PHD MEETING IN TREST 2015

3rd to 5th November 2015

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CONTENT:

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Program

Tuesday 3.11.2015

8:30	***Departure***	
12:00-13:00	***Lunch***	
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Molecular and Cellular Physiology		
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13:35-14:05	Dept. 17	K. Bardova, M. Cerna, J. Hansikova, P. Kucharikova
14:05-14:20	Dept. 75	A. Dvorak
14:20-14:50	***Coffee break***	
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15:20-15:35	Dept. 16	B. Husekova
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17:20-17:30	Opening speech	Jan Kopecky
17:30-19:00	Poster session	
19:00	***Dinner***	

Wednesday 4.11.2015

8:00-9:00	***Breakfast***	
Neurobiology		
9:00-9:25	Dept. 15	P. Adamek, N. Kalynovska, P. Mrozkova
9:25-9:40	Dept. 14	P. Zimcik

9:40-10:00	Dept. 24	Z. Novosadova, L.Olejnikova
10:00-10:30	***Coffee break***	
10:30-11:05	Dept. 31	A. Hynkova, B. Krausova, M. Ladislav, K. Lichnerova, K. Skrenkova
11:05-12:00	Dept. 32	E. Antosova, I. Fajnerova, H. Hatalova, K. Holubova, M. Chvojkova, L. Kleteckova, A. Pistikova, I. Vojtechova, H. Buchtova
12:00-13:00	***Lunch***	
13:00-17:00	***Walk***	
16:30-17:00	***Coffee break***	
17:00-17:40	Dept. 33	P. Fabera, A. Posusta, P. Vlk, K. Vondrakova, J. Kudlacek
17:40-18:00	Dept. 35	M. Radova, J. Ziak
18:00-18:20	Dept. 34	S. Kortus, M. Levakova
19:00	***Dinner***	

Thursday 5.11.2015

8:00-9:00	***Breakfast***	
Cardiovascular Physiology		
9:00-9:15	Dept. 25	A. Vavrinova Louckova
9:15-9:30	Dept. 80	J. Hrdlicka
9:30-9:45	Dept. 81	B. Sankova
9:45-10:15	Lecture	Bohuslav Ostadal
10:15-10:45	***Coffee break***	
10:45-11:15	Lecture	Tomas Mracek
11:15-12:00	Ceremony - Results Announcement	
12:00-13:00	***Lunch***	
13:30	***Departure to Prague***	

List of departments

Abstracts

TRPM4 ION CHANNEL BINDS PIP2 AND PIP3

K. Bousova [1], M. Jirku [1], L. Bumba [2], J. Vondrasek [3], L. Bednarova [3], J. Teisinger [1]

[1] Department of Protein Structure, Institute of Physiology Academy of Sciences of the Czech Republic, [2] Institute of Microbiology Academy of Sciences of the Czech Republic, [3] Institute of Organic chemistry and Biochemistry Academy of Sciences of the Czech Republic

Transient receptor potential melastatin-4 (TRPM4) is a calcium-activated non-selective ion channel serving as monovalent ion transporter. Receptor plays a plethora of roles in cell sensors systems - participates in ongoing processes in neurons, cardiomyocytes, pancreas cells and T-cells. It has been proven link between defects in the TRPM4 receptor and progressive familial heart block type 1B. The regulation of most TRP channels is inter alia mediated by intracellular proteins and other signal molecules. The direct binding of phosphatidylinositol- 4,5 bisphosphate (PIP2), a minor phospholipid component of cell membranes, to TRP channels and its unique role in receptor modulation have been described previously [1]. We have utilized biochemical and molecular modelling methods to study the interactions of the proximal N-terminal region of TRPM4 with PIP2 and its homolog phosphatidylinositol- 3,4,5 trisphosphate (PIP3). Basic amino acid residues R755 and R767 were determined to be involved in the interaction with PIP2 and PIP3. This is a first report dealing with PIP2 and PIP3 binding at the N-terminus of the TRPM4 receptor. It can be assumed that any binding site for PIP2 is always also for PIP3. These findings provide new insight into the ligand binding domains of the TRPM4 channel.

[1] T. Rohacs, Phosphoinositide Regulation of TRP Channels, Handb Exp Pharmacol 223 (2014) 1143-76.

Supported by GACR 301/10/1159, GACR 207/11/0717 and GAUK 842313.

A NOVEL PHOSPHATIDYLINOSITOL-4,5,-BISPHOSPHATES BINDING SITE ON THE N-TERMINUS OF THE TRPM1 CHANNEL

M. Jirku [1,2], K. Bousova [1], L. Bumba [3], J. Vondrasek [4], L. Bednarova [4], J. Teisinger [1]

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Transient receptor potential melastatin 1 (TRPM1) channel belongs to the superfamily of ion channels that respond to various physiological stimuli like chemosensation, termosensation and mechanosensation. TRP channels have six transmembrane domains with a pore region between the fifth and the sixth segment. Cytosolic N-/C-tails are responsible for regulation of TRPs, which carry binding sites for signal molecules. (1,2) TRPM1 channel is ubiquitously expressed in most eukaryotic cells and is involved in many cellular processes like transduction of sensory signals and regulation of Ca^{2+} and Mg^{2+} homeostasis. Mutations in TRPM1 gene effect on cell cycle in human skin cells. It seems that a loss of TRPM1 in human melanocytes correlates with increased aggressiveness in melanoma and the homozygous loss of TRPM1 in bipolar cells in retina could be a possible explanation for congenital stationary night blindness in humans. (3,4)

We studied possible interactions between phosphatidylinositol-4,5-bisphosphate (PIP2) and the N-terminus (NT) of TRPM1. Using bioinformatic approach we identified PIP2 binding site in A451-N566 region. This domain contains several basic amino acids which can interact with anionic phospholipids. Alanine substitution mutagenesis screening revealed the crucial amino acids for these interactions. The equilibrium dissociation constants were estimated using surface plasmon resonance measurement. We identified PIP2-binding site and found mutations that decreased the affinity of TRPM1-NT/PIP2 interaction. Based on this results we concluded that basic residues play crucial role in TRP channels binding to PIP2. Moreover, we have provided the structural insight to TRPM1-NT/PIP2 interaction using computer ligand docking.

[1] Duncan L.M., Deeds J., Hunter J., Shao J., Holmgren L. M., Woolf E. A., Tepper R. I., Shyjan A. W. Cancer Res 58:1515-20,1998

[2] Holakovska B., Grycova L., Jirku M., Sulc M., Bumba L., Teisinger J. J Biol Chem 287:16645-55, 2012

[3] Hammock L., Cohen C., Carlson G., Murray D., Ross J. S., Sheehan C., Nazir T. M.,Carlson J. A. J Cutan Pathol 33: 599-607, 2006

[4] Nakamura M., Sanuki R., Yasuma T. R., Onishi A., Nishiguchi K. M., Koike C., Kadowaki M., Kondo M., Miyake Y., Furukawa T. Mol Vis 16 425-37, 2010

Supported by GAUK 238214 and GACR P304/12/G069 - Project of Excellence in the Field of Neuroscience.

MOLECULAR MECHANISM OF 14-3-3 PROTEIN DEPENDENT REGULATION OF CASPASE-2

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14-3-3 proteins are a family of conserved regulatory molecules that are expressed in all eukaryotic cells. They have the ability to specifically recognize and bind phosphoserine/phosphothreonine-containing as well as unphosphorylated motifs. Through these binding interactions 14-3-3 proteins serve as molecular chaperons that modulate the enzyme activity, the subcellular localization or the structure of multitude of functionally diverse signaling proteins. 14-3-3 proteins thus play an important role in crucial cellular processes such as regulation of signal transduction, apoptosis, cell cycle control, nutrient-sensing pathways or neurohormone synthesis regulation. The interactions between 14-3-3 proteins and their binding partners have to be strictly controlled.

Caspases are cysteine proteases activated during the programmed cell death - apoptosis. They cleave multiple proteins to allow the ordered dismantling of cells that are undergoing death. Caspases are present as inactive zymogens in healthy cells. Their activation or arresting in the inactive form is a key event in the initiation and execution of apoptosis. Caspase-2 is one of the first mammalian caspases discovered and the most evolutionarily conserved of the caspases. Caspase-2 promotes apoptosis induced by diverse stimuli. The control of caspase-2 activation and inhibition is caused by phosphorylation and the 14-3-3 protein binding. 14-3-3 protein binding to pro-caspase-2 prevents kinase maturation and consequently inhibits the apoptosis. This mechanism was described in *Xenopus laevis* oocytes and mouse eggs (1).

Because of the caspase-2 contribution to malignant cell survival, transformation and tumor development it is exciting to clarify the mechanism of 14-3-3 protein-mediated inhibition of caspase-2 in human. We have identified two key phosphorylation sites within the caspase-2 proenzyme whose phosphorylation is required for 14-3-3 protein/caspase-2 complex assembling. We would like to realize a biophysical characterization of this interaction.

[1] Nutt LK et al. Developmental Cell 16:856-866, 2009

Supported by GA ČR P207/11/0455 and 14-10061S.

STRUCTURAL STUDIES OF ASK1-TBD: TRX1 COMPLEX

Salome Kylarova[1,2], Dalibor Kosek[1,2], Tomas Obsil[1,2], Veronika Obsilova[1]

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Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family that activates the c-Jun N-terminal kinase (JNK) a p38 MAP kinase pathways, in response to diverse stresses. ASK1 activates apoptosis in various cells and thus it plays an important role in the development of cancer, cardiovascular or neurodegenerative diseases. ASK1 is regulated by many factors, which suppress its proapoptotic activity. Our main interest is the biophysical characterization of complexes with its key regulators such as 14-3-3 protein or thioredoxin (Trx). [1].

To better understand the role of Trx binding in the inhibition of ASK1, we performed structural characterization of the isolated Trx-binding region of ASK1 (ASK1-TBD) and its complex with reduced TRX1 using fluorescence spectroscopy, circular dichroism and small-angle X-ray scattering. It has been shown that ASK1-TBD is a compact monomeric and rigid domain that under reducing conditions forms with TRX1 a stable and well defined complex with 1:1 molar stoichiometry. We showed that the catalytic motif $W^{31}CGPC^{35}$ of TRX1 is essential for the interaction and TRX1 interacts with the region of ASK1-TBD located in the vicinity of Cys 250. Moreover, TRX1 binding does not induce significant structural change of ASK1-TBD [2].

To elucidate the role of cysteine residues of both proteins in the interaction under reducing and oxidative conditions we used analytical centrifugation and the spectrophotometric assay with Ellman´s reagent.

[1] Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, Ichijo H. EMBO J 17: S2596-2606, 1998

[2] Kosek D, **Kylarova S,** Psenakova K, Rezabkova L, Herman P, Vecer J, Obsilova V, Obsil T. J Biol Chem 279, S24463-24474, 2014

Supported by the Czech Science Foundation (Project 14-10061S) and The Czech Academy of Sciences (Research Projects RVO: 67985823 of the Institute of Physiology).

FUNCTIONAL ABLATION OF TMEM70 ALTERS BIOGENESIS OF ATP SYNTHASE AND LEADS TO EMBRYONAL LETHALITY IN MICE

J. Kovalcikova[1], M. Vrbacky[1], H. Nuskova[1], K. Chawengsaksophak[2], I. Beck[2], R. Sedlacek[2], P. Hozak[2], D. Sedmera[1], F. Papousek[1], J. Houstek[1]

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TMEM70 is a transmembrane protein localized in the inner mitochondrial membrane and involved in the biogenesis of the eukaryotic ATP synthase. *TMEM70* mutations cause isolated deficiency of ATP synthase often resulting in a fatal neonatal mitochondrial encephalocardiomyopathy, lactic acidosis and 3-methylglutaconic aciduria in patients.
To clarify the exact function of this factor, we generated *Tmem70* knockout mice by embryonic stem cell technology. While the heterozygous mice were viable and developmentally normal, the homozygous embryos were distinctly growth retarded and died during the embryonic development about 9.5 days *post coitum*. Confocal microscopy revealed delayed development of the cardiovascular system and electron microscopy indicated disturbed mitochondrial morphology in the homozygous when compared to the wild type embryos. Blue native electrophoresis demonstrated isolated defect of ATP synthase in the homozygous embryos with the content of fully assembled F_1Fo ATP synthase decreased to less than 20% of wild types. In contrast, comparison of the viable heterozygous and wild type mice aged 5 and 14 weeks did not show any significant differences in the heart and liver content of respiratory chain complexes, oxygen consumption, ATP synthase assembly and ATPase hydrolytic activity. On the other hand, we observed decreased fractional shortening, the parameter of the heart function, in heterozygous compared to wild type mice.
In conclusion, this first direct demonstration of the biological role of TMEM70 in experimental animals shows that *Tmem70* deficiency in the mouse has lethal consequences that are analogous to *TMEM70* dysfunction in humans.

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TISSUE- AND SPECIES-SPECIFIC DIFFERENCES IN CYTOCHROME C OXIDASE ASSEMBLY INDUCED BY SURF1 DEFECTS

N. Kovarova[1], P. Pecina[1], H. Nuskova[1], M. Vrbacky[1], M. Zeviani[2,3], T. Mracek[1], C. Viscomi[3], J. Houstek[1]

[1]Dept. of Bioenergetics, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic, [2]Molecular Neurogenetics Unit, Instituto Neurologico "C. Besta", Milan, Italy, [3]MRC-Mitochondrial Biology Unit, Wellcome Trust MRC Bldg, Addenbrookes Hospital Hills Rd, Cambridge, U.K.

