA and 5-HT neuronal terminals.

The inhibitory effect of spice extracts on the formation of amyloid fibrils using trypsin in aqueous ethanol

Phanindra Babu Kasi[1], Márta Kotormán[1], Attila Borics[2], Titanilla Szögi[3], Lívia Fülöp[3]

[1]Department of Biochemistry and Molecular Biology, Faculty of Science and Informatics, University of Szeged, Középfasor 52, H-6726 Szeged, Hungary
[2]Laboratory of Chemical Biology, Biological Research Centre of Hungarian Academy of Sciences, Temesvári krt. 62, H-6726 Szeged, Hungary
[3]Department of Medical Chemistry, University of Szeged, Dóm t. 8, H-6720 Szeged, Hungary

phansi93@gmail.com

The formation of amyloid fibrils have been associated with several human diseases, including Alzheimer's and Parkinson's diseases, spongiform encephalopathy and type II diabetes. Amyloid formation might be a general property of the polypeptide backbone as all proteins can form long-unbranched, beta-sheet rich amyloid fibrils in vitro under appropriate conditions. Spices contain important bioactive compounds without undesirable side effects, which are necessary for the prevention and cure of various diseases. 52 Phenolic compounds were identified in culinary herbs and spices. The aromatic rings of polyphenols may competitively interact with aromatic residues in amyloidogenic proteins, prevent the p-p interaction, and block the self-assembly process. The phenolic hydroxyls of polyphenols may inhibit amyloid fibril formation via binding the hydrophobic residues in amyloidogenic proteins. Here we report the inhibitory effect of some spice extracts on the formation of amyloid fibrils using trypsin as a model protein in 60 % ethanol at pH 7.0. Inhibition of aggregation and fibrillation of PMS-trypsin was determined using based on turbidity measurement, aggregation kinetics assay, amyloid specific dye Congo red (CR), electronic circular dicroism (ECD) and transmission electron microscopy (TEM). Chemical modification with PMSF inactivates trypsin irreversibly. Thus the autolysis of enzyme at pH 7.0 does not affect the results. The experiments revealed that the greatest anti-fibrillation activity was exerted by chili extract from all the spice extracts investigated. We demonstrated that chili extract significantly inhibits fibril formation as well as the inhibitory effect is dose dependent.

Postnatal development of the orbitofrontal cortex volume is not affected in the animal model of attention-deficit hyperactivity disorder (ADHD)

Małgorzata Kolenkiewicz[1], Anna Kozłowska[2], Maciej Równiak[3]

[1]Department of Pathophysiology, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, Warszawska Av, 30, 10-082 Olsztyn, Poland
[2]Department of Comparative Anatomy, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, pl. Łódzki 3, 10-727 Olsztyn, Poland
[3]Department of Human Physiology, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, Warszawska Av, 30, 10-082 Olsztyn, Poland

malgorzata.kolenkiewicz@uwm.edu.pl

The prefrontal cortex (PC) is one of the structures implicated with the attention-deficit hyperactivity disorder (ADHD). Some data suggest that PC is very important in the control and organization of goal-directed behaviors or planning and selection of appropriate actions. Additionally, PC is composed by a few regions and one of them is the orbitofrontal cortex (OFC). In mammals, damage to OFC is characterized among others by disinhibition of motor behaviors, abnormalities in socio-emotional behaviors and spontaneity, what is very characteristic for ADHD. The aim of this study was to compare volumes of the orbitofrontal cortex in the spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY).

The areas of orbitofronal cortex were visualized on frozen sections using pan-neuronal marker and immunohistochemistry. Morphometric measurements were obtained manually and volumes of these parts were analyzed using Cavalieri method in 4-10-week old SHR rats (animal model of ADHD) and WKY rats used as control animals.

The results show that the volumes of orbitofronal cortex are insignificantly larger in WKY than SHR rats at all developmental stages. There are also no significant volumetric defences in WKY and SHR rats as far as right and left hemispheres are concerned.

In conclusion, present results indicate that development of the orbitofronal cortex is not affected in individuals with ADHD, thus most probably sources of ADHD symptoms are abnormalities in the neurotransmission systems.

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Volumetric brain differences during postnatal development in the animal model of attention-deficit hyperactivity disorder (ADHD) and hypertension

Anna Kozłowska[1], Maciej Równiak[2], Małgorzata Kolenkiewicz[3]

[1]Department of Human Physiology, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, Warszawska Av, 30, 10-082 Olsztyn, Poland
[2]Department of Comparative Anatomy, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, pl. Łódzki 3, 10-727 Olsztyn, Poland
[3]Department of Pathophysiology, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, Warszawska Av, 30, 10-082 Olsztyn, Poland

kozlowska.anna@uwm.edu.pl

Spontaneously hypertensive rats (SHR) are animal model of and attention-deficit hyperactivity disorder (ADHD) and hypertension. Both these syndromes develop in juvenile animals. ADHD, which is characterised by hyperactivity and inattention develops at 4th week of life while hypertension, which is characterised by enlarged cerebral ventricles starts later at 5th-6th week of life. These observations suggested that there may be morphological abnormalities in SHR rats in many brain areas.

To test this hypothesis the volumes of various parts of the brain were analyzed using Cavalieri method in 4-10-week old SHR and Wistar Kyoto (WKY) rats used as control animals. Morphometric measurements were manually obtained on the frozen sections stained by immunohistochemistry.

The results show that from 5th week of life at all stages studied, the brain, both hemispheres, brainstem and cerebellum were smaller in SHR rats when compared to the WKY strain. However, the most pronounced size differences were observed since 7th week of life. On the other hand, from 6th week of life the ventricles were approximately two-fold larger in SHR than WKY rats, suggesting that there is less brain tissue in SHR than in WKY rats.

In conclusion, present results indicate that during the period of ADHD development there are volumetric abnormalities in brain regions studied, however, these abnormalities are even more evident from 7th week of life, when hypertension develops. Thus, both ADHD and hypertension may be the factors of various volumetric abnormalities observed in the brain of SHR rats.