In this study we focused on distinct biochemical phenotype of cytochrome *c* oxidase (COX) deficiency in mouse and humans due to the absence of SURF1 protein, an important ancillary factor of COX biogenes. While mutations inhuman *SURF1* gene lead to a fatal neurodegenerative mitochondrial disorder Leigh syndrome, *SURF1*$^{-/-}$ knockout in mouse results in surprisingly mild COX deficiency and no neurodegenerative changes [1, 2]. The aim of our study was to find out interspecies differences in the impaired process of COX biogenesis, from early assembly intermediates to formation of COX supercomplexes (SC) with other respiratory enzymes. This was achieved by investigating *SURF1*$^{-/-}$ mouse tissues and fibroblasts in comparison with patient fibroblasts lacking SURF1 protein due to *SURF1* gene mutations. Our study revealed considerably decreased COX monomer and COX activity in *SURF1* patient fibroblasts compared to *SURF1*$^{-/-}$ mouse tissues/fibroblasts. Assembled COX was present mainly in I-III$_2$-IV$_n$ SC in *SURF1* patient fibroblasts where the prominence of COX assembly defect was also apparent from high accumulation of COX assembly intermediates. In contrast, *SURF1*$^{-/-}$ mouse tissues/fibroblasts showed much lower accumulation of COX assembly intermediates and very low amount of I-III$_2$-IV$_n$ COX SC. We subsequently characterized kinetics of COX biogenesis in *SURF1* patient and *SURF1*$^{-/-}$ mouse fibroblasts by doxycycline reversible arrest of mitochondrial translation and ^{35}S-labeling of mtDNA encoded proteins. Doxycycline inhibition and gradual recovery to steady state revealed rather stable proportion between COX monomer and SC in human control cells, while in *SURF1* patient cells COX monomer markedly decreased and formation of SC was preferred. In *SURF1*$^{+/+}$ and *SURF1*$^{-/-}$ mouse cells, however, the recovery proceeded mainly to the level of COX monomer. Pulse-chase metabolic labeling clearly showed higher stability of COX monomer and faster degradation/depletion of accumulated COX assembly intermediates in *SURF1*$^{-/-}$ mouse fibroblasts, while more persistent COX assembly intermediates prevailed over the gradually decreasing signal of COX monomer in *SURF1* patient cells. Our experiments demonstrate crucial importance of the SURF1 protein for effective COX biogenesis in human cells, whereas its absence seems better tolerated in mouse cells and tissues.

[1] Kovarova N, Cizkova Vrbacka A, Pecina P, Stranecky V, Pronicka E, Kmoch S, Houstek J. Biochim Biophys Acta 1822(7): 1114-1124, 2012

[2] Dell'Agnello C, Leo S, Agostino A, Szabadkai G, Tiveron C, Zulian A, Prelle A, Roubertoux P, Rizzuto R, Zeviani M. Hum Mol Genet 16(4): 431-444, 2007

Supported by the Grant Agency of the Czech Republic (14-36804G), Ministry of Education, Youth and Sports of the Czech Republic (ERC CZ: LL1204, RVO:67985823), the Grant Agency of the Ministry of Health of the Czech Republic (NT12370-5) and ERC Advanced Grant FP7-322424.

CENRAL CHOLINERGIC SYSTEM DECLINE IN APPSWE/PS1DE9 MICE MODEL IS NOT CAUSED BY LOWER MRNA EXPRESSION

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Institute of Physiology CAS, Department of Neurochemistry

Pathology of Alzheimer disease (AD) is closely connected with memory loss and decline of cognitive function. Amyloid plaques and neurofibrillary tangles are major pathological hallmarks found in advanced stage of AD patiens' brains. Main component of amyloid plaques are β-amyloid (Aβ) fragments which in not fully understood way triger and drive progression of AD. Double transgenic APPswe/PS1dE9 mice tribe is characterized by overproduction of Aβ and amyloid plaques forming in early stage of live, thus provides sensible *in-vivo* AD animal model. We have previously informed about significant decrease of presynaptic (vesicular acetylcholine transporter and choline acetyltransferase) and postsynaptic (muscarinic receptors) cholinergic proteins and weakening of their functions (1, 2, 3) in APPswe/PS1dE9 mice central nervous system. Using qPCR we have investigated whether these changes correlates with mRNA levels of those cholinergic proteins. However, we didn't find any significant difference in mRNA levels which indicates that changes in cholinergic system of AD mouse model are not caused by lower gene expression but rather by posttranscriptional events.

[1] Machova E., Jakubik J., Michal P., Oksman M., Iivonen H., Tanila H., Dolezal V. Neurobiol Aging 29:368-78, 2008

[2] Machova E., Rudajev V., Smyckova H., Koivisto H., Tanila H., Dolezal V. Neurobiol Dis 38:27-35,2010

[3] Janickova H, Rudajev V, Zimcik P, Jakubik J, Tanila H, El-Fakahany EE, Dolezal V. Neuropharmacology 67:272-83, 2013

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THE CANCER CHEMOTHERAPEUTIC DRUG PACLITAXEL MODULATES TRPV1 ACTIVITY ON PRESYNAPTIC ENDINGS OF SPINAL CORD NEURONS BY TLR4 ACTIVATION

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The chemotherapeutic drug paclitaxel is widely used in the clinical practice to treat several types of malignant tumours. The treatment is frequently accompanied with development of persistent painful peripheral neuropathy. This type of pain is often resistant to standard analgesics. In our study we tested hypothesis that these adverse effects could be at least partially mediated due to activation of Toll-like 4 (TLR4) and Transient Receptor Potential Vanilloid 1 (TRPV1) receptors at the spinal cord level. Whole-cell patch clamp recordings of miniature (mEPSC), spontaneous (sEPSC) and dorsal root stimulation evoked (eEPSC) excitatory postsynaptic currents were made from superficial dorsal horn neurons in acute spinal cord slices prepared from adult male mice C57BL/6 and from young male Wistar rats (P19–P21). Our data demonstrated that acute application of low concentration paclitaxel (50 nM) induced significant increase in the frequency of mEPSC. This effect was prevented by the TRPV1 antagonist SB366791 (10 μM) pretreatment. However, the paclitaxel application did not influence frequency or amplitude of the sEPSC and eEPSC. These results indicate modulation of TRPV1 receptors activity at presynaptic endings by paclitaxel (1). In other set of experiments we study the effect of acute paclitaxel treatment on desensitization and tachyphylaxis of TRPV1 receptors. We tested the effect of paclitaxel on repeated capsaicin evoked response in superficial dorsal horn neurons recorded as mEPSC frequency. Under control conditions the mEPSC frequency of the second response to low concentration capsaicin (0.2 μM) was significantly smaller when compared with the first one (32.6 %). Paclitaxel application 10 minutes before the second capsaicin application prevented the decrease of mEPSC frequency and the second response was not different from the first one (90,8 %). This effect was prevented by coapplication of the paclitaxel with TLR4 antagonist LPS-RS (2 μg/ml). These results demonstrate that functional interaction between TLR4 and TRPV1 receptors may play an important role in modulation of nociceptive synaptic transmission in spinal cord dorsal horn after paclitaxel treatment and these changes were also confirmed by our collaborators in human DRG neurons (1). TRPV1 receptors thus play an important role in the development of chemotherapy induced neuropathic pain and modulation of nociceptive signalling at the spinal cord level. Understanding of these mechanisms is needed to improve analgesic treatment of the chemotherapy-induced neuropathic pain in patients.

[1] Li Y, Adamek P, Zhang H, Tatsui C, Rhines L, Li Q, Mrozkova P, Zhang H, Kosturakis A, Cassidy R, Cata J, Sapire K, Harrison D, Kennarmer-Chapman R, Jawad A, Ghetti A, Yan J, Palecek J, and Dougherty P. J Neurosci 35(39):13487–13500, 2015

Supported by GACR 15-11138S, MSMT LH12058, MSMT LH15279, GACR P304/12/G069, CZ.1.07/2.3.00/30.0025, CZ.1.05/1.1.00/02.0109, RVO67985823, and GAUK 138215

PAIN MODULATION BY PROINFLAMMATORY CYTOKINES AND TRPV1 RECEPTORS IN A MODEL OF PACLITAXEL INDUCED PERIPHERAL NEUROPATHY

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[1]Institute of Physiology, Academy of Science of the Czech Republic, [2]Department of Physiology, Faculty of Science, Charles University

Chemotherapy-induced peripheral neuropathy often represents a dose-limiting negative side effect for the use of paclitaxel in clinical practice. However, the underlying mechanism of the disorder remains still poorly understood. Accumulating evidence from animal models implicated a number of peripheral and central proinflammatory processes to play an important role. In our experiments we have focused on the function of the spinal cord TNFalpha, CCL2 and TRPV1 receptors in a model of paclitaxel induced neuropathy.

The model of peripheral neuropathy was established in adult male Wistar rats by i.p. injection of paclitaxel solution (5 x 2 mg/kg) on five alternate days. On days 10 and 21 after the first injection, lumbar DRG and spinal cord tissues were collected and further used for Western blot and RT PCR experiments. The acute effect of paclitaxel application was tested on spinal cord slices from 21 days old rats under in vitro conditions.

Paclitaxel treatment significantly increased protein expression of TRPV1 receptors, glial cell marker GFAP, protein levels of TNFalpha and CCL2 in lumbar DRGs at day 10 and with exception of TNFalpha also at 21 days after the first injection of paclitaxel. In the spinal cord dorsal horn paclitaxel induced TRPV1 overexpression only at day 10, but not at day 21. Increased levels of GFAP, TNFalpha and CCL2 were present on day 21, but not at day 10. Surprisingly, mRNA levels of TRPV1 and proinflammatory markers did not change during both tested periods (day 10 and 21).

In vitro spinal cord slice preparation was used to study molecular mechanisms of paclitaxel-induced cellular changes within the central nervous system. In the first experiments, c-Fos expression was evaluated in dorsal horn neurons after incubation with 100 nM paclitaxel for 60 min. The paclitaxel application induced a significant increase in c-Fos expression within the superficial area of the spinal cord dorsal horn. This increased expression of c-Fos protein was significantly attenuated by 10 min preincubation of the spinal cord slices with TRPV1 antagonist (SB 366791 and AMG 9810).

Our data indicate an important role of TRPV1 receptors in the cellular mechanisms of paclitaxel-induced neuropathy and possible modulation of their function by proinflammatory cytokines.

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MODULATION OF NOCICEPTIVE SYNAPTIC TRANSMISSION BY PAR2 RECEPTORS AT SPINAL CORD LEVEL UNDER INFLAMMATORY CONDITION

P. Mrozkova,[1,2] J. Palecek[1]

[1]Department of Functional Morphology, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic, [2]Faculty of Science, Charles University in Prague, Prague, Czech Republic

Modulation of synaptic transmission in the spinal cord dorsal horn plays a key role in the development of pathological pain states and chronic pain diseases. Protease-activated receptors (PARs) are a family of four G-protein-coupled receptors (PAR1-4) activated by proteases. The role of PAR2 receptors in pain perception is well established in the peripheral tissues. However, the role of PAR2 receptors on the central branches of DRG neurons in the spinal cord is not fully understood. The present study aimed to study the role of PAR2 in nociceptive processing and modulation of synaptic transmission in the superficial dorsal horn (DH) neurons in a model of carageenan induced peripheral inflammation.

Whole-cell patch clamp recordings of miniature – mEPSCs and spontaneous - sEPSCs were made from superficial DH neurons in acute spinal cord slices prepared from Wistar rats 21 days old, with the presence of strychnine (5uM) and bicuculline (10uM), at -70mV holding potential. TTX (0.5uM) application was used for mEPSC detection. Peripheral inflammation was induced by application of 3% mixture of carrageenan and kaolin into the paw 24h before the experiment.

Application of PAR2 agonist SLIGKV-NH_2 (PAR2 AP, 100μM) in slices from naive animals induced significant inhibition of mEPSC frequency from the control level, while under the inflammatory conditions it induced significant increase of mEPSC frequency. This effect of the PAR2 agonist application was significantly different between the naive and inflammatory groups ($p<0,001$). PAR2 AP application induced significant increase of sEPSC frequency in both the naive and also under the inflammatory conditions. Application of the inactive peptide VKGILS-NH_2 (100uM) did not evoke any change in the mEPSCs, sEPSC frequency. Each neuron was tested for the presence of capsaicin (0.2 uM) evoked response in the end of the experiment. Out of the 70 neurons tested in this study 86% of them responded with increased EPSC frequency after capsaicin application.

Our results suggest that presynaptic PAR2 receptors may play an important role in modulation of nociceptive synaptic transmission in the spinal cord dorsal horn particularly under conditions of peripheral inflammation. Further experiments are needed to fully evaluate the role of spinal cord PAR2 receptors in pain modulation.

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TRANSPORT SYSTEMS FOR POTASSIUM UPTAKE IN *CANDIDA* SPECIES

B. Husekova, H. Elicharova, H. Sychrova

Department of Membrane Transport, Institute of Physiology Academy of Sciences of the Czech Republic, v.v.i.,

Potassium plays a major role in regulation of cell volume, stable membrane potential and intracellular pH in most organisms. It is also required for enzyme activation. Thus the cells of all organisms need to accumulate high concentrations of potassium.

The risk of fungal infections has increased in recent years because of various factors. More people are becoming immunocompromised due to chemotherapy or transplantations, and resistance to antifungal drugs is growing. *Candida* species are the most common cause of invasive fungal infections from all fungal pathogens. Pathogenic *Candida* species compete for the necessary amount of K^+ with the cells of their hosts. Therefore *Candida* had to evolve very efficient potassium uptake systems. These fungi-specific K^+ uptake systems are different from systems in mammalian cells thus they become interesting targets of chemotherapeutics in human fungal infections. In *Candida albicans,* we can find genes encoding three types of putative K^+ uptake systems, differing in their transport mechanisms. The first one is the Trk protein, which is the most commonly occurring transporter of K^+ in yeasts. It is a high-affinity K^+ uniporter that enables growth at micromolar concentrations of K^+. The second putative transporter is Hakp which is a K^+- H^+ symporter. *ACU1* gene encodes rare ATPase which mediates a high-affinity K^+ uptake. The sequence of ACU-ATPase, which was found in *C.albicans* genome, was considered a pseudogene. To characterize the transport properties of individual *C. albicans* potassium transporters we use the heterologous expression of coding sequences in *S. cerevisiae* BYT12 cells lacking their own systems for K^+ uptake (*trk1 trk2*). We show that *Ca*Trk1 and *Ca*Acu1 are localized in the plasma membrane of *S. cerevisiae* cells. The activity of *Candida* transporters is different however, all three proteins (*Ca*Trk1, *Ca*Hak1, *Ca*Acu1) are uptake systems for the accumulation of necessary K^+.

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EARLY DIFFERENCES IN METABOLIC FLEXIBILITY BETWEEN OBESITY-RESISTANT AND OBESITY-PRONE MICE

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Decreased metabolic flexibility supports development of adverse consequences of obesity. Metabolic flexibility to glucose is traditionally assessed using indirect calorimetry (INCA), which also allows for measuring energy expenditure. The aims of this study were to (i) characterize metabolic flexibility of obesity-resistant A/J and obesity-prone C57BL/6J mice at weaning, i.e. during the switch from lipid to carbohydrate intake and before the dissociation in body weight; (ii) to compare INCA with glucose tolerance test (GTT) approach.

A/J and C57BL/6J mice were maintained at 20°C and weaned to chow diet at 30 days of age. During the first day after weaning, using separate subgroups of fasted mice (n=8), either GTT (oral, OGTT; and intraperitoneal, IGTT; using 1-3 μg glucose/g body weight, BW) or INCA oral gavage with 1-7.5 μg glucose/g BW; or a fasting/refeeding protocol) were performed, either at 20°C or 34°C (to exclude interference of thermogenesis), using mice of both genders.

Comparable results were obtained using (i) both OGTT and IGTT with1 μg glucose/g BW at 20°C; (ii) INCA with 7.5 μg glucose/g BW at 34°C; and (iii) INCA during fasted/re-fed transition at 34°C. Results furthermore indicated lower ability to switch between metabolic substrates associated with low glucose tolerance and relative hyperglycemia in C57BL/6J as compared with A/J mice.

We have found lower glucose tolerance using GTT and lower metabolic flexibility using INCA in C57BL/6J versus A/J mice. These differences between strains may be linked to the differential genetically-determined propensity to obesity of the mice.

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RE-ESTERIFICATION OF FATTY ACIDS IN ADIPOSE TISSUE MACROPHAGES

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Lipid metabolism of adipose tissue is a complex interplay between many cell types, especially between adipocytes and immune cells like adipose tissue-resident macrophages. Our goal is to characterize complex metabolism of adipose tissue – re-esterification of fatty acids during lipolysis in adipocytes and adipose-tissue macrophages.

Mature adipocytes were isolated using collagenase from epididymal adipose tissue of adult C57BL/6J mice and RAW 246.7 macrophages/monocytes were polarized into the M1 or M2 state. Adipocytes were incubated in the presence of macrophages for 2 hrs. Then, cells and media samples were extracted for lipidomic profiling using Folch or Bligh Dyer methods. The profiles of acylcarnitines, phospholipids and acylglycerols of macrophages were subsequently analyzed by RP-LC-MS/MS methods on Thermo UltiMate3000 RSLC coupled to triple quadrupole mass spectrometer.

We observed that both M1 and M2 macrophages oxidize fatty acids when exposed to adipocytes, but M2 cells rely more on fatty acid oxidation than M1. Also, M1 macrophages accumulate lipids in lipid droplets while M2 tend to utilize fatty acids for energy. M2 macrophages produce more substrates for fatty acid re-esterification via glyceroneogenesis, while M1 use the glycolytic pathway. Changes in phospholipids and acylglycerol profiles illustrate remodeling of acyl chains and different pathways of fatty acid metabolism in M1 and M2 macrophages.

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DEVELOPMENTAL CHANGES OF AMP-ACTIVATED PROTEIN KINASE IN MURINE SKELETAL MUSCLE: STRAIN-SPECIFIC DIFFERENCES

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AMP-activated protein kinase (AMPK) is a multisubunit protein, which plays a key role in control of skeletal muscle metabolism. Namely, lipid and glucose metabolism are modulated at both gene and protein levels. Surprisingly little is known about changes in AMPK subunits expression and AMPK activity in skeletal muscle during the early postnatal development, between birth and weaning. The aims of this study were to (i) characterize the activity and protein level of AMPKα1 and AMPKα2 isoforms, and expression of the genes encoding its catalytic subunits in murine skeletal muscle during postnatal development, namely between birth and weaning; and (ii) to assess influence of gender and genetic background of mice on AMPK developmental changes. Male (M) and female (F) pups of the obesity-prone mice C57BL/6 (B/6) and obesity-resistant mice A/J were born and maintained at temperature closed to thermoneutrality (30 °C), mothers were fed Chow diet, and mice were weaned at 28 days (D) of age. At 10D, the activity of AMPKα1 was significantly higher in comparison with AMPKα2 in all tested groups (A/J F, A/J M, B/6 F, B/6 M). Between 10D and 28D, the activity of AMPKα1 decreased in mice of both strains except for A/J F. In A/J mice at 28D, activity of AMPKα2 was higher than that of AMPKα1. Total activity of AMPK (α1+α2) in B/6 mice decreased significantly between 10D and 28D but it stayed constant in A/J mice. Total protein level between 15D and 28D of AMPK (α1+α2) decreased in B/6, but significant only in B/6 F. Expression of AMPKα1 gene was constant in both A/J and B/6 mice between 10D and 28D. Expression of AMPKα2 gene increased between 5D and 28D in both strains. During lactation, i.e., the period of the switch from high- to low-fat intake, strain-specific changes in AMPK activity in murine skeletal muscle were observed. While in the obesity-resistant A/J mice the activity stayed constant, it declined in the obesity-prone B/6 mice. The developmental change in AMPK activity resulting decrease in activity of AMPKα1 isoform. However changes in activity and protein level are not coresponding to AMPKα1 gene expression. The kinase activity exhibit the correlation with protein level only in B/6 mice (AMPKα1 $r_s = 0,85$; AMPKα2 $r_s = 0,63$), but not in A/J mice. Changes in AMPK activity in skeletal muscle during early postnatal development may affect propensity to obesity in adulthood, depending on the genetic background of the mice.

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EARLY PHASE OF METFORMIN ACTION IN DIETARY-OBESE MICE: LACK OF INVOLVEMENT OF AMPK AND POSSIBLE INTERACTION WITH N-3 FATTY ACIDS

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Long-chain *n*-3 polyunsaturated fatty acids (omega-3) act as natural hypolipidaemics and prevent development of cardiovascular disease in humans. We have shown that omega-3 could prevent impairement of hepatic insulin resistance in mice fed high-fat diet (HFD), depending on functional AMP-activated protein kinase (AMPK). Metformin is a first-line oral antidiabetic drug with blood glucose-lowering effect and beneficial impact on lipid metabolism. The precise mechanism of metformin action still remains unknown. Existing data suggest that metformin lowers glycaemia via mild suppression of mitochondrial complex I activity, leading to a suppression of hepatic glucose production and concomitant AMPK activation. However, AMPK-independent mechanisms of metformin action may also exist. We sought to learn whether pre-treatment of dietary-obese mice with omega-3 could enhance early phase of metformin action, and whether AMPK is involved. Adult male B6 mice were fed a corn oil-based HFD (35 % lipids wt/wt) for 6 weeks. Mice were then randomly divided into two groups and fed for 2 weeks either the HFD or HFD-based diet containing omega-3 concentrate replacing 15 % (wt/wt) of dietary lipids (HFD-F). At the end of dietary intervention, mice were treated with a single dose of metformin (400 mg/kg body weight) or NaCl (placebo) administered by oral gavage, and 30 min later some mice were subjected to oral glucose tolerance test (OGTT), while the remaining mice were killed in order to collect liver and skeletal muscle for the AMPK activity assay and western blot analysis. A similar experimental setup, with a lower metformin dose (60 mg/kg), was used to investigate the involvement of AMPK in metformin action using transgenic mice with a whole-body inactivation of α2 subunit of AMPK (AMPKα2-KO). Glucose levels at 30th and 60th minute after glucose administration during OGTT, as well as an incremental area under the glycaemic curve (AUC; marker of glucose intolerance), were decreased (1.7-fold, 2.0-fold and 2.9-fold, respectively; $p<0.05$) after the single dose of metformin as compared to the saline-treated mice. Two-week consumption of HFD-F diet lowered AUC as compared to the HFD diet-fed mice (1.5-fold, $p<0.05$), the omega-3 treatment tended to augment effect of metformin, but the effect of the interaction between omega-3 and metformin was not statistically significant (3.2-fold vs. the HFD group; 1.1-fold vs. the HFD + metformin group). No differences in AMPK activity between the subgroups were detected. There were no significant differences in the OGTT in the response to metformin between wild type and AMPKα2-KO mice. We demonstrated acute dose-dependent hypoglycaemic effect of metformin during OGTT in dietary-obese mice. Although two-week-supplementation by omega-3 lowered the response to glucose challenge, significant synergistic effect of omega-3 and metformin was not found in this experimental setup. There was also no difference between wild type and AMPKα2 deficient mice suggesting that AMPK was not essential for the acute blood glucose-lowering effect of metformin. Possible AMPK-independent interaction between omega-3 and metformin in their effects on glucose homeostasis is likely and requires further characterization.

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IMPACT OF STRESS ON EXPRESSION OF ENZYMES AND NEUROPEPTIDES ASSOCIATED WITH HPA AXIS REGULATION IN LEWIS AND FISHER RATS

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Enzyme 11β-hydroxysteroid dehydrogenase type 1 (11HSD1) converts inactive forms of glucocorticoids to their biologically active counterparts. Our previous results showed that social stress upregulated this enzyme in structures involved in HPA axis regulation but not in principal components of the HPA axis and thus the changes of 11HSD1 expression might play a role in HPA axis regulation. In recent study we expanded our interest in two directions. Using variable stress paradigm we investigated stress induced alteration of expression of genes encoding 11HSD1 and neuropeptides associated with HPA axis regulation in two rat strains, which differs in reactivity of HPA axis (the stress hypo-responsive Lewis (LEW) rats and the hyper-responsive Fisher 344 (F344) rats). Specifically, we focused on corticotropin-releasing hormone (CRH), urocortins 2 and 3 (UCN 2, 3), oxytocin (OXT) and pituitary adenylate cyclase-activating peptide (PACAP). Recent results showed in accordance with our previous findings stress-induced upregulation of 11HSD1 expression in central amygdala and ventral CA1 and CA2 subfields of hippocampus in F344 rats and in prelimbic and infralimbic prefrontal cortex and lateral amygdal of LEW rats. In the hypothalamic paraventricular nucleus, the expressions of OXT, CRH, UCN3 and PACAP were increased in response to stress followed by expression of CRH receptor type 2 in both strains. In contrast, stress revealed strain specific alteration of CRH, UCN 2 and UCN 3 expression in amygdala even if expression of PACAP and its receptor PAC1 was increased in both strains. Our results provide evidence that inbred F344 and LEW rats differ not only in the activity of the HPA axis but they exhibit strain- and stress-dependent differences in expression of genes encoding 11HSD1 and neuropeptides associated with the HPA axis activity.

THE QUANTITATIVE PROTEOMIC STUDY OF HUMAN SALIVA SAMPLES OBTAINED FROM CARIES-FREE AND CARIES-SUSCEPTIBLE PEOPLE

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Only a minor part of the population is resistant to tooth caries (about 10% people in the age 30) (1). Our study was aimed to proteomics of saliva and to find the differences in the abundances of the responsible proteins between caries-resistant and caries-susceptible people. Only few studies compared the protein saliva composition of people with carious teeth and people with no caries (2,3). Human saliva proteom differs in poputation (gender, age). Therefore we used in our study only females with the similar age (age 26-36). The proteins of oral fluids were separated by two-dimensional gel electrophoresis and the resulting protein maps were quantitatively evaluated. Spots exhibiting statistically significant changes were excised and analyzed by nano-liquid chromatography coupled to a Q-TOF mass spectrometer. Thus we revealed two proteins (Ig lambda-3chain C region (LAC3) and Zinc-alpha-2-glycoprotein (ZA2G)) with significantly higher expression in the caries-susceptible group and one protein (Cystatin-SN (CYTN)) with significantly higher expression in the caries-free group. Our results demonstrates that the observed differences in the protein levels might have influence on the anticaries resistance. This result should be further verified on a larger group of respondents. This study comparing proteomes of whole saliva from carious-resistant and carious-susceptible people brings new findings to the saliva protection against tooth caries. These proteins could play specific role in the pathophysiology of dental caries.

[1] JÁGR M, ECKHARDT A, PATARIDIS S, BROUKAL Z, DUŠKOVÁ J, MIKŠÍK I: Proteomics of Human Teeth and Saliva. Physiol Res. (2014) 63: 141-154

[2] PREZA D. Acta Odontol Scand 67: 161-169, 2009

[3] VITORINO R. Eur J Oral Sci114: 147-153, 2006

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CHANGES IN FEEDING REGIME AFFECT CIRCADIAN CLOCKS DIFFERENTLY IN THE PANCREAS AND IN THE LIVER

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Many processes in our body exhibit circadian rhythms. These rhythms are driven by a system composed of the central pacemaker in the brain which entrains the peripheral oscillators. Food intake represents one of the cues which entrain the peripheral clocks. Restriction of food availability to improper time of the day (restricted feeding, RF) phase shifts circadian oscillations of clock gene expression in the liver; however, the effect of RF on the pancreatic clock was not well documented. Mice were subjected to RF regime (6-h food availability during the daytime) for 10 days and their behavioral activity was observed. Daily expression profiles of clock genes Per1, Per2, Cry1, Bmal1, Rev-erbα and clock-controlled gene Dbp were analyzed in the liver and pancreas. In control ad libitum fed animals, circadian rhythms in expression of all studied genes were present both in the liver and pancreas. As expected, RF affected the locomotor activity and significantly phase-shifted expression of all analyzed genes in the liver. However, in the pancreas, none of the studied genes showed significant phase-shift. Instead, due to RF the expression of Cry1 in the pancreas was completely arrhythmic. Additionally, the level of gene expression was changed by RF differently in the liver than in the pancreas. Therefore, at the tissue level, the clock in the pancreas responded differently to RF than that in the liver, possibly due to other type of metabolic inputs. Our study analyzed for the first time in detail the response of the pancreatic clock to temporal change in feeding regime and revealed its specific features compared to the hepatic clock. The data may contribute to our understanding of the mechanism how circadian disruption impacts on pancreas-related pathology.