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Ischemia/reperfusion-induced translocation of protein kinase C beta II to mitochondria results in phosphorylation of respiratory complex I subunit

Olga Krupska[1], Ewa Sitkiewicz[2], Łukasz Charzewski[3], Krystiana Krzyśko[3], Barbara Zabłocka[1], Małgorzata Beręsewicz[1]

[1]Molecular Biology Unit, Mossakowski Medical Research Centre, PAS, Warsaw, Poland
[2]Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland
[3]Department of Physics, University of Warsaw, Warsaw, Poland

mberesewicz@imdik.pan.pl

Currently, using gerbil model of cerebral ischemia, we focus on understanding the role of the different isoforms of protein kinase C (PKC) associated with mitochondria in the processes leading to endogenous neuroprotection. In this model, cerebral ischemia results in selective death of pyramidal cells in hippocampal CA1 region, while the adjacent region CA2-4, DG remains relatively resistant. Using this model, we showed that ischemia/reperfusion (I/R) injury results in translocation of protein kinase C beta isoform II (PKC beta II) from cytoplasm to mitochondria but only in CA2-4, DG. We claim that described translocation is responsible for endogenous neuroprotection in this region. However, the exact mechanism(s) underlying PKC beta II-induced neuroprotection remain(s) unknown. Thus, we hypothesized that the I/R-induced translocation of PKC beta II likely results in phosphorylation-dependent activation/inhibition of specific mitochondrial proteins what in turn guarantees neuroprotection by modifying mitochondrial function.

Using pull down method followed by mass spectrometry, we identified NDUFS1, the 75kDa subunit of respiratory complex I, as a potential PKC beta II partner. This protein-protein interaction was confirmed by co-immunoprecipitation method as well as by proximity ligation assay. Moreover, in silico analysis revealed that NDUFS1 due to its amino acid linear sequence could be potentially phosphorylated by PKC beta II. Preliminary in vitro data demonstrate that PKC beta II specifically phosphorylates NDUFS1.

On this basis, we speculate that PKC beta II-mediated phosphorylation of NDUFS1 might be involved in PKC beta II-induced neuroprotection. The contribution of NDUFS1 in this process likely preserves mitochondrial function and attenuates production of reactive oxygen species during I/R.

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Biomimetic microenvironment for preconditioning of human Wharton's Jelly Mesenchymal Stem Cells

Wioletta Lech, Marzena Zychowicz, Krystyna Domanska-Janik, Leonora Buzanska

Stem Cell Bioengineering Unit, Mossakowski Medical Research Centre, Polish Academy of Sciences, 5 Pawinskiego Street, 02-106 Warsaw, Poland

lech.wioletta@gmail.com

The natural stem cells microenvironment determines their survival and further differentiation. In order to provide biomimetic microenvironment in vitro for preconditioning of human WJ-MSC, similar to endogenous oxygen level and 3D structures were applied.

The aim of this study was to test the effect of different oxygen concentration and 3D scaffolds on WJ-MSC proliferation, viability, and gene expression profile.

WJ-MSC isolated from human umbilical cords were cultured under 21% O_2 and 5% O_2 as well as 2D and 3D conditions. The samples were characterized with qRT-PCR for the expression of cytokines and proliferation rate of cell cultures was estimated. In WJ-MSC grown in 3D structures the live/dead cells presence were analyzed.

The results have indicated that different microenvironment conditions affect analyzed stem cells properties. In tested scaffolds the increased expression of several cytokines, e.g. BDNF, NGF was observed.

The WJ-MSC proliferation rate revealed rising tendency in both 2D and 3D conditions, however it was significantly higher under 5% O_2. The scaffold type did not make significant difference in proliferation rate at 5% O_2, while in 21% O_2 diverse cell response was observed.

The analysis of scaffolds for live/dead cells indicated the presence of live cells throughout the experiment. We have observed cells growing protrusions and interconnecting to build up a network.

Our results suggests that optimizing and standardizing conditions for generation of therapeutically competent cell population should involve 3D culture and adjusting oxygen level to physiological normoxia.

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Transfection with liver-type glutaminase (GAB) enhances sensitivity of human glioblastoma cell lines to hydrogen peroxide-induced oxidative stress

Ewelina Majewska[1], Monika Szeliga[1], Javier Marquez[2], Jan Albrecht[1]

[1]Department of Neurotoxicology, Mossakowski Medical Research Centre, Polish Academy of Sciences, 5 Pawinskiego Street, 02-106 Warsaw, Poland
[2]Department of Molecular Biology and Biochemistry, Faculty of Sciences, Campus de Teatinos, University of Malaga, 29071 Malaga, Spain

emajewska@imdik.pan.pl

Glutamine (Gln) plays a crucial role in the metabolism of tumors of different origin including glioblastoma (GBM). Deregulated expression/activity of glutaminase (GA, EC 3.5.1.2), an enzyme converting Gln to glutamate (Glu) and ammonia is a characteristic feature of many cancer cell lines and tumors. GA is encoded by two genes: GLS encoding kidney-type isoforms (KGA and GAC) and GLS2 encoding liver-type isoforms (GAB and LGA). Kidney-type isoforms are correlated with high rate of cell proliferation, whereas expression of liver-type isoforms is characteristic for resting or quiescent cells. In GBM GLS expression is abundant, while GLS2 transcripts are hardly detectable. Transfection of human GBM T98G cells with GAB sequence decreased their survival, proliferation, migration ability and sensitized them to damage caused by hydrogen peroxide. Here we assessed the influence of transfection with GAB on the sensitivity of other human GBM cell lines: U87MG, U251MG and LN229 to hydrogen peroxide measuring cell viability using the MTT assay.

GAB transfection significantly increased the sensitivity to hydrogen peroxide in each of the cell lines, albeit the response differed from one cell line to the other. Studies on the sensitization mechanism are under way.

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Effects of the psychiatric risk gene Cacna1c on mitochondrial function in neuronal and glial cells

Susanne Michels[1], Goutham K. Ganjam[1], Helena Martins[2], Moria D. Braun[3], Theresa M. Kisko[3], Gerhard M. Schratt[2], Rainer K.W. Schwarting[3], Markus Wöhr[3], Holger Garn[4], Carsten Culmsee[1]

[1]Institute of Pharmacology and Clinical Pharmacy, Philipps-University, Karl-von-Frisch-Straße 1, 35043 Marburg, Germany
[2]Institute of Physiological Chemistry, Philipps-University, Karl-von-Frisch-Straße 1, 35043 Marburg, Germany
[3]Experimental and Biological Psychology, Philipps-University, Gutenbergstraße 18, 35037 Marburg, Germany
[4]Institute of Laboratory Medicine and Pathobiochemistry, Philipps-University, Hans-Meerwein-Straße 3, 35043 Marburg, Germany

susanne.michels@pharmazie.uni-marburg,de

To date, the pathophysiology of affective disorders is still largely unknown. Multiple lines of evidence indicate the involvement of neuroinflammation, oxidative stress and resulting mitochondrial dysfunction in the neurobiology of psychiatric diseases. Further, several genome wide association studies have identified CACNA1C, which codes for the alpha1C subunit of the L-type calcium channel (LTCC) Cav1.2, as one of the strongest genetic risk factors for affective disorders.