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MATERNAL SYNCHRONIZATION OF THE SUPRACHIASMATIC NUCLEI IN RAT DURING ONTOGENESIS

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Entrainment of the circadian system with external environment is crucial for its proper function. Photic stimuli from external environment are dominant entraining cues for the central clock in the suprachiasmatic nuclei (SCN), however, before the photic entrainment develops during early ontogenesis, the clock is dependent on maternal cues. The strength of the maternal entrainment has not been fully understood. The aim of our study was to examine whether the SCN of pups could be entrained by altered behavior and feeding rhythms of a foster mother with an aberrant circadian phenotype (the spontaneously hypertensive rats, SHR) at the developmental stage when light already entrains the clock. Pregnant Wistar rats and SHR were kept under light-dark regime LD12:12 h and one day after delivery, the entire litters of pups were transferred to foster mothers of the different rat strain. The pups' SCN were sampled in 4-h intervals throughout the day at postnatal day 10 (P10) and P30 and mRNA levels of clock genes *Per2*, *Bmal1* and *Rev-erbα* were determined by *in situ* hybridization. At P10, the clock gene expression rhythms were phase-advanced in Wistar rat pups reared by SHR mother but no shift was present in SHR pups reared by Wistar rat mother. At P30, the maternal entrainment was lost. These results demonstrate that at P10, aberrant maternal cues may entrain the SCN even though the clock is already entrained by light.

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EXPRESSION OF GENES OF CATECHOLAMINERGIC SYSTEM IN ADRENAL MEDULLA AND SYMPATHETIC GANGLIA OF SPONTANEOUSLY HYPERTENSIVE RAT: IMPORTANCE OF THE SELECTION OF APPROPRIATE REFERENCE GENES

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Spontaneously hypertensive rats (SHR) represent a widely used experimental model of human essential hypertension. Catecholaminergic system (adrenal medulla and sympathetic ganglia) plays an important role in regulation of blood pressure and hypertension development. The available articles concerning mRNA expression level of catecholaminergic system genes in SHR are often contradictory. This could be due to various reference genes used as internal controls.
The aim of our study was to find the suitable reference gene for gene expression profiling in adrenal medulla and sympathetic ganglia of SHR and Wistar-Kyoto (WKY) rats, which would enable the comparison of mRNA expression of genes of catecholaminergic system between the two strains.

Expression of mRNA was measured by quantitative real-time PCR in adrenal medulla and superior cervical ganglia of 4-week-old (young) and 24-week-old (adult) SHR and WKY rats. We focused on the expression of genes involved in catecholamine biosynthetic pathway, genes related to catecholaminergic vesicles and genes involved in catecholamine removal from the synaptic cleft. We evaluated 12 reference genes by software Normfinder and compared them as an internal control.

Combination of reference genes *Hprt1* and *Ywhaz* in adrenal medulla and *Gapdh* and *18S* in sympathetic ganglia were chosen as the best ones. Numerous tissue-, age- and strain-dependent differences in the expression of genes of catecholaminergic system were detected. Most of alterations in both tissues were associated with biosynthetic pathway, which seems to be downregulated in 4 week old SHR. This attenuation is preserved during aging in sympathetic ganglia and becomes even more significant in adrenal medulla. Expression of genes related to vesicles is also attenuated during aging in adrenal medulla of SHR.
Using various reference genes for the standardization of our data strongly influenced obtained results and their interpretation, which indicates importance of appropriate internal control.

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THE N-TERMINAL ANKYRIN REPEATS DIFFERENTIALLY REGULATE THE TRPA1 CHANNEL GATING

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The ankyrin transient receptor potential channel TRPA1 is a Ca^{2+}-permeable cation channel whose polymodal activation results from a complex synergy between distinct activation sites. This sensory-neuron-specific channel is gated in response to a variety of pungent chemicals, such as isothiocyanates or cinnamaldehyde derivatives, which probably covalently interact with the reactive cysteine residues within the N-terminal part of the channel. In addition to these chemicals, TRPA1 channel is strongly modulated by permeating Ca^{2+} and, in the absence of any agonist, it can be activated by depolarizing voltages. Although a cryo-EM structure of human TRPA1 with 4.24 Å resolution has been recently published (1), the distal parts of the cytoplasmic C- and N-termini remain unresolved.

To address the functional role of the N-terminus, we performed mutagenesis studies targeting the highly conserved ankyrin repeat (AR) tetrapeptide motifs S/TPLH. Our results suggest that local conformational stability of the most conserved ARs differentially regulates TRPA1. Moreover, a short range stability of AR12 seems to be crucial for TRPA1 channel functioning because even a highly conserved mutation S448T increases its voltage, chemical and calcium sensitivity. We also found that specific mutations in the most conserved ARs alter the voltage- (AR2) and calcium- (AR6) induced responses, most likely through affecting the allosteric coupling required for TRPA1 channel activation.

[1] Paulsen et al., Nature 520(7548):511-7, 2015

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NEUROSTEROIDS WITH LIPHOPHILIC D-RING MODIFICATIONS ARE MORE POTENT INHIBITORS OF NMDA RECEPTORS THAN ENDOGENOUS PREGANOLONE SULPHATE

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N-methyl-D-aspartate receptors (NMDARs) are glutamate-activated ion channels permeable to Ca^{2+} involved in excitatory synaptic transmission and synaptic plasticity. However, their excessive activation leads to excitotoxicity that may contribute to the pathology of certain neurodegenerative diseases. The activity of NMDARs may be affected by a variety of allosteric modulators, including endogenous neurosteroid pregnanolone sulphate (PAS) that inhibits the activity of NMDARs in a use-dependent but voltage-independent manner and has a neuroprotective effect, which make it a promising therapeutic target. Our previous experiments suggested the interaction of the steroid with the plasma membrane as a route for inhibitory neurosteroids to reach their binding site on NMDARs, located in the extracellular vestibule of the receptor's ion channel pore formed by the M3 membrane domains (1). Our further goal was to identify key structural features of neurosteroids crucial for their inhibitory effect on NMDARs. It has been established that for inhibitory action of neurosteroids on NMDARs, the charged group at the carbon C3 of the steroidal A-ring and the bended steroid structure associated with a 5β-stereochemistry were required (2). The acetyl moiety at the carbon C17 of the steroidal D-ring is, apart from the sulphate group at C3, another significant structural determinant of PAS. The aim of our study was, therefore, to determine whether the substitution of the 17-acetyl moiety can affect the ability of the derivatives to modulate the function of NMDARs. We performed electrophysiological recordings on human embryonic kidney cells (HEK 293) expressing recombinant GluN1/GluN2B receptors to assess the IC50 values for 16 derivatives of PAS with modifications at the carbon C17. Using computational analysis we investigated lipophilic qualities of the tested PAS derivatives (values of logP ranging from 2.5 to 5.9). Our results show that the modifications at C17 can significantly increase the potency of all tested derivatives to inhibit NMDAR responses compared to the naturally occurring PAS with an IC50 = 31.1 ± 5.9 μM. In addition, we found a strong correlation (R2 = 0.8) between the IC50 and the logP values for these derivatives. The most lipophilic derivative substituted at the C17 by *i*-butyl was, as well, the most potent inhibitor with an IC50 = 84 ± 1 nM. These results suggest that besides the specific interaction of C17 substituents with the NMDAR, there is also non-specific effect of accumulation of the steroid in plasma membrane on the potency of these compounds to inhibit the NMDARs. Detailed knowledge of structure-activity relationships between neurosteroids and NMDARs is important for the development and design of new drugs that can be potentially used for the treatment of diseases associated with disorders of the NMDAR system.

[1] Vyklicky V, Krausova B. Sci Rep 5:10935,2015

[2] Weaver CE, Land MB. J Pharmacol Exp Ther 293:747-754,2000

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THE ROLE OF M3-S2 INTERDOMAIN LINKERS OF NMDA RECEPTOR FOR THE ION-CHANNEL OPENING

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Excitatory signal transduction in the mammalian brain is mostly mediated by glutamate activating ionotropic glutamate receptors (iGluR). The iGluRs are pharmacologically subdivided on AMPA, kainate and NMDA receptors exhibiting vastly different functional properties. Each receptor is composed of four protomers having a conserved domain organization.
The NMDA receptors contain two obligatory glycine-binding (GluN1) and two alternative glutamate/glycine-binding (GluN2/3) subunits. Extracellularly the most distal part to the membrane is the amino-terminal domain connected to the ligand-binding domain (LBD), which is linked to the transmembrane domain (TMD) by three linkers. Moreover, TMD communicates with intracellular space by the carboxy-terminal domain.

Each subunit of iGluR contains three small polypeptide linkers connecting two functionally distinct parts of receptor - LBD and TMD. The linker connecting third helix of the TMD (M3) and bottom domain of the LBD (S2) is central to the opening of the ion channel. According to the proposed mechanism the gating is initiated by ligand interaction with its binding pocket within the LBD. This interaction leads to conformation change of the LBD providing energy to the pore opening. According to the current knowledge linkers play a significant role in the transfer of this conformation energy (1, 2).

Individual rat NMDA receptor subunits possess linkers with distinct amino acid sequence which might be coupled to different functional properties of the channel. Moreover, the model of channel opening has shown the different contribution of GluN1-1a and GluN2B subunits in the course of opening. We were studied the role of M3-S2 interdomain linkers of both NMDA receptor subunits for the ion-channel opening. Using electrophysiology recording we found that (i) small shortening of the linker (in the length of one amino acid) have usually no effect on receptor function. (ii) In addition, in the GluN1-1a subunit shortening of the linker near to ion channel vestibule impact more radically the receptor function then analogous shortening of the linker of the GluN2B. It suggests that the role of GluN1-1a at normal circumstances dominates in initiation of opening while the GluN2B takes over approaching the fully open state.

[1] Mayer, M. L. & Armstrong, N. Annu Rev Physiol 66:161–81,2004

[2] Mayer, M. L. Nature 440:456–62,2006

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TWO N-GLYCOSYLATION SITES IN THE GLUN1 SUBUNIT ARE ESSENTIAL FOR RELEASING N-METHYL-D-ASPARTATE (NMDA) RECEPTORS FROM THE ENDOPLASMIC RETICULUM

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NMDA receptors (NMDARs) comprise a subclass of neurotransmitter receptors whose surface expression is regulated at multiple levels, including processing in the endoplasmic reticulum (ER), intracellular trafficking via the Golgi apparatus, internalization, recycling and degradation. With respect to early processing, NMDARs are regulated by the availability of GluN subunits within the ER, the presence of ER retention and export signals and posttranslational modifications, including phosphorylation and palmitoylation. However, the role of *N*-glycosylation, one of the most common posttranslational modifications, in regulating NMDAR processing has not been studied in detail. Using biochemistry, confocal and electron microscopy, and electrophysiology in conjunction with a lentivirus-based molecular replacement strategy, we found that NMDARs are released from the ER only when two asparagine residues in the GluN1 subunit (Asn-203 and Asn-368) are *N*-glycosylated. Although the GluN2A and GluN2B subunits are also *N*-glycosylated, their *N*-glycosylation sites do not appear to be essential for surface delivery of NMDARs. Furthermore, we found that removing *N*-glycans from native NMDARs altered the receptor affinity for glutamate. Our results suggest a novel mechanism by which neurons ensure that postsynaptic membranes contain sufficient numbers of functional NMDARs.

THE *N*-GLYCOSYLATION OF THE GLUN3A SUBUNIT IS CRITICAL FOR SURFACE DELIVERY OF THE GLUN1/GLUN3A RECEPTORS

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N-methyl-D-aspartate receptors (NMDARs) play a crucial roles in glutamatergic neurotransmission, synaptic plasticity and learning. GluN3A is a nonconventional subunit which endows NMDARs with altered functional properties compared with NMDARs composed of only GluN1 and GluN2 subunits. Many studies presented biochemical data that the GluN1 and GluN2 subunits are extensively *N*-glycosylated, both in arteficial and native expression systems. Recently, we revealed that the major types of the NMDARs, the GluN1/GluN2A and GluN1/GluN2B, are efficiently released from the endoplasmic reticulum only when two asparagines within the GluN1 subunit, the N203 and N368, are glycosylated. However, to our knowledge there are no studies about roles that *N*-glycosylation plays in the trafficking of the GluN3A-containing receptors nor about the glycans attached to this subunit. Using confocal microscopy and biochemistry, we showed that the presence of at least three conventional *N*-glycosylation sites (out of 12 sites present within the extracellular parts of the GluN3A) is critical for the surface delivery of the GluN1/GluN3A receptors in heterologous COS-7 cells and cultured hippocampal neurons. Furthermore, our deglycosylation biochemical analysis revealed that native GluN3A subunit is endoglycosidase H-resistant, in contrast to the GluN3A subunit expressed in the heterologous cells. Thus, our findings identify novel mechanism by which a neuron can ensure that the proper numbers and types of the NMDARs are present on the cell surface.

PROTECTIVE EFFECT OF CAEDTA IN HYPOBARIC HYPOXIA INDUCED NEUROINFLAMMATION AND NEURONAL DAMAGE

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Hippocampus is very sensitive to global reduction in oxygen levels of the body and thus even brief exposure to oxygen-deprived environments can lead to severe damage, of this area in the brain, resulting from the death of neurons or loss of connections etc. Hypoxic exposure to hippocampus induces cell death and injury leading to release of ions and molecules, which evoke inflammatory processes that ultimately cause secondary neuronal damage. During these inflammatory processes, levels of several cytokines, including interleukin 6 (IL-6), IL-1β etc., changes. This project explored whether treatment with CaEDTA, a well-known chelator, demonstrate any neuroprotective effects in hypobaric hypoxia induced neuroinflammation, which might include decrease in the levels of pro-inflammatory cytokines. BALB/c male mice (30-35g) were exposed to hypobaric hypoxic conditions corresponding to an altitude of 25,000 ft for three days (6 hours/day) in a specially designed hypoxic chamber (Seven Stars, India). The animals were randomly divided into the following groups: Normal (group 1), Normal + CaEDTA (1.25mM; group 2), Intermittent hypoxia (group 3) and Intermittent hypoxia + CaEDTA (1.25mM; group 4). CaEDTA, dissolved in normal saline, was administered via ip (intraperitoneal) route to group 2 and 4, at a dose of 5.12mg/kg body weight. Group 1 and group 3 were given equal volume of saline. The mRNA expression analysis was performed for the following genes: IL-6, IL-1β, tumour necrosis factor alpha (TNF-α) and hypoxia-inducible factor 1-α (HIF-1a) and immunofluorescence was performed for IL-6. The results highlighted that animals exposed to hypobaric hypoxia had increased level of cytokines including TNF-α, IL-1β, IL-6 and HIF-1a; however, animals treated with CaEDTA showed reduced level of these cytokines, in mRNA analysis. Similar results were obtained in immunofluorescence. These data suggest that excessive release of Zn^{2+} during hypobaric hypoxia is chelatable by CaEDTA and this may, secondarily, decrease the levels of proinflammatory cytokines.

IMMUNODETECTION OF NEURAL SUBSTRATE OF MAGNETORECEPTION IN C57BL/6J MICE

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The ability to perceive the Earth's magnetic field has been demonstrated in a variety of animals, including representatives of all five classes of vertebrates. The physiological mechanisms underlying magnetic field sensation, however, remain largely unknown. Behavioral, physiological, neuroethological studies and studies using early response genes as neuronal activation markers indicated that a major role in the perception and processing of magnetic information play trigeminal, vestibular and visual systems. Subsequently, magnetic information seem to be integrated with multimodal sensory and motor information within the hippocampal-entorhinal system. In the majority of studies, however, birds have been used as model organisms. In this work I analyzed the neural substrate of magnetic compass orientation in the mouse strain C57BL/6J using markers c-Fos and Egr1. I found that all the aforementioned systems contain neurons responsive to the experimental magnetic fields. This finding demonstrates a complex processing of the magnetic information at level of the central nervous system.

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IMPAIRED COGNITIVE COORDINATION ON A ROTATING ARENA AFTER SYSTEMIC DIZOCILPINE (MK-801).

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Disruption of cognitive abilities such as learning, memory and spatial navigation accompanies a number of neuropsychiatric disorders including schizophrenia. Cognitive symptoms are also most difficult to target by available pharmacotherapy. Behavioral and molecular evidence point to a disruption of NMDA receptors and glutamatergic and dopaminergic neurotransmission. Impaired cognitive coordination has been proposed as a core cognitive deficit in schizophrenia. Noncompetitive NMDAR antagonists are used to model schizophreniarelated symptoms in humans and in experimental animals. Place avoidance on a rotating arena is used to model cognitive functions disrupted in an animal model of schizophrenia. The goal of this thesis is to show whether the deficit in place avoidance is due to disrupted cognitive coordination or another effect of NMDAR antagonism such as hyperlocomotion, general learning deficit, or altered sensitivity.

FROM ANIMAL MODELS TOWARDS SCHIZOPHRENIA: SPATIAL NAVIGATION IN VIRTUAL REALITY ENVIRONMENTS

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Cognitive deficit is considered one of the core symptoms of schizophrenia and related psychotic disorders that affects several cognitive domains (1). Cognitive functioning, such as spatial navigation, offers very useful methodological approach for comparative studies. Deficit in visuo-spatial abilities has been demonstrated in schizophrenia patients (2) and similar behavioral changes were observed in animal models of schizophrenia (3). In order to assess complex spatial abilities in schizophrenia and compare their results with data obtained in previous animal studies, we designed two virtual reality tasks adopted from the animal research. We recruited a study group (SZ) of 33 first-episode schizophrenia patients and a control group of 33 healthy volunteers (HC) matched for age, sex, education and gaming experience. At baseline day all participants completed a standard neuropsychological assessment and SZ symptoms were evaluated by PANSS and GAF scales. The second day all participants underwent a short pre-training followed by two virtual tasks: 1. Stable arena, the vFGN (virtual Four Goals navigation) task, is inspired by the Morris water maze hidden goal paradigm. 2. Rotating arena, the vAAPP (virtual Active Allocentric Place Preference task), is inspired by the Carousel maze modified from avoidance to a preference task. Twenty-two subjects from the SZ group were tested one year after the first hospitalization using the above described battery in order to test the stability of the cognitive deficit after antipsychotic (AP) treatment. Results of both virtual tests showed significant decline in spatial abilities in SZ compared to HC in all test phases. According to the PCA analysis, data obtained in the stable arena form a cluster together with learning and memory tests, while the rotating arena seems to be more related to performance tests dependent on flexibility, timing and psychomotor speed. Cognitive impairment observed in the group of SZ patients in both standard and virtual tests persists also after one year of AP treatment. Visuo-spatial deficit observed in SZ patients is in agreement with the results of standard neuropsychological assessment, and with the findings of other spatial studies in schizophrenia patients and animal models of schizophrenia.

[1] Elevag B, Goldberg TE. Crit Rev. Neurobiol. 14: S1-21,2004

[2] Hanlon FM, Weisend MP, Hamilton DA, Jones AP, Thoma RJ, Huang M, Martin K, Yeo RA, Miller GA, Canive JM. Schizophr. Res. 87:S67-80,2006

[3] Lobellova V, Entlerova M, Svojanovska B, Hatalova H, Prokopova I, Petrasek T, Vales K, Kubik S, Fajnerova I, Stuchlik A. Behav Brain Res. 246:S55-62,2013

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REVERSAL LEARNING IMPROVEMENT AFTER ADULT NEUROGENESIS REDUCTION IN DENTATE GYRUS: EVIDENCE FOR PATTERN INTEGRATION FUNCTION.

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Adult neurogenesis in dentate gyrus causes a significant addition of new neurons into hippocampal network. Functional importance of these neurons is still unknown. Most theories suggest new neurons function in pattern separation, but empirical evidence is inconclusive. Another theory states that these neurons serve an opposite function - a pattern completion. This latter theory is only one based on unique properties of these new cells and on mathematical models that include neurogenesis. We used a versatile carousel maze to test reversal learning in rats with intact and reduced neurogenesis. Neurogenesis was reduced by repeated administration of cytostatic temozolomide (TMZ, 10mg/kg). First, rats were taught to avoid a 60 degree sector of arena by administration of mild electric shock upon entering. After five sessions this shock sector was relocated to the opposite side of the arena. Number of entrances in the first reversal session was a key measure of cognitive flexibility. Results show that acquisition learning was same for both intact rats and rats treated with TMZ. However, TMZ treated animals had significantly less entrances in a first reversal session than control group. Our surprising results indicate that rats with reduced neurogenesis are better in carousel maze reversal task compared to control animals. We explain this by new neurons serving a pattern integration function – making events which happen in temporal proximity more similar with an addition of new neurons. This result is in line with mathematical models which predict that when two environments (acquisition and reversal) are similar, new neurons make it more difficult to correctly distinguish these two environments.

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RAPAMYCIN BLOCKS THE ANTIDEPRESSANT EFFECT OF KETAMINE IN TASK-DEPENDENT MANNER.

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Objective: The aim of our study was to test whether ketamine produces an antidepressant effect in animal model of olfactory bulbectomy and assess the role of mTOR pathway in ketamine´s antidepressant effect.
Methods: Bulbectomized (OBX) rats and sham controls were assigned to 4 subgroups according to the treatment they received (ketamine, saline, ketamine+rapamycin, saline+rapamycin). The animals were subjected to open field (OF), elevated-plus maze (EPM), passive avoidance (PA), Morris water maze (MWM) and Carousel maze (CM) tests. Blood samples were collected before and after drug administration for analysis of phosphorylated mTOR level. After behavioral testing, brains were removed for evaluation of BDNF in prefrontal cortex (PFC) and hippocampus.
Results: Ketamine normalized hyperactivity of OBX animals in EPM and increased the time spent in open arms. Rapamycin pre-treatment resulted in elimination of ketamine effect in EPM test. In CM test, ketamine+rapamycin administration led to cognitive impairment not observed in saline, ketamine or saline+rapamycin treated OBX rats. Prefrontal BDNF content was significantly decreased and level of mTOR was significantly elevated in OBX groups.
Conclusions: OBX animals significantly differed from sham controls in most of the tests used. Treatment had more profound effect on OBX phenotype than controls. Pre-treatment with rapamycin eliminated the anxiolytic and antidepressant effect of ketamine in task-dependent manner. The results indicate that ketamine+rapamycin application resulted in impaired stress responses manifested by cognitive deficits in active place avoidance (CM) test. Intensity of stressor (mild vs. severe) used in the behavioral tests had opposite effect on controls and on OBX animals.

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TRIMETHYLTIN MODEL OF NEURODEGENERATION

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Trimethyltin is an organometal compound which can be used as a pharmacological model of neurodegeneration. Its intraperitoneal application to the rat causes limbic system degeneration (including the hippocampus and amygdala), resulting in histological and behavioral changes. The trimethyltin model shares many features with human neurodegenerative disorders, including neuroinflammation, oxidative stress, cognitive impairment and pattern of progression. This model is therefore considered as an appropriate model of neurodegeneration (1). However, the effect of trimethyltin is strain- and age-dependent and the current knowledge about its appropriate dosing and timing of subsequent tests is rather inconsistent.

The aim of this study is, therefore, to validate the trimethyltin model of neurodegeneration in the rat. We targeted to induce behavioral and histological changes in the rats without reaching unwanted serious toxic effect of trimethyltin and to optimize the choice and timing of behavioral tests. In the next step we will test the effectivity of selected neuroprotectants in this model.

Wistar and Long-Evans male rats of different ages were used. After pilot experiments, 11-13 weeks old Wistar rats were chosen as the most appropriate. Trimethyltin chloride was dissolved in saline and applied intraperitoneally (8 mg/kg). Control animals received saline. Because of possible agression caused by trimethyltin the rats were housed individually. Behavioral functions of the rats were tested in the Morris water maze (hippocampus-dependent test; 14 days after application of trimethyltin) and fear conditioning test (amygdala-dependent test; 21 days after application) and active allothetic place avoidance task (AAPA; hippocampus dependent test). Four weeks after application of trimethyltin the rats were perfused. Histological staining (Nissl) of their brains was done.

The application of trimethyltin led to retarded learning in the Morris water maze. In the fear conditioning test, the freezing time of the trimethyltin treated animals tended to be decreased, but the difference was not significant. To compare, the learning ability of both control and trimethyltin treated animals in the AAPA was impaired, most probably due to their individual housing. Because of this known methodological limitation we excluded AAPA testing from this project. Histological staining showed that trimethytin caused an impairment in CA3 hippocampal areas.

We established the trimethyltin model of neurodegeneration in the rat, using the dose 8 mg/kg and 11-13 weeks old male Wistar animals. The main methods used in this project will be Morris water maze for testing of cognitive functions and histological staining, including Nissl, for detection of brain tissue damage. We are going to test the neuroprotective effectivity of NMDA receptor antagonists in this model.

[1] Geloso MC, Corvino V, Michetti F. 2011. Trimethyltin-induced hippocampal degeneration as a tool to investigate neurodegenerative processes. Neurochem Int 58(7):729-38.

NEUROPROTECTIVE EFFECT OF 3A5B – PREGNANOLONE GLUTAMATE IN THE EARLY STAGE OF THE IMMATURE RAT ISCHEMIA. HISTOLOGICAL AND NEUROCHEMICAL PROPETIES.

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Hypoxic-ischemic damage is the most common form of perinatal brain damage. This pathological state in early age leads to permanent neurological consequences. A key role in the development of these consequences plays a complex molecular processes running to massive release of glutamate. The most of NMDA glutamate receptor antagonists have neurotoxic effect on an immature brain. The neuroactive steroid 3α5β-pregnanolone glutamate (PG), synthetic analog of naturally-occuring 3α5β-pregnanolone sulphate, is a NMDA receptor negative modulator acting via use-dependent mechanism and GABA-A receptor positive modulator. In our previous research we demonstrated absence of neurotoxic effect of PG on the immature brain (1). Therefore we used PG as a potential neuroprotective agent that may reduce glutamatergic excitotoxicity in the brain afflicted by ischemia. Twelve days old rats were under isoflurane (2%) anesthesia fixed in stereotaxic and head skin was carefully cut up. Focal cerebral ischemia was induced by injection of endothelin 1 (ET-1; 40pmol; 1 μl) in the right dorsal hippocampus (AP 3.7; L 3.3; h 3.5 mm individually recalculated). One minute after infusion, skin was glued and anesthesia was terminated. Five minutes after the end of ET-1 injection PG (1mg/kg or 10mg/kg) was administrated intraperitoneally. Controls received intrahippocampal injection ET-1 or phosphate buffer (pH 7.4) followed by application of β-cyclodextrin (CDX). Twenty-four hours after intrahippocampal injection pups were overdosed with urethane (2 g/kg, i.p) and transcardially perfused with 4% paraformaldehyde. Brains were cryoprotected, frozen and sectioned in the coronal plane (50 μm). To evaluate degree of damage in the afflicted hippocampus Fluoro Jade-B (FJB), Iba-1, GFAP and PVA-positive interneurons staining were used. Our results confirm a neuroprotective effect of the PG treatment in used model of the focal cerebral ischemia in immature rats. Systemic administration of PG leads to reduction of the ischemia induced injury in neural tissue. This statement is supported by reduced activation of microglia, reduced astrogliosis, protection of PVA-positive interneurons and reduced neurodegeneration.