We investigated the effects of modified Cacna1c gene expression on glutamate-induced oxidative stress and LPS-stimulated neuroinflammation in HT22 cells and primary astrocytes/microglia, respectively. HT22 cells were transfected with Cacna1c siRNA or treated with the LTCC blocker nimodipine. The glial cells were obtained from neonatal Cacna1c+/– rats. Morphological alterations after stimulation were studied via impedance readout in all cell types. Mitochondrial respiration and bioenergetics were assessed using a Seahorse XFe96-Analyzer. Additional mitochondrial parameters, such as ROS formation, membrane potential, and Ca^{2+} levels, were analyzed in HT22 cells. In the supernatants of activated microglia nitric oxide and pro-inflammatory cytokine release were determined.

Overall, we found that reduced Cacna1c expression mediated neuroprotective effects at the level of mitochondria. We also revealed differences between the LPS-challenged heterozygous Cacna1c and wild-type glial cultures regarding morphological appearance, metabolic status, and secretion of inflammatory mediators.

These findings suggest that Cacna1c plays a role in neuroinflammatory and oxidative stress pathways with particular impact on mitochondrial integrity and function. The molecular mechanisms underlying the effects of Cacna1c regulation on mitochondrial performance and the link to the development of mood disorders remain to be elucidated.

Alterations in serum BDNF and catecholamines during exercise to volitional exhaustion - the influence of normobaric hypoxia and endurance training

Zofia Piotrowicz[1], Miłosz Czuba[1], Józef Langfort[1,3], Małgorzata Chalimoniuk[2]

[1]Department of Sports Theory, The Jerzy Kukuczka Academy of Physical Education, Katowice, Poland
[2]Department of Cellular Signalling, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland
[3]Department of Experimental Pharmacology, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland

zofia.piotrowicz@wp.pl

Both acute and chronic aerobic exercises increase circulating BDNF concentrations in sedentary subjects and in athletes. We wanted to know: 1) whether there is an association between exercise, peripheral BDNF and catecholamines levels during normobaric hypoxia, and 2) whether this phenomenon is modulated by state of training.

Ten healthy young males and 10 elite cyclist performed cycloergometer incremental exercise to volitional exhaustion (EVE) at normoxic and hypoxic conditions. The normobaric hypoxic conditions was suited to 2000 m (FiO_2 = 16,4%, H-2000), and 3000 m altitude (FiO_2 = 14.7 %, H-3000).

Basal serum level BDNF was low in elite endurance trained cyclists compared to matched-young no-trained men. A distinct responses of serum BDNF levels to EVE in sedentary young men, e.g. no changes or increased BDNF levels were observed in normoxic conditions whereas BDNF levels were elevated when EVE was performed in H-3000. In athletes no changes in serum BDNF during EVE was seen whilst in hypoxic condition serum BDNF was elevated.

In normoxic, H-2000 and H-3000 conditions, basal serum DA and ADR concentrations were lower cyclists compared to no-trained young men. EVE did not change DA levels whereas ADR levels were elevated in both group in response to EVE in normoxic condition. H-2000 and H-3000 conditions elevated level in serum DA and ADR after EVE test and during 30 min of recovery period.

The results of this study indicate that endurance training upregulates the serum BDNF level in hypoxic conditions probably by means of modulation of catecholamines metabolism.

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Antinociceptive effects of novel histamine H3R receptor antagonist in mouse model of neuropathic pain

Katarzyna Popiolek-Barczyk[1,2], Dorota Łażewska[1], Gniewomir Latacz[1], Wioletta Makuch[2], Ewelina Rojewska[2], Katarzyna Kieć-Kononowicz[1], Joanna Mika[2]

[1]Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Krakow, Poland
[2]Institute of Pharmacology, Polish Academy of Sciences, Department of Pain Pharmacology, Krakow, Poland

katarzyna.popiolek@gmail.com

A promising target for the development of new analgesics is histaminergic system, since expression of histamine H3R receptor has been reported in regions related with nociceptive transmission. The aim of our study was to examine the influence of single and/or repeated intraperitoneal (i.p.) and/or intrathecal (i.th) administration of newly synthesized H3R (E-98) antagonist on mechanical (von Frey) and thermal (cold plate) stimuli in mice following chronic constriction injury (CCI) of the sciatic nerve. Additionally, we evaluated the effects of H3R antagonist on morphine effectiveness. At the spinal cord level we analyzed the changes in immunological factors (interleukins, chemokines), receptors (H3R, MOP) and glial markers (Iba1, GFAP) after repeated E-98 administration. We revealed that E-98 time-dependently attenuated nociceptive responses after single intraperitoneal (1, 5, 10, 20, 40, 60 mg/kg), as well as intrathecal (30 µg/ 5 µl) injections. Moreover, single injection of E-98 (10 mg/kg; i.p.) significantly potentiated morphine (5 mg/kg; i.p.) analgesia at day 7^{th} after nerve injury. Our experiments demonstrated that when injected chronically E-98 also showed analgesic action as measured at day 3^{rd} and 6^{th} after injury. We investigated that those antinociceptive effects were correlated with spinal changes in neuro-immune factors balance. Our work revealed a therapeutic utility for new H3R (E-98) antagonist during neuropathy. Targeting H3R can potentiate morphine analgesia, which is in agreement with the multimodal pain therapy.