PG could represent a novel drug acting as GABA-A and NMDA receptor allosteric modulator to treat perinatal ischemia.

[1] Kleteckova L, Tsenov G, Kubova H, Stuchlik A, Vales K, Neuroscience Letters

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EFFECT OF HIGH SALT (NACL) INTAKE AND HYPERTENSION ON NEUROGENESIS IN THE HIPPOCAMPUS IN SALT-RESISTANT AND SALT-SENSITIVE DAHL RATS

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Dentate gyrus of hippocampus is one of the few places with ongoing neurogenesis into adulthood. These neurons are functional and are incorporated into hippocampal network. There are numerous environmental factors affecting rate of neurogenesis, both positively and negatively. Because hippocampus is sensitive to change in blood pressure, it is viable that adult neurogenesis may also be affected by hypertension. Hypertension is one of the putative factors in development of age related cognitive deficits and dementia, therefore it is possible that they are related to altered adult neurogenesis. Also, main factor of development of hypertension is western style diet. In this experiment we used a special animal model of hypertension induced by high salt diet to assess rate of neurogenesis – salt-sensitive and salt-resistant strains of Dhal laboratory rats. High salt content induces hypertension in salt-sensitive strain, but not in salt-resistant strain. Rats were either on high salt (5%) or low salt (0.5%) diets for a period of five weeks. After this period animals were sacrificed and BrdU+ cells, marker of neurogenesis, were assessed. Results show that only salt-sensitive strain on a high salt diet showed a reduced rate of neurogenesis in dentate gyrus. Importantly, in animals resistant to high salt change neurogenesis was not observed compared to the same strain on low salt diet. We can conclude that hypertension, but not high salt diet, is responsible for a reduction of neurogenesis.

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THE ROLE OF ATERIOR CINGULATE CORTEX IN REMOTE MEMORY, COGNITION AND ANXIETY

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It is generally known that a hippocampus plays a crucial role in spatial orientation, memory and cognition. However, an interconnection between the hippocampus and cortical structures of a brain is essential for these functions and spatial memories storage. Anterior cingulate cortex (ACC) is probably important for consolidation of long-term spatial memory. It might also play a role in behavioural flexibility, i.e. reaction to environmental changes. Moreover, ACC could be linked to emotionality due to the connections to other structures of limbic brain system. The aim of this study was to test the hypothesis that ACC is an essential structure for the above mentioned functions. In adult rats, we made permanent lesions of ACC by quinolinate (0.09 M, volume 0.2 μl - 4 injection sites in each hemisphere, 0.05 μl/min); control animals (sham) received saline. The animals were tested one month after the training in the Morris water maze (MWM, test of precise spatial navigation) and Carousel maze (CM, test of cognitive coordination) tasks for remote memory retrieval. We also studied cognitive flexibility by changing the position of the to-be-found platform (MWM) or the to-be-avoided sector (CM). In addition, we tested the animals in the elevated plus maze and light-dark test which both measure anxiety. We found a deficit in retrieval of remote memory in the water maze, but not in the Carousel maze. It is possible that memories, which needed cognitive coordination, are stored preferentially in a different brain structure. Cognitive flexibility was not influenced either in one of these tasks. The groups of rats with lesions did not differ in anxiety level compared to the controls. In conclusion, it seems that spatial memories consolidated in remote memory in the ACC. However, this structure is probably essential neither for cognitive flexibility nor for anxiety.

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THE CORRELATION OF GABA AND ADENOSINE CONCENTRATIONS WITH CHANGES IN HIPPOCAMPAL EXCITABILITY AFTER STATUS EPILEPTICUS IN IMMATURE RATS

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Purpose: Status epilepticus (SE) induced in immature rats may result in development of spontaneous seizures. Majority of spontaneous seizures is generated in hippocampus therefore we started to study hippocampal excitability after SE. The functional changes were correlated with concentration of neurotransmitters (GABA, adenosine) in this structure.
Methods: LiCl-pilocarpine SE was elicited in P12 rats and hippocampal epileptic afterdischarges (ADs) were studied in P15, P18, P25, P32 rats with electrodes implanted into dorsal hippocampus. Control animals received saline instead of pilocarpine. All age groups consisted of 10-14 animals. Threshold intensities were found and the rats were stimulated (2-s series of 60 Hz pulses of 1-ms duration) at 20-min intervals six times. Thresholds and duration of ADs were evaluated. The hippocampus of P15 and P32 rats (both controls and SE) was analyzed for concentration of GABA and adenosine by HPL chromatography.
Results: Threshold for hippocampal ADs was significantly higher in P15 SE rats in comparison with controls. The two older groups did not exhibit a difference between SE and controls but 32-day-old group revealed significantly lower threshold for SE rats than for controls. Corresponding changes were found in AD duration – shorter ADs were recorded in P15 SE rats whereas ADs in P32 SE rats were significantly longer in comparison with appropriate controls. These findings correlated with concentration of neurotransmitters - increased GABA in P15 SE rats and decreased adenosine in P32 SE rats in contrast with their age-matched controls.
Conclusion: Our data demonstrated that during 20 days after SE complex changes of hippocampal excitability took place. P32 rats revealed higher excitability of dorsal hippocampus than appropriate controls. This increased hippocampal excitability might be a background of changes in GABA and adenosine after SE in this structure.

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SPATIO-TEMPORAL PROFILE OF SEIZURE INITIATION IN TEMPORAL LOBE EPILEPSY OF HIPPOCAMPAL ORIGIN

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Purpose: Despite decades of study of the pathophysiology of TLE, the role of the individual limbic structures in seizure genesis is still not well understood. Traditional models of TLE (kainate, pilocarpine) do not allow for determination of the causal role of each limbic structure in ictogenesis due to widespread damage across the limbic system induced by the initial status epilepticus. The goal of this study was to elucidate the spatio-temporal profile of seizure initiation in TLE induced in the dorsal hippocampus.
Method: TLE was induced in seven adult rats by injection of 10 ng of tetanus toxin into the right dorsal hippocampus. Following the injection, animals were implanted with bipolar recording electrodes in the following structures of both hemispheres: amygdala, dorsal hippocampus, ventral hippocampus and piriform, perirhinal and entorhinal cortices. The animals were video-EEG monitored for four weeks.
Results: In total 140 seizures (20 seizures per animal) were analyzed. The average duration of each seizure was 53.2±3.9 s. Seizure onsets were classified as hypersynchronous with high-amplitude spikes initiated simultaneously across several sampled structures. Analysis of seizure onset revealed that the majority initiated in the ipsilateral (41 %) and contralateral (21 %) ventral hippocampi. These structures had a significantly higher probability of seizure initiation than other studied structures. The involvement of other limbic structures varied between individual animals. Only 7 % of seizures initiated in the injected dorsal hippocampus.
Conclusion: This study demonstrates the involvement of multiple limbic structures in seizure initiation in TLE induced in the dorsal hippocampus. Furthermore, it confirms the significance of the epileptogenic network concept to understand TLE ictogenesis, in which the ventral hippocampi play the dominant role.

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SURFACE ELECTROMYOGRAPHIC SIGNAL IN HUMAN-MACHINE AND COMPUTER INTERACTION

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Surface detection of electrical activity of muscles is point of interest for more than 4 decades. Electromyography (EMG) is commonly used for diagnostic and therapeutical purposes, sport applications and recently also for human-computer interaction. This work concerns with better utilization of the EMG signal and development of fast online analysis methods. Algorithms are optimized and embedded into small integrated portable stand-alone USB device, which is capable to process and analyze the signal in realtime onboard. Device provides continuous quantitative estimate of muscle activation and can emulate various types of virtual switches. The recognized control signals are used for general control of a computer and can be transformed to text input as well (using specialized visual feedback). In stand-alone battery powered mode the equipment allows direct engine/servo control or provide inputs for an artificial devices. Solution was designed with respect to the reduction of false-positive detection caused by activity of other muscle groups and noise or artifacts.

The device was tested by healthy subjects and also by a disabled person. Input method and responsibility of system were positively evaluated. One of our studies [1] also demonstrated that the system and user interface can be used for effective text input, editing and other features of contemporary computers in disabled people.

[1] Pošusta A., Sporka A., Poláček O., Rudolf Š., Otáhal J., J Multimodal User Interfaces, 2015 [in press]

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UTILIZATION OF CUSTOM MULTICHANNEL IMPLANT IN EXPERIMENTAL EPILEPSY RESEARCH

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Multichannel recordings of brain electrical activity represent state-of-the art techniques which allow us to gain insight into the network organization of the brain and complex interactions between neurons and neuronal populations. These techniques enable recording at various spatial scales ranging from single neurons to large neuronal populations of both hemispheres. Multichannel recording techniques play crucial role in modern epilepsy research. Obtained results advance our understanding of the network mechanisms involved in seizure genesis and development of epilepsy. The main aim of this study is to design, optimize and apply multichannel electrode implant for recording of spontaneous and evoked cortical activity in chronic animal model of neocortical epilepsy. For this purpose we have designed implant which components can be built using 3D printer. After the printing the implant is assembled manually. The multichannel array of electrodes allows long-term monitoring of spontaneous neocortical activity combining field potentials and multiunit activity. Pilot data reveal the microscopic organization of neocortical epileptic network, which is characterized by presence of micro- and macro- interictal epileptiform discharges. Developed implant is also characterized by high level of versatility when recording electrodes can be combined with optical stimulation or local drug delivery systems.

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NEUROCHEMICAL CHANGES IN THE ENDOTHELIN-1 MODEL OF FOCAL CEREBRAL ISCHEMIA IN IMMATURE RATS

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Clinical and experimental evidence recently showed that Focal Cerebral Ischemia (FCI) can lead to disturbances of consciousness, behavioral or learning disorders, and in some cases, even to death. Likewise, studies demonstrated that in some cases FCI is accompanied by acute electrographic seizures. Especially in perinatal period the development of seizures represent one of the important consequence of FCI. However, using endothelin-1 (ET-1) model of FCI in immature rats (p12) we demonstrated that there is dissociation between ischemia induced processes and seizure development. To bring more light, in present study we focused on mechanisms that associated with mentioned phenomenons. Using microdialysis we sequentially collected samples in a close proximity of ischemic brain area during 2h. Followed uHPLC-MS were used to determine metabolites changes associated with ischemic cascade and seizure development. Endothelin-1 induced FCI in immature rat brain caused 48% decrease of glutamate, on the other hand in adult brain this amino acid increased. Ischemia resulted in decrease of extracellular GABA (82%) and pyruvate (39%). On the other hand dopamine and lactic acid increased about ~176% and ~570% respectively first hour after ischemia. An expanded analysis focused on inflammatory markers (Leukotriene B4 – LTB4 and Prostaglandin E2 – PGN E2) pointed that inflammatory process might play an important role in the development of seizures after ET-1 infusion. We found that levels of LTB4 and PGN E2 were significantly ($p<.05$) increased (about ~136% and ~68% respectively) second hour after ischemia. Based on our findings, we hypothesize, that the seizure development in the ET-1 model of focal cerebral ischemia in immature rats is certainly associated with two processes: lactate acidosis and leukocyte recruitment. However, other factors (glutamate-GABA ratio, oxidative stress, etc.) cannot be excluded.

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COMPUTATIONAL ESTIMATION OF CALCIUM FLUXES IN ISOLATED MAGNOCELLULAR NEURONS

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The magnocellular vasopressin (AVP) and oxytocin (OT) neurones exhibit specific electrical behavior, synthesize AVP and OT peptides, and secrete them into the neurohypophysial system in response to various physiological stimulants. The electrical activities of these neurones are regulated by the release of AVP and OT either somato-dendritically or when applied to supraoptic neurones or slices preparations *in vitro*. In these neurones, both AVP and OT bind to specific autoreceptors which induce distinct Ca^{2+} signals and regulate cellular events. We demonstrate that freshly isolated single SON neurones from the adult non-transgenic and transgenic rats exhibited distinct spontaneous $[Ca^{2+}]_i$ oscillations. In transgenic rats (AVP-eGFP and OT-mRFP), the type of neuron was identified under fluorescence microscope with GFP or RFP filters; in non-transgenic rats, the type was identified by specific $[Ca^{2+}]_i$ responses to either AVP or OT. The vast majority (more than 80%) of both OT and AVP neurons exhibited oscillations. In AVP-eGFP or AVP-sensitive neurones, AVP either triggered oscillations in silent neurons or modified the oscillating pattern when the neurones were already oscillating. The oscillations were disrupted upon exposure to hypertonic or hypotonic solutions (325, 275 $mOsmol/l^{-1}$, respectively). Furthermore, none of the blockers of the intracellular transduction pathways of the phospholipase C and adenylyl cyclase affected these oscillations. We found that the mechanism of these oscillations depends on several components localized at the plasma membrane: the high-voltage-dependent calcium channels dominated by R-type channel, the Na^+/Ca^{2+} exchanger, and the Ca^{2+}-activated pump. Mitochondrial oxidative activity is required to sustain the oscillations. Together, these results unveil for the first time that both AVP and OT neurones maintain, via Ca^{2+} signals, their remarkable intrinsic *in vivo* physiological properties in an isolated condition.

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ESTIMATION OF NEURONAL RESPONSE LATENCY: METHODS AND ACCURACY

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Neuronal response latency denotes the time delay between the stimulus onset and the beginning of the evoked response of a neuron. It is believed to reflect the intensity of the presented stimulus. In our work we study two closely related aspects of response latency. Firstly, we investigate estimation methods that could be applied to extract latency from experimental data. Secondly, we are interested in the maximum achievable accuracy with which the stimulus intensity can be decoded from latency by neurons.

In the last twenty years, many methods dealing with latency estimation from experimental data were proposed (4). Since the studied responses are mostly excitatory, this is also an inherent assumption of most of the estimation methods. Therefore we focused specifically on the case of inhibitory response and proposed several estimators for this particular case (3). Unlike in traditional approaches, our estimators are based on measurements of the time interval between the stimulus onset and the first consecutive spike observed in repeated trials. Several parametric models using stochastic point processes are proposed and subsequently utilized for implementation of estimation methods, such as the maximum-likelihood method, the method of moments and the method using Laplace transform.

If the maximum achievable estimation accuracy of the stimulus intensity is plotted against the range of stimulus intensities, it can reveal for which stimuli is the neuron in question optimally tuned. For evaluation of the estimation accuracy, Fisher information can be applied (1,2). Under certain regularity conditions, it is the inverse of the variance of the best estimator of the stimulus intensity. Fisher information is investigated in basic discharge patterns modelled by a Poisson and a renewal process and the impact of spontaneous activity and a delay of the response is studied.

[1] Jenison RL. Neurocomputing 40:239-248, 2001.

[2] Lansky P., Greenwood P. Neural Comp 17:2240-2257, 2015.

[3] Levakova M, Ditlevsen S, Lansky P. Biol Cybern 108:475-493, 2014.

[4] Levakova M, Tamborrino M, Ditlevsen S, Lansky P. Biosystems, in press.

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THE ROLE OF CRMP2 AND PIN1 IN AXON GROWTH AND REGENERATION *IN VITRO* AND *IN VIVO*.

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Regulation of neuron growth and guidance is essential for normal development and function of the nervous system. Its defects are associated with neurodevelopmental disorders like autism or epilepsy. However molecular mechanisms which control precise guidance of axons and dendrites to their final destination are so far largely unknown. An important role in axon growth and regeneration plays regulation of cytoskeleton (microtubules) by microtubule-associated protein CRMP2 which is a member of Semaphorin3a signalling cascade. In its active, nonphosphorylated form CRMP2 binds heterodimers of tubulin and promotes their polymerization and axon growth. Recently we showed that isomerase Pin1 stabilizes CRMP2A isoform of CRMP2 in distal parts of axons and thereby stimulate growth of axon (1). Moreover downregulation of Pin1 leads to a lower level of CRMP2 and decrease of axon outgrowth.

In my PhD project we aim to test a role of Pin1 and CRMP2A in axon growth and regeneration *in vitro* and *in vivo* in a mouse model of spinal cord injury (SCI). Within the project, we used in vitro Sema3A collapse assay to characterize the effect of CRMP2A and CRMP2B isoforms on Sema3A growth/collapse of peripheral nerves. Furthermore, lentiviral vectors expressing Pin1 and CRMP2A or silencing Pin1 were cloned and purified and are currently used to study their effect on neuronal regeneration in mice after SCI.

Furthermore, we would like to test library of chemical compounds by high-throughput screening in order to find compounds which could improve neuronal growth. These compounds could be possibly used in SCI therapy too**.** We isolated and cultured cortical and hippocampal neurons from early postnatal mice (P4). For visualization of only neuronal cells we are using Thy1-YFP-16 transgenic mice (Jackson Laboratories), which express yellow fluorescent protein under neuron specific Thy1 promoter in cortical and hippocampal neurons (2).

The proposed project will provide a new insight into the function of CRMP2 and Pin1 in axon growth and regeneration. Furthermore our project can bring a new insight into the regulation of neuron regeneration *in vivo* with potential therapeutic use.

[1] Balastik, M., Zhou, X.Z., Alberich-Jorda, M., Pazyra-Murphy, M.F., Cosker, K.R., Weissova, R., Ziak, J., Machonova, O., Kozmikova, I., Chen Ch., Pastorino, L., Luo, M., Asara J.M., Cole, A., Sutherland, C., Segal, R.A. and Lu, K. P. Prolyl isomerase Pin1 regulates axon guidance by stabilizing CRMP2A selectivity in distal axons. Cell Reports (in press).

[2] Feng, G., Mellor, R.H., Bernstein, M., Keller-Peck, C., Nguyen, Q.T., Wallace, M., Nerbonne, J.M., Lichtman, J.W., and Sanes, J.R. (2000). Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. Neuron *28*, 41–51.

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DENDRITIC SPINES ANALYSIS IN PIN1-/- MICE

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Peptidyl-prolyl cis-trans isomerase Pin1 catalyzes conformational changes of phosphorylated Ser/Thr-Pro peptide bonds in some proteins. It was hypothesized that Pin1 protects against age-dependent neurodegeneration, as mice lacking Pin1 develop retinal degeneration and age-dependent neuropathy accompanied by motor and behavioral deficit. Previously, we showed that Pin1 is expressed in developing axons and regulates axonal growth. Interestingly, Pin1 is present also in dendritic spines and regulates their proteosynthesis. We hypothesize, that Pin1 acts as a regulator of dendritic spine length/density in postnatal and ageing neurons. We investigated dendritic spine length and density in different parts of cortex in $Pin1^{-/-}$ mice using biolistic neuron staining approach. Animals were divided into 3 age-dependent groups: 7 weeks old (young), 6 months old (middle) and 2 years old (old). At least 3 $Pin1^{-/-}$ and control mice were in each group. Cortical neurons in 250 um thick slices were labelled with DiI and/or DiO lipophilic fluorescent dyes and dendritic segments were scanned. Through all age groups, dendritic spine length in $Pin1^{-/-}$ mice decreased by 25% in basal dendrites of motor cortex. This effect was most profound in young animals. Although spine length declined with age in control animals, no changes were observed in $Pin1^{-/-}$ mice. Surprisingly, spine density was higher in young $Pin1^{-/-}$ comparing to controls. In both groups, spine density declined with age. As the observed effects (shorter and more dense spines in $Pin1^{-/-}$ mice) were present either through all ages or diminished with ageing, the reported neuronal pathology in aging $Pin1^{-/-}$ mice is not associated with changes in dendritic spines. Moreover, the most significant differences were observed in young animals. This suggests that Pin1 may play a role in processes of spines development or maintenance.

PHYSIOLOGICAL LEVELS OF 2-HYDROXYGLUTARATE REGULATE PROLIFERATION OF PRIMARY FIBROBLASTS

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Cancer cells with upregulated c-Myc become addicted on glutamine which is converted to 2-oxoglutarate (2-OG) and supports cell growth through conversion to citrate in the reductive carboxylation pathway. Moreover, mutated isocitrate dehydrogenase 1/2 (IDH1/2) in numerous cancer cell lines convert 2-OG to oncometabolite D-2-hydroxyglutarate (2-HG). It has been demonstrated that 2-HG elevation maintains cancer cells de-differentiated and increases their proliferation. One such pathway reportedly proceeds through competitive inhibition of multiple 2-OG-dependent dioxygenases, causing modulation of hypoxia inducible factor 1α (HIF1α) stabilization, and global change in DNA and histone methylation pattern.

The aim of this study was to investigate the presence of reductive carboxylation (RC) and role of 2-HG in primary non-transformed cells in comparison with cancer cells.
Rat primary fibroblasts and human SHSY5Y neuroblastoma cell line were subjected to treated with hypoxia, serum withdrawal or by addition of 2-HG to medium. Cellular concentrations of intermediary metabolites and their incorporations of 13C from L-Glutamine-1-13C were measured with gas chromatography - mass spectrometry.

The comparable rates of reductive carboxylation and 2-HG synthesis were found in both cell lines. The ratio of 2-HG/2-OG in fibroblasts dropped in the absence of growth factors and increased in hypoxia, while the response of SHSY5Y was opposite. Hypoxia and 2-HG addition enhanced the growth of fibroblasts, while serum withdrawal decreased it. Rat spleen tissue rich in proliferating cells contained higher 2-HG/2-OG ratio than heart and liver. Addition of 2-HG increased the stabilization of HIF1α.

Finally, RC and 2-HG concentration were measured in five different non-transformed cell lines and six cancer cell lines. Interestingly, statistically significant decrease of 2HG/2OG ratio was found in healthy cells, while dramatic changes of RC rate and 2-HG synthesis were not found in the individual cells. Value of 2HG/2OG in non-transformed cells was close to 1 but in cancer cells had a value greater than 5.

In conclusion, physiological levels of 2-HG in primary cells are regulated in response to physiological stimuli and modulate cell proliferation probably through the inhibition of 2-OG-dependent dioxygenases. Interestingly, the responsibility of 2-HG levels to various stimuli differs in primary and cancer cells.

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EFFECT OF CONTINUOUS NORMOBARIC HYPOXIA AND MODERATE EXERCISE TRAINING ON POSTINFARCTION HEART FAILURE IN RATS

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Adaptation to chronic hypoxia and exercise training are known to protect the heart against acute ischemia/reperfusion injury. Much less is known about potential therapeutic effect of these interventions on myocardial infarction (MI). The aim of this study was to find out whether chronic hypoxia or moderate exercise training can attenuate the progression of postinfarction heart failure. MI was induced in two-month-old male rats by coronary artery occlusion. Seven days after surgery, the MI rats were randomly assigned to three groups: i) sedentary controls kept at room air, ii) exposed to continuous normobaric hypoxia (12% O2, 3 wks) or iii) trained on a treadmill (15 m/min, 60 min/day, 5 days/wk, 3 wks). Echocardiography examination of the left ventricle (LV) was performed 3 days before MI and 7, 14 and 28 days after MI. MI resulted in a gradual increase in systolic and diastolic diameter (LVDs, LVDd) and a decrease in relative posterior wall thickness (RWT) compared to sham-operated animals. Fractional shortening (FS) decreased from 42,8 % before MI to 15,1 % on day 28 post-MI. Chronic hypoxia attenuated ventricular dilatation without significantly affecting FS. Moderate exercise training had no effect on LV geometry and function. Our data suggest that prolonged exposure to continuous hypoxia has certain potential to attenuate the progression of unfavourable changes in ventricular geometry induced by MI in rats.

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FUNCTIONAL DEPLOYMENT OF VENTRICULAR CONDUCTION SYSTEM IN MOUSE DURING DEVELOPMENT

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Individual compartments of cardiac conduction system (CCS) became functional in order which correlates with cardiac morphogenesis. Ventricular CCS compartments mature with ventricular septation and it is accompanied by shift of activation pattern from primitive base to apex, which follows blood flow, to advanced apex to base. There are some important differences between avian and murine CCS. From the functional point of view, the main contrast is the appearance of mature activation sequence well before time of ventricular septation completion (embryonic day [ED] 13.5 in mouse) and also that there was never reported activation originating from the base of heart.

Function of CCS was studied by optical mapping and monitored parameters were speed of electrical impulse propagation, location first activation site on ventricular epicardial surface together with direction of action potential spread [evaluated as activation patterns, namely activation utilizing primary ring, left and right apical breakthroughs, only right or only left apical breakthrough - corresponding from ED14.5 with left and right bundle branches, respectively].
By measurement of time necessary for activation of the left ventricle from ED9.5 to ED18.5 we observed remarkable acceleration between ED9.5 and ED11.5 where activation time dropped to a half. This was due to decrease in frequency of primary ring activation pattern with a slow speed of action potential propagation compared to activation from apex to base. Primary ring is a primitive, temporary preferential activation pathway located in future interventricular septum and was the most common activation pattern for ED9.5 and 10.5; at ED11.5 one third of hearts was activated through primary ring and at ED12.5 it was recorded only sporadically. Activation from apex to base appeared from ED9.5, where it first originated from the primitive left ventricle; for mature activation originating from apex in the later stages was the most typical activation from left and right or only right apical breakthrough (since ED11.5). Appearance of these apical breakthrough sites correlated with expression of Cx40 in ventricular trabeculae. This analysis of normal development of CCS was useful for interpretation of changes observed in Cx40 deficient mice as well as in other transgenic strains with CCS phenotype.

Key words: optical mapping, conduction system development

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Poster session

NANOFIBROUS POLYMER MEMBRANES MODIFIED WITH FIBRIN AND COLLAGEN STRUCTURES AS CARRIERS FOR SKIN CELLS

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Our study contributes to the tissue engineering, mainly to the construction of appropriate scaffolds for regeneration of damaged skin. This research aims to treat skin burns, bedsores and other skin defects. Simultaneously, it brings valuable insights for basic research in the field of molecular mechanisms of adhesion, proliferation and phenotypic maturation of cells and the control of the cell behaviour through the cell extracellular matrix, represented by synthetic nanofibrous material. Nanofibrous polylactic-co-glycolic acid (PLGA) membranes were prepared by needle-less electrospinning technology. These membranes were further modified with cell adhesion-mediating biomolecules, e.g. collagen, fibronectin and fibrin in order to increase their affinity to colonizing cells. Adhesion, growth and differentiation of keratinocytes (HaCaT) and fibroblasts, i.e. major cell types of epidermis and dermis, were evaluated on these nanofibrous membranes.The results show that the membrane modification using fibrin structures improved adhesion and proliferation of human dermal fibroblasts. The collagen structure on the surface of membranes improved the adhesion and proliferation of human HaCaT keratinocytes. Furthermore, fibrin structure stimulated fibroblasts to produce collagen, which is a major component of extracellular matrix (ECM) in the natural skin dermis. Fibronectin enhanced cell attachment to the membranes. Therefore, we can conclude that nanofibrous PLGA membrane covered with protein layer, fibrin or collagen appear to be a promising solution for the construction of temporary skin tissue carriers. In the next part of our research, we would like to construct whole skin carrier containing of both cell types. Human dermal fibroblasts or adipose-derived stem cells will be used as a feeder for human dermal keratinocytes.