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Correlation between ceramides and lipid peroxidation marker 8-isoprostane in brain of rats treated with streptozotocin

Sławomir Prokopiuk[1], Anna Sadowska[1], Diana Sawicka[1], Małgorzata Żendzian-Piotrowska[3], Adrian Chabowski[2], Mateusz Maciejczyk[2], Kamil Bienias[1], Halina Car[1]

[1]Department of Experimental Pharmacology, Medical University of Bialystok, Szpitalna 37, 15-295 Bialystok, Poland
[2]Department of Physiology, Medical University of Bialystok, Mickiewicza 2c, 15-089 Bialystok, Poland
[3]Department of Hygiene and Epidemiology, Medical University of Bialystok, Mickiewicza 2c, 15-089 Bialystok, Poland

s_prokopiuk@o2.pl

Diabetes type 1 is characterized by insulin insufficiency and increased glucose level, which disturb brain activity leading to cognitive dysfunctions or/and neurodegeneration. Brain structures are differently susceptible to high levels of glucose in diabetes type 1. One of the factors responsible for alterations of the central nervous system in diabetes type 1 are ceramides. There are several reports suggesting that ceramides may also be important players in diabetes. Our previous study showed increased ceramide levels in the hippocampus and prefrontal cortex of rats with diabetes type 1 induced by streptozotocin (STZ). Additionally, lipid peroxidation plays significant role in the pathogenesis of diabetes type 1. Increased levels of 8-isoprostane – major peroxidation product, and modified antioxidant ability are important in leading to disturbances during diabetes. Many studies have shown a relationship between diabetes and increased levels of 8-isoprostane in the brain.

Streptozotocin rat model of diabetes type 1 was applied. Gas-liquid and thin-layer chromatography were used to measure the level of ceramides. Lipid peroxidation parameter (8-isoprostane) was determined colorimetrically using commercial kits.

Two weeks after STZ administration, lowered amount of ceramides in hippocampus and enhanced level of ceramides in prefrontal cortex were noted. In both brain structures lipid peroxidation parametr – 8-isoprostane was upregulated. Lipid peroxidation marker correlated positively with the level of ceramides in selected structures.

Ceramide concentrations correlate with lipid peroxidation parameter in hippocampus and prefrontal cortex of rats with experimental model of diabetes type 1.

Profiling the gene expression alterations following minocycline administration in a rat model of neuropathic pain

Ewelina Rojewska[1], Anna Piotrowska-Murzyn[1], Michal Korostynski[2], Ryszard Przewlocki[2], Barbara Przewlocka[1], Joanna Mika[1]

[1]Department of Pain Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna Street, 31-343 Krakow, Poland
[2]Department of Molecular Neuropharmacology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna Street, 31-343 Krakow, Poland

ewelinarojewska@tlen.pl

Studies conducted in recent years have shown that multiple endogenous factors regulate neuropathic pain, and neuroimmune interactions play significant roles in this process. The molecular mechanisms underlying neuropathic pain are constantly being studied to create new opportunities to prevent or alleviate neuropathic pain. The aim of our study was to determine gene expression changes induced by chronic constriction injury (CCI) that are modulated by minocycline, an inhibitor of microglia activation. Genes associated with minocycline efficacy in neuropathic pain should provide insight into identify novel therapeutic targets. We have performed screening for differentially expressed genes in the spinal cord in the rat CCI model. Out of 22,500 studied transcripts, abundance levels of 93 transcripts were altered following CCI. The percentage analysis revealed that 54 transcripts were not being affected by repeated administration of minocycline (30 mg/kg, i.p.), but expression of 39 transcripts was modulated following minocycline treatment. To further research we have selected one gene – Kmo. It remains unclear how Kmo inhibitors influence the development of neuropathic pain. Therefore, our studies were designed to address this issue. Using qRT-PCR, we assessed how chronic administration of minocycline influenced the nerve injury-induced increase in the Kmo mRNA levels in the spinal cord and in the DRG. Additionally we evaluated whether administration of the Kmo enzyme inhibitor Ro61-8048 influences pain development and the levels of glia-released pronociceptive factors in a rat model of neuropathy. Further studies are needed to elucidate the role of the individual genes and in therapeutic role of minocycline in neuropathy.

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Developmental abnormalities in the striatum volume in the juvenile spontaneously hypertensive rats (SHR)

Maciej Równiak[1], Małgorzata Kolenkiewicz[2], Anna Kozłowska[3]

[1]Department of Comparative Anatomy, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, pl. Łódzki 3, 10-727 Olsztyn, Poland
[2]Department of Pathophysiology, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, Warszawska Av 30, 10-082 Olsztyn, Poland
[3]Department of Human Physiology, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, Warszawska Av 30, 10-082 Olsztyn, Poland

mrowniak@uwm.edu.pl

Attention-deficit hyperactivity disorder (ADHD) is the most common childhood neuropsychiatric disorder which is characterized by motor hyperactivity, inattention and mental problems. Some neuroimaging studies indicated that the striatum volume is reduced in children with ADHD, however, there is no data which part of the striatum is reduced. Thus, we investigated volume differences of three parts of the striatum at various ages before puberty in SHR rats (animal model for ADHD) versus control strain, Wistar-Kyoto rats (WKY).

The volumes of parts of the striatum were compared using Cavalieri method in SHR and WKY rats at the ages from 4 to 10 weeks. Morphometric measurements of these regions were manually obtained on the frozen sections stained by immunohistochemistry.

The results show that mean striatum volumes are significantly smaller in the SHR rats at the age of 5, 6 and 7 weeks of life, when compared with their WKY counterparts. These size differences are mostly caused by bilaterally lowered volumes of the caudate-putamen complex (CPU) and globus pallidus (GP) in the SHR rats, as the volumes of the right and left nucleus accumbens (AC) are similar in both rat strains.

Concluding, present results indicate that the striatal volume abnormalities in the SHR rats are observed in the period when ADHD develops in these animals. As the volumes of both, input (CPU) and output (GP) stations of the striatum are reduced, both these factors may account for hyperactivity observed in SHR rats and patients with ADHD.

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Brain volume in Wilson's disease is correlated with neurological impairment and copper metabolism

Lukasz Smolinski[1], Tomasz Litwin[1], Barbara Redzia-Ogrodnik[2], Karolina Dziezyc[1], Anna Czlonkowska[1]

[1]Institute of Psychiatry and Neurology, 2nd Department of Neurology, Sobieskiego 9, 02-957, Warsaw, Poland
[2]Institute of Psychiatry and Neurology, Department of Radiology, Sobieskiego 9, 02-957, Warsaw, Poland

luke.smolinski@gmail.com

Brain atrophy is a prominent neuroimaging feature of Wilson's disease (WD). However, it is usually assessed qualitatively, and the relationship between quantitative measures of brain atrophy, neurological impairment, and copper metabolism in WD has not been investigated. Therefore, we aimed to address this issue.

We retrospectively analysed 47 newly diagnosed WD patients in whom brain MRI and copper metabolism studies were performed before treatment initiation. Neurological deficits were assessed on the Unified Wilson's Disease Rating Scale (UWDRS). Brain parenchymal fraction (BPF, i.e. brain volume normalized for intracranial volume) was calculated for each participant with Statistical Parametric Mapping software (v.12). Copper metabolism consisted of serum concentrations of ceruloplasmin, total copper, and non-ceruloplasmin bound copper (NCC). Statistical analysis included correlations between UWDRS, copper metabolism, and BPF.

UWDRS scores correlated significantly with BPF ($r = –0.631$, $p < 0.001$), and both UWDRS and BPF correlated with age at diagnosis ($r = 0.392$, $p = 0.006$ for UWDRS; $r = –0.690$, $p < 0.001$ for BPF). The relationship between BPF and UWDRS remained significant after controlling for age and gender ($r = –0.535$; $p < 0.001$). Moreover, after accounting for gender and age at diagnosis, both UWDRS and BPF correlated with the serum NCC concentration ($r = 0.285$; $p = 0.037$ for UWDRS; $r = –0.295$; $p = 0.032$ for BPF). Ceruloplasmin and total serum copper were not significantly related to UWDRS scores nor to BPF.

In conclusion, brain atrophy is related to neurological impairment in patients with WD and is associated with serum NCC concentration.

Inflammatory autoimmune disorders and lateralization in humans

Beata Sokołowska[1], Teresa Sadura-Sieklucka[2], Angelika Błażejewska[3], Krystyna Księżopolska-Orłowska[2]

[1]Bioinformatics Laboratory, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland
[2]Rehabilitation Clinic, Professor E. Reicher National Institute Geriatrics Rheumatology and Rehabilitation, Warsaw, Poland
[3]Faculty of Physics, University of Warsaw, Warsaw, Poland

beta.sokolowska@imdik.pan.pl

Better understanding of the immune and inflammation processes in neurodegenerative and other inflammatory disorders, such as rheumatoid arthritis (RA), is fundamental not only to the description of complex pathological mechanisms but also to the design of more optimal active and passive immunotherapy and/or physiotherapy. RA is a progressive inflammatory autoimmune disease with symmetrical articular and systemic effects. Although the cause of RA is not well known there are premises that molecular and genetic contributions are significant and complex. RA affects 0.5-1% population and 60% of the patients have wrist problems. Limitation of the hand function and change in its shape typically lead to partial withdrawal from social life and professional activity of the patients. That's why it is important to look for more effective rehabilitation/therapy procedures which could delay development of deformation and dysfunction. Wrist orthoses are recommended to patients with RA, but more popular are wrist splits in contrast to immobilization of orthoses. The purpose of using the first one is to protect the wrist activity of daily living. The second one reliefs pain and reduces signs of inflammation. Let's note that laterality in humans is important in manual activities, typically carried out with the right (dominant) hand, or with different contributions from the two hands. We study beneficial effects of rehabilitation on the hand function [Rheumatology 2016;54,6:285-290] and significant influence of the lateral preferences on the effects in the patients.

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The blocking of Rho-ROCK signalling pathway and/or combination therapy with chitosan implants support the overgrowth of damaged axons after spinal cord injury

Andrea Stropkovska, Katarina Bimbova, Nadezda Lukacova

Institute of Neurobiology, Slovak Academy of Sciences, Soltesovej 4-6, Kosice, Slovak Republic

stropkovska@saske.sk

Rho-ROCK signalling pathway play a role in the pathophysiology of spinal cord injury (SCI). Its activation triggers growth cone collapse leading to a significant barrier to axon regeneration, and the activation of cell death pathways.The aim of our study was to block RhoA–ROCK signalling pathway in rat SCI model (Th9 compression; 40g/15 min) using RhoA-kinase inhibitor (Y-27632), and to limit the development of cytoskeletal changes. To bridge the lesion, chitosan porous scaffold (ChPS) was implanted into the site of injury 14 days after SCI. The tissue sparing, overgrowth of axons and the expression of neurofilaments were identified along the cranio-caudal axis in 2 mm sections from the lesion. Eight weeks after SCI the lesion spread up to 8 mm. In addition, we noted extensive loss of neurofilament positive axons in all white mater columns. Our results show that the Y-27632 enhanced the plasticity and effectively promoted the overgrowth of axons. The implantation of ChPS bridged the lesion, but the GAP-43 or neurofilament positive axons were disorganized and were not able to transverse the lesion area. Treatment with Y-27632, or Y-27632 combined with ChPS decreased the extent of lesion, increased the expression of neurofilaments and significantly promoted the expression of growth associated protein (GAP-43) when compared with untreated SCI rats. Although a slight progress in recovery rate of the hindlimbs was seen at 1st and 2nd week after treatments, the difference in outcome between the groups was not significant at the end of survival period.

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Exposure to silver nanoparticles changes the properties of microvessels in adult rat brain

Grzegorz Sulkowski[1], Beata Dąbrowska-Bouta[1], Jolanta Orzelska-Górska[2], Małgorzata Frontczak-Baniewicz[3], Lidia Strużyńska[1]

[1]Laboratory of Pathoneurochemistry, Department of Neurochemistry, Mossakowski Medical Research Centre, Polish Academy of Sciences, 5 Pawińskiego str., 02-106 Warsaw, Poland
[2]Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, 4a Chodźki str., 20-093 Lublin, Poland
[3]Electron Microscopy Platform; Mossakowski Medical Research Centre, Polish Academy of Sciences, 5 Pawińskiego str., 02-106 Warsaw, Poland

gsulkowski@imdik.pan.pl

Neurotoxicity of silver nanoparticles has been confirmed in a lot of in vitro and in vivo studies using different experimental models. However, the mechanisms of the toxic action have not been fully clarified. Since nanoparticles have the ability to enter the brain and significantly accumulate in this organ, it is important to investigate their neurotoxic mechanisms.

In the current study we exposed adult rats to a low dose (0.2 mg/kg. b.w.) of small (10 nm) citrate-stabilized silver nanoparticles (AgNPs). We examined the effect of prolonged exposure on blood-brain barrier (BBB) ultrastructure and expression of tight junctions protein components as opposed to the ionic silver.

Administration of AgNPs over a two-week period resulted in changes in BBB ultrastructure and integrity. TEM analysis revealed accumulation of AgNPs inside endothelial cells of microvessels, mainly in lysosomes. Ultrastructural features of enhanced permeability of cerebral microvessels were observed such as enhanced activity of pinocytotic vesicular system and swollen perivascular astrocytic end-feets. This suggests uptake of fluid and its transfer to parenchyma which further results in perivascular edema. Additionally, we observed changes in the level of mRNA of the main tight junction proteins such as claudine, ocludine, and ZO1 as well as PDGF and its receptor PDGFbR which constitute the signaling pathway between endothelial cells and pericytes. All these characteristic protein components are responsible for the integrity of BBB.

The results of the current study demonstrate that exposure of adult rats to AgNPs induces BBB dysfunction leading to the enhanced permeability of cerebral microvessels.

Impact of elastin-derived peptides (EDPs) on matrix metalloproteinases -2, -9 (MMPs-2, -9) mRNA expression in mouse astrocytes in vitro

Konrad A. Szychowski, Marcin L. Leja, Urszula E. Binduga, Jakub Tobiasz, Jan Gmiński

Department of Public Health, Dietetics and Lifestyle Disorders, Faculty of Medicine, University of Information Technology and Management in Rzeszow, Sucharskiego 2, Rzeszow 35-225, Poland

konrad.szychowski@gmail.com

Elastin is an essential protein of mammalian organisms, which provides elasticity to many connective tissues. Degradation products of elastin, elastin-derived peptides (EDPs) are involved in various physiological and pathological processes. EDPs are detectable in cerebrospinal fluid of both group of healthy subjects and patients with ischemic and hemorrhage stroke. Matrix metalloproteinases (MMPs) are involved in various physiological as well as pathological processes and are engaged in remodeling and degradation of basement membrane and compounds of extracellular connective tissue matrix. Furthermore, MMPs are involved in healing process after stroke. Therefore, the aim of this research was to investigate the impact of elastin-derived peptide (VGVAPG) on mRNA expression of metalloproteinases-2, -9 (MMPs -2,-9) in mouse astrocytes in vitro. The cultures of cortical astrocytes were prepared from Swiss mouse embryos on 17/18 days of gestation. The cells were cultured in phenol red-free DMEM/F12 medium supplemented with 10% FBS and were exposed to 50 nM, 1 or 50 μM of VGVAPG peptide for 3 and 6 h. Afterwards, mRNA was collected and gene expression was measured by qPCR method. The results showed that after 3 h of exposure to studied peptide gene expression was not changed. However, after 6 h of exposure to VGVAPG peptide, expression of both studied MMPs-2, -9 in concentration 1 and 50 μM, significantly decreased. To conclude, VGVAPG decreased mRNA expression of both MMPs-2, -9 in mouse cortical astrocytes in vitro, which suggests initiation of healing process.

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Alterations in genes expression of sphingolipids metabolism enzymes in animal model of Alzheimer's disease

Przemysław L. Wencel[1], Henryk Jesko[2], Robert P. Strosznajder[1]

[1]Laboratory of Preclinical Research and Environmental Agents, Department of Neurosurgery, Mossakowski Medical Research Centre, Polish Academy of Sciences, PL-02106 Warsaw, Poland
[2]Department of Cellular Signaling, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland
pwencel@imdik.pan.pl

Bioactive sphingolipids are suggested to be involved in regulation of neurotransmission, signal transduction, cell survival and death. Imbalance between sphingolipids such as ceramide and sphingosine-1-phosphate (S1P) can promote generation of the amyloid peptides and oxidative stress, leading to neuronal death. In this study we evaluated transcription of genes involved in regulation of sphingolipids metabolism in animal model of AD.

3, 6 and 12 month old (3M, 6M, 12M) FVB/APP+ transgenic mice (Tg) with London APP mutation were used in this study. Mice without mutation (APP-) were used as a control. Brain cortex was isolated and biochemical and qPCR methods were applied.

Our results indicate several significant changes of sphingolipids metabolism enzymes gene expression in Tg mice comparing to appropriate control. It was found that alkaline ceramidase (Acer3) gene expression was downregulated in 3M and 12M Tg mice. Also, gene expression of ceramide synthase (Cers4) was significantly reduced in 3M and 6M Tg mice. We have also observed significant decrease in gene expression of ceramide kinase (Cerk) in 12M Tg mice. The expression of both sphingosine kinases: Sphk1 and Sphk2, was downregulated in APP+ 12M mice. Moreover, downregulation of Sphk1 gene expression was observed in 6M Tg mice. Concomitantly, we have also observed decreased expression of S1P receptor 1 (S1pr1) in APP+ mice.

Our data indicated significant alterations of genes expression involved in regulation of homeostasis between bioactive lipids: ceramide and S1P in animal AD model. Observed changes of Cers, Acer, Cerk and Sphks transcription may have important implications in cells' signaling and their neuronal fate.

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The effect of FTY720 on genes expression of sphingolipids metabolism enzymes in brain cortex of Alzheimer's transgenic mice

Przemysław L. Wencel[1], Henryk Jesko[2], Robert P Strosznajder[1]

[1]Laboratory of Preclinical Research and Environmental Agents, Department of Neurosurgery, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland
[2]Department of Cellular Signaling, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland
pwencel@imdik.pan.pl

Alterations of sphingolipids, the main components of lipid rafts, play an important role in Alzheimer's disease (AD) pathology. Bioactive sphingolipids such as ceramides and sphingosine-1-phosphate (S1P) plays crucial role in regulation of cells survival and death. In this study we examined the influence of S1P receptors modulator (FTY720) on expression of genes involved in sphingolipid metabolism in animal model of AD.

3, 6 and 12 month old (3M, 6M, 12M) FVB/APP+ transgenic (Tg) mice with London APP mutation were used in this study. Mice without mutation (APP-) were used as a control. Animals received i.p. injections of FTY720 (1 mg/kg b.w.) for 2 weeks. Brain cortex was isolated and biochemical and qPCR methods were applied.

Our results indicate that in all Tg age groups FTY720 upregulates gene expression of alkaline ceramidase (Acer3)-the enzyme which regulate degradation of ceramide. Simultaneously similar changes were observed in gene expression of enzyme which synthesizes ceramide – ceramide synthase-4 (Cers4). FTY720 concomitantly activates transcription of ceramide kinase (Cerk) in 12M APP+ mice. Moreover, the injection of FTY720 significantly upregulates gene expression of sphingosine kinases: Sphk1 and Sphk2 in 6M and 12M Tg animals. Finally, FTY720 elevates expression of S1P receptor 1 (S1pr1) in 6M Tg mice with a tendency to increase it in the other Tg groups.

Summarizing, FTY720 exerts significant effects on the expression of genes related to sphingolipids metabolism and its neuroprotective effect may be connected with activation of transcription of Sphk1, Sphk2 and Cerk genes. The enhancement of expression of these kinases may maintain the levels of S1P the important signaling molecule and regulator of memory processes.

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Dexamethasone differentially modulates the cell surface sialylation pattern and immune Siglec-F receptor binding in immunogenic and moderate immunogenic glioma cells

Przemysław Wielgat[1], Robert Czarnomysy[2], Adam Hołownia[3], Halina Car[1]

[1]Department of Clinical Pharmacology, Medical University of Bialystok, Waszyngtona 15A, 15-274 Białystok, Poland
[2]Department of Synthesis and Technology of Drugs, Medical University of Bialystok, Kilińskiego 1, 15-089 Białystok, Poland
[3]Department of Pharmacology, Medical University of Bialystok, Mickiewicza 2C, 15-222 Białystok, Poland
pwielgat@gmail.com

Glucocorticosteroids, including dexamethasone, are commonly used to control tumor-induced edema in brain cancer patients. They are also known as potent modulators of adhesive and antigenic properties of mammalian cell membranes that are crucial for basic processes such as immune recognition, cell growth and proliferation as well as apoptosis. There are increasing evidences that crosstalk between glioma and immune cells results in inhibiting immune function and promotion of glioma invasion. Sialic acid-binding lectins (Siglecs) belong to immunoglobulin superfamily that recognize terminal sialic acid residues of glycoproteins and thereby mediate immunosuppressive signaling by inhibitory ITIM motifs. Siglec-F is a CD33-related siglec that recognizes and binds to α2,3-, α2,6- and weakly α2,8-linked sialic acids. In this study we analyzed the effect of dexamethasone on cell surface sialylation pattern and recognition of sialylated structures by Siglec-F receptor in moderate immunogenic GL261 glioma cells and immunogenic SMA560 glioma cells. Detection and differentiation of sialic acids was analyzed by western blot and flow cytometry using specific plant lectins (MAA and SNA) and anti-PSA-NCAM antibody. We found dose-dependent effect of dexamethasone on α2,6- and α2,8-, but not α2,3-linked sialic acids, in GL261 and SMA560 glioma cells. Flow cytometry analysis showed that dexamethasone significantly decreased binding of Siglec-F protein to the immunogenic SMA560 cells, but not GL261 cells, in dose-dependent manner. Our data suggest that glucocorticosteroid-induced alterations in cell surface sialylation pattern and recognition of Siglec receptors family may mediate glioma-promoting function of immune cells and give new view on corticosteroid therapy in brain cancer patients.

Interleukin 6 deficiency improves long-term spatial memory in young adult mice

Maria M. Winnicka, Izabela Bialuk

Department of General and Experimental Pathology, Medical University of Białystok, Mickiewicza 2c, 15-222 Białystok, Poland
M.WIN@poczta.fm

Interleukin 6 (IL-6) is a cytokine constitutively expressed within the central nervous system. Increasing with age and in neurodegenerative disorders, such as Alzheimer disease, IL-6 expression indicates that it may be involved in pathomechanism of age and neurodegeneration related memory impairment.

The aim of this study was the evaluation of IL-6 involvement in long-term spatial memory formation in IL-6-deficient and control mice performed in Morris water maze (MWM). As all cognitive effects may be altered by motor activity and anxiety level, a day before MWM the locomotor and exploratory activities, as well as the anxiety level were evaluated in a holeboard test and in an elevated plus maze, respectively. The experiments were performed on twenty 4-month-old C57BL/6J IL-6-deficient and 20 age-matched male wild type mice.

While IL-6 deficiency did not change learning ability in tested animals, it significantly improved memory retention measured by escape latency time and number of crossings over the previous platform location evaluated 7 days after the last learning day. Because there were no differences between genotypes with regards to the locomotor and exploratory activities, as well as in the anxiety level, observed effect was memory specific.

These results indicate that in young adult mice the physiological level of IL-6 is involved in the myriad of learning and memory formation mechanisms, and that this cytokine plays a role as a negative regulator of cognitive processes. Therefore, IL-6 signaling may constitute a potential target for treatment of age and neurodegeneration related cognitive decline.

Analysis of polymorphisms of ABCB1/MDR1 gene in epilepsy patients

Katarzyna Wize[1,2], Urszula Łagan-Jędrzejczyk[2], Marta Kowalska[2], Jolanta Dorszewska[2], Wojciech Kozubski[3]

[1]Students Scientific Neurobiological Association, Poznan University of Medical Sciences, 49 Przybyszewskiego Street, 60-355 Poznan, Poland
[2]Laboratory of Neurobiology, Department of Neurology, Poznan University of Medical Sciences, 49 Przybyszewskiego Street, 60-355 Poznan, Poland
[3]Chair and Department of Neurology, Poznan University of Medical Sciences, Poznan University of Medical Sciences, 49 Przybyszewskiego Street, 60-355 Poznan, Poland

katarzynaw93@gmail.com

Epilepsy is one of the most common neurological disorder that affects 50 million people around the world. Epilepsy is characterized by the occurrence of seizures. It is considered as a chronic disorder, which requires long-lasting antiepileptic drugs. It is postulated that polymorphisms in ABCB1/MDR1 gene encoding glycoprotein P are one of the causes of in effectiveness in treating. The aim of the study was to analyse polymorphisms in ABCB1/MDR1 gene in epilepsy patients and control group. Epilepsy patients were divided into categories according to the type of seizures (focal or generalized ones) and also kind of used treatment (monotherapy and polytherapy).

39 patients were selected for this study and 38 individuals were used as a control group. G2677(A/T) polymorphism was analysed by HRM method, while C1236T and C3435T were examined by PCR-RFLP and electrophoresis. The obtained results were confirmed by sequencing.

The studies showed no difference in frequency of occurrence of G2677(A/T), C1236T and C3435T polymorphisms between the study group the controls. Futhermore, the first time described rare TA genotype of G2677(A/T) and previously described TT C3435T ABCB1/MDR1 gene were found only in epilepsy patients. There have been no evidence for association of analyzed polymorphisms with type of seizures. However, CT genotype of C1236T ABCB1/MDR1 ($p<0,05$) was more frequent in patients on AED monotheraphy.

The ABCB1/MDR1 gene polymorphisms analysis may bring new information into the pathogenesis of epilepsy and in the future may contribute to improving the effectiveness of treatment and improving the quality of patients' life.

Cardiovascular and respiratory effects of endomorphin-1 injection into the femoral vein of anaesthetized rats

Piotr Wojciechowski, Katarzyna Kaczyńska

Laboratory of Respiration Physiology, Mossakowski Medical Research Centre PAS, Pawińskiego 5, 02-106 Warsaw, Poland

pwojciechowski@imdik.pan.pl

Endomorphin-1 (EM-1) is an endogenous opioid tetrapeptide showing high mu opioid receptor selectivity and affinity. It is distributed among central and peripheral nervous system in the areas associated with central cardio-respiratory regulation. Systemic injection of this compound in anaesthetized rats elicits hypotensive responses. However, its effects on respiratory system in unconscious animals are focused primarily on the changes in the ventilation rather than in the shape of the respiratory pattern.

Urethan-chloralose anaesthetized male Wistar rats breathed spontaneously room air via tracheal tube and were treated with an intravenous injection of EM-1 (50 µg/kg) in control conditions, after bilateral dissection of cervical vagi nerves, and after pretreatment with peripherally active opioid receptor antagonist – naloxone methiodide (2 mg/kg).

Bolus injection of 50 µg/kg of endomorphin-1 into the femoral vein evoked apnoea. After the cessation of breathing tidal volume (VT) and respiratory frequency (F) were transiently diminished, then the following breathing was of increased VT and F of baseline values. The changes in VT and F affected minute ventilation (VE) resulting in a biphasic response, an ephemeral VE decrease after the apnoea followed by a transient VE increase. In intact rats EM-1 challenge evoked short-lived hypotension and bradycardia. Midcervical vagotomy and pre-challenge with naloxone methiodide reversed the cardio-respiratory effects of EM-1 injection.

Our experiments suggest that EM-1-induced arrest of breathing and its cardiovascular effects are a result of stimulation of µ-opioid receptors present within the lung vagi.

The effect of methamphetamine acute and chronic combination on DNA damage in the mouse cortex

Adam Wojtas[1], Anna Górska[1], Katarzyna Kamińska[1], Grzegorz Kreiner[2], Krystyna Gołembiowska[1]

[1]Department of Pharmacology, Institute of Pharmacology of the Polish Academy of Sciences, Smętna 12, 31-343 Kraków, Poland
[2]Department of Brain Biochemistry, Institute of Pharmacology of the Polish Academy of Sciences, Smętna 12, 31-343 Kraków, Poland

adam_wojtas@interia.pl

Methamphetamine (METH) is a strong central nervous system stimulant that is used as a recreational drug. In low doses, METH can elevate mood, concentration and energy. At higher doses it induced psychosis, aggressiveness, neurotoxicity, breakdown of skeletal muscle and seizures. METH is also known to have a very high addiction liability. The abuse of METH is growing problem worldwide since the popularity among young people attending private clubs.

Amphetamines block reverse transport of dopamine (DA) and serotonin (5-HT) and increase synaptic levels of monoamines. METH has high affinity at dopamine transporter (DAT), but negligible one at serotonin transporter (SERT). METH given chronically in animals potently increases DA release but its effect on 5-HT release in the brain is much weaker. METH blocks also vesicular monoamine transporter (VMAT2) and increases intracellular level of DA. Doses effects on DA release and storage induce generation of free radicals. Insufficient ability of brain antioxidant systems leads to development of oxidative stress. The aim of our study was to find out effect of acute and chronic treatment with METH (5 mg/kg) in the mouse brain cortex. The oxidative damage of DNA was assessed with the Comet Assay. It was found that METH produced marked damage of DNA with the effect more potent after chronic treatment. Obtained results may explain neurotoxic properties of this stimulant.

Total Glu, Gln and GABA content in the hippocampus in two rat models of autism: in vivo and ex vivo magnetic resonance study

Elzbieta Zieminska[1], Robert Filipkowski[1], Rafal Polowy[1], Jaroslaw Orzel[1], Beata Toczylowska[2], Michal Gorka[3], Jerzy W. Lazarewicz[1]

[1]Mossakowski Medical Research Centre, Polish Academy of Sciences, Pawinskiego 5, 02-106 Warsaw, Poland
[2]Nalecz Institute of Biocybernetics and Biomedical Engineering Polish Academy of Sciences, Ks. Trojdena 4, 02-109 Warsaw, Poland
[3]Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Zwirki & Wigury 93, 02-089 Warsaw, Poland

elziem@imdik.pan.pl

The disorders of the glutamatergic neurotransmission have been implicated in the pathogenesis of autism. In this study changes in the brain content of glutamate (Glu), glutamine (Gln) and GABA were evaluated in the rat models of pharmacologically-induced autism using Magnetic Resonance Spectroscopy (MRS) and Nuclear Magnetic Resonance (NMR). The rat females at the 11th day of gestation were fed with 800 mg/kg of valproic acid (VPA) or 500 mg/kg of thalidomide (THAL). The pups at PND 9 were submitted to ultrasonic vocalization (USV) test, and at PND 30, to MRS studies using the 7T Bruker BioSpec 70/30 Avance III system. Then the amino acids from homogenates of rat hippocampi were extracted for NMR studies using the HCl-Bligh and Dyer procedure. All NMR spectra were acquired on a Avance III HD 500MHz (Bruker) spectrometer. The results of USV tests showed that the pups prenatally treated with VPA, and to a greater extent with THAL, less frequently produced USV calls. MRS studies demonstrated increase by 20% in Glu content in the hippocampus of male rats from both, VPA- and THAL-treated groups. NMR studies showed gender-dependent differences in Glu content in VPA-group (by 36%) and THAL-group vs control (by 16%); increased level of Gln in males from both groups (by 47 and 74%) and increased level of GABA in male VPA-treated rats (by 86%). These results are consistent with a hypothesis on the role of the imbalance in excitatory glutamatergic vs inhibitory GABAergic neurotransmission in the pathogenesis of autism.

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