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HUMAN ADIPOSE STEM CELLS AS A POTENTIAL SOURCE FOR CONSTRUCTION OF VASCULAR AND BONE TISSUE REPLACEMENTS

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Nowadays, the stem cells seem to be a promising source for regenerative medicine and tissue engineering. In comparison to differentiated cells, the stem cells have higher potential to replicate, are less prone to senescence, and it is possible to differentiate them into various cell types. The stem cells are present not only in embryonal and fetal tissues, but they were also proved to be a component of adult tissues, e.g. bone marrow, peripheral blood, skeletal muscle tissue, adipose tissue, and many others. Human adipose stem cells (hASCs) are very suitable for tissue engineering as they are abundant in many patients and can be easily harvested by minimally invasive surgery such as high and low pressure liposuction.

In my research work I would like to concentrate on differentiation of hASCs into hard tissue cell types, i.e. osteoblasts, as well as into soft tissue type, namely the vascular smooth muscle cells (VSMCs). The differentiation of hASCs into endothelial cells will be also tried out, although this differentiation is considered to be difficult due to the transition from fibroblastoid to epithelial morphology. The hASCs will be harvested in cooperation with the Clinic of Plastic Surgery at Bulovka Hospital in Prague. The differentiation into various cell types will be involved by three different conditions, namely by usage of suitable biomaterial scaffolds, by optimal composition of the culture medium and by exposure to appropriate mechanic stress in a dynamic bioreactor. As biomaterials for osteogenic differentiation, hard mechanically resistant biomaterials will be used, e.g. metals coated with diamond, other carbon or ceramic nanoparticles. The biomaterials for vascular prostheses are usually composed of synthetic polymers with various surface modifications. As cell culture media, we intend to use mainly commercially available media that are chemically defined and enriched with suitable growth and differentiation factors for each cell type. One of the appropriate mechanical stimulations for osteogenic cell differentiation is vibrational stress. The exposure to uniaxial cyclic strain stress seems to be suitable for differentiation into VSMCs.

We expect that our research work will be of great importance for improving the currently-used tissue replacements, and particularly for constructing novel replacements based on advanced material scaffolds influencing the differentiation of stem cells.

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MODULATION OF NOCICEPTIVE SYNAPTIC TRANSMISSION IN SPINAL CORD DORSAL HORN

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Pharmacological modulation of synaptic transmission in spinal cord is one of the most promising future targets for effective and save analgesia. Process of nociceptive transmission at spinal cord level is influenced by dynamic changes in excitability of local neuronal circuits, presynaptic afferent endings, neuron-glia and neuroimmune interactions, descendent facilitatory and inhibitory pathways. Imbalance in spinal pain processing system may lead to development of pathological states of increased sensitivity, hyperalgesia and allodynia.

Transient receptor potential vanilloid 1 (TRPV-1) channels play a key role in pain perception in peripheral nociceptors. In the spinal cord dorsal horns are TRPV1 receptors located on presynaptic endings of C and Aδ-fibers. Their activation leads to depolarization and calcium influx which has facilitatory effect on glutamate release. Results from our laboratory showed that under pathological conditions, sensitivity of spinal TRPV1 receptors is increased and they are responsive to low concentrations of endogenous agonists.

The main goal of my study on the spinal nociceptive transmission topic, is to elucidate interactions of TRPV-1 with cannabinoid and opioid receptors. Some of the endocannabinoids, like anandamide may activate both cannabinoid and TRPV-1 receptors. The aim of my experiments will be to test the effect of anandamide application on the synaptic transmission at the spinal cord dorsal horn.

We will use behavioral, immunohistochemical and electrophysiological methods. One of the main approaches will be recording of postsynaptic currents from superficial dorsal horn neurons in spinal cord slices from control animals and also after induction of peripheral inflammation. Our preliminary data suggest that anadamide application on spinal cord slices *in vitro* may have rather complex effects on the frequency of mEPSCs, depending on the specific neuronal population.

Our results will provide new information on the interaction of the vanilloid and cannabinoid system at the spinal cord level and may bring new approaches for analgesic treatment.

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INDUCIBILITY OF TRIGLYCERIDE TURNOVER IN WHITE FAT – A MARKER OF LEAN PHENOTYPE

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White adipose tissue (**WAT**) plays a crucial role in storage of energy in form of triglycerides (**TG**). Another role of WAT is producing numerous signaling molecules, which influence metabolism of many other organs. Metabolic activity of WAT is relatively low, however several pieces of evidence indicate that UCP1-independent activation of lipolysis of TG and fatty acids (**FA**) re-esterification substrate cycle (**TG/FA cycle**) in adipocytes could influence total energy balance. Two approaches were chosen to study metabolic changes in WAT: 1) combined intervention using *n*-3 polyunsaturated fatty acids (***n*-3 PUFA**) and calorie restriction (**CR**) in dietary obese mice and 2) cold exposure using obesity-resistant **A/J** and obesity-prone B57BL6/J (**B6/J**). In both experiments genes involved in mitochondrial biogenesis, FA oxidation and glyceroneogenesis were increased. The activity of futile TG/FA cycle,measured by ^{2}H-NMR was also elevated. These changes in WAT metabolism were linked with anti-inflammatory effect of the combined intervention (*n*-3 PUFA + CR). **PPARγ** signaling was activated in response to formation of lipid mediators such as 15d-PGJ2. **PPARα** and **PGC-1** are also involved in the changes in the transcriptional program in white adipocytes. The changes at the level of gene expression are reflected in the induction of mitochondrial β-oxidation.

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DETECTION OF ION CURRENTS FROM THE MEMBRANE OF THE MECHANICALY DISSOCIATED GARDEN SNAIL *HELIX POMATIA L.* NEURONS USING PATCH CLAMP METHOD

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Membrane receptors and ion channels of molluscan neurons are usually studied on isolated single cells dissociated by enzymatic treatment. However, it has been reported that enzymatic digestion might negatively affect the functionality of membrane proteins. Therefore, in this study we used an alternative methodology, i.e, the isolation of neurons from the ganglion complex of garden snail *Helix pomatia (L.)* by mechanical dissociation. The yield and the quality of mechanically dissociated neurons were determined by counting the number of neurons and their physical characterization such as measurement of diameter of the cell body and identification of the presence or absence of axons. The functionality of isolated neurons was investigated by recording of membrane currents mediated by voltage-dependent potassium in the presence or absence of tetraethylamonium (TEA) and 4-amiophyridine (4-AP) using whole-cell patch clamp method. Patch clamp recordings showed that mechanically isolated neurons exhibited potassium channel-mediated outward current which was decreased in the presence of both potassium channel blockers, TEA or 4-AP, indicating the expression of functional potassium channels in the cell membrane. Altogether, these results suggest that it is possible to mechanically dissociate significant number of viable and functional single neurons from *Helix pomatia* (*L*.) without the use of enzymes.

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SPONTANEOUS CALCIUM PERMEABILITY OF IONIC CHANNEL OF P2X RECEPTOR AFTER SUBSTITUTION OF CONSERVED TYROSINE IN THE 1st TRANSMEMBRANE DOMAIN

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Purinergic P2X receptors (P2XRs) are an ATP-gated channel family composed of seven members, termed P2X1-7, that are organized as trimeric homomers or heteromers. All subunits have two transmembrane domains (TM1 and TM2), a large extracellular loop, and intracellular N- and C- termini. The TM1 and TM2 adopt α-helical structures and helices of different subunits move relative to each other during channel opening and closing. The TM2 region appears to play a dominant role in receptor functions, including the fixation of receptors in the membrane, channel assembly, gating, ion selectivity, and permeability for divalent ions. The TM1 helix does not directly form the pore but is instead positioned more peripherally than TM2. This domain could make a significant contribution to control the ion permeability, and it may also contribute to the control of Ca^{2+} permeability and the transition of the channel pore from a relatively selective state to a dilated state. Each transmembrane domain contains two amino acids conserved across all P2X subunits. One of these residues in the TM1 of P2X2 receptor is Tyr43. In previous experiments, electrophysiological measurements demonstrated that substitution of conserved Tyr43 with alanine causes increase in receptor sensitivity to agonists and prolongation of receptor deactivation after agonist washout. This work was focused on clarifying the role of conserved tyrosine in the process of opening of the channel in the absence of agonist. We measured permeability of several P2X receptor-channels for Ca^{2+} by the Ca^{2+} imaging method in transfected GT1 neurons that lack endogenous P2 receptors. Permeability was measured in the wild type P2X2, P2X3, P2X4 and P2X7 receptors and their mutants after substitution of conserved tyrosine with alanine. Changes in intracellular calcium were measured in the presence and in the absence of ATP, and in the presence of 5 mM Ca^{2+}.in the extracellular solution. We found that substitution of the TM1 tyrosine with alanine increased the resting membrane permeability for Ca^{2+} ions in the absence of ATP in the P2X2 and P2X4 receptors. In the case of P2X3 receptor this effect could not be examined because of fast desensitization of this receptor which is completed within 2 s. In the P2X7 receptor, which exhibit several specific properties including pore dilations in the prolonged presence of agonist, this effect was not observed. These results indicate that conserved tyrosine in TM1 of slowly desensitizing P2X2 and P2X4 receptors stabilizes the closed state of the ion channel and prevents spontaneous pore dilation.

THE EFFECT OF NEWLY SYNTHETISED NEUROSTEROIDS ON NMDA RECEPTORS

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N-methyl-D-aspartate (NMDA) receptors are a subtype of ionotropic glutamate receptors, which participate in fast excitatory synaptic transmission and play a key role in synaptic plasticity. NMDA receptor overexcitation leads to cell death that underlies many serious neurological and psychiatric disorders. NMDA receptors can be modulated by numerous compounds, including neurosteroids, which are endogenous steroids produced in the central nervous system. Pregnanolone sulphate (3α5βS) is a naturally occurring neurosteroid that blocks NMDA receptor by a voltage-independent "foot in the door" mechanism (1,2). Compounds which share this mechanism of action have a use-dependent onset but a use-independent offset of block. We have recently found that neurosteroids with D-ring modifications maintain biological activity and some of these modifications produce even more potent inhibitors than the endogenous 3α5βS (3). This demonstrates that the acetyl moiety on the D-ring of 3α5βS is not necessary for the inhibitory action of this neurosteroid on NMDA receptors. To further reveal the fundamental structural features of 3α5βS that are crucial for its inhibitory activity, we have synthesized a neurosteroid analogue (2R,4aS,4bS,8aR,10aR)-4β,14β-dimethyltetradecahydrophenanthren-2-yl sulphate (PAS-D) lacking the D-ring of the steroidal core. The results of our experiments show that PAS-D, like 3α5βS, blocks NMDA receptors in a voltage-independent manner and with a "foot in the door" type of blockade. The IC50 value of PAS-D on GluN1-1a/GluN2B was estimated to be 12 ± 1 μM, which is almost 4x times lower than the IC50 of 3α5βS ($44{,}49 \pm 8$ μM) (1). In contrast to 3α5βS which is twofold more potent at inhibiting responses mediated by GluN1/GluN2C-D receptors than those mediated by GluN1/GluN2A-B receptors (1), our experiments do not show any significant subunit selectivity of PAS-D on NMDA receptors composed of GluN1-1a/GluN2A-D subunits. We conclude that the inhibitory effect of neurosteroids on NMDA receptors does not require the D-ring, however, the D-ring may have some effects on affinity and subunit specificity.

[1] Petrovic M, Sedlacek M, Horak M, Chodounska H & Vyklicky L. J Neurosci 25(37):S8439-8450,2005

[2] Vyklicky V, Krausova B, Cerny J, Balik A, Zapotocky M, Novotny M, Lichnerova K, Smejkalova T, Kaniakova M, Korinek M, Petrovic M, Kacer P, Horak M, Chodounska H & Vyklicky L. Sci Rep:S1-15,2015

[3] Kudova E, Chodounska H, Slavikova B, Budesinsky M, Nekardova M, Vyklicky V, Krausova B, Svehla P, Vyklicky L. J Med Chem 59:S5950-5966,2015

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HUMAN SPATIAL NAVIGATION STRATEGIES IN VIRTUAL ENVIRONMENTS

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Spatial cognition is a field that combines cognitive psychology and neurology. In my master thesis I designed several experiments based on the Carousel maze task with the use of game engine Unity3D, which allowed me to take advantage of controlled and realistic experimental environment in virtual reality. These experiments tested the process of accessing two discrete spatial reference frames: room-centred and object-centred. The research tried to answer whether these reference frames interface during the learning phase and whether their access is simultaneous or sequential. Previous research, both psychological [1, 2] and physiological [3], suggested that internal representations of stable and moving reference frames exist independent of each other and subjects require some time to switch between them. But the researchers focused on discrete representations rather than encouraging the merge. Experiments in my thesis were designed in a way that promoted the use of both at the same time. Obtained data were nevertheless concordant with previous findings and imply that the two representations seem to coexist independently, even when the merge is encouraged. Subjects tend to manifest longer reaction times during the spatial task when they need to change the reference frames in order to provide a correct answer. But the data also suggest that the switch from the object-centred reference frame does not occur prior to the task itself, which is conflicting with previous studies.

[1] Wang, R. F., & Brockmole, J. R. (2003). Human navigation in nested environments. Journal of Experimental Psychology: Learning, Memory, and Cognition, 29(3), 398–404.

[2] Brockmole, J. R., & Wang, R. F. (2002). Switching between environmental representations in memory. Cognition, 83(3), 295–316.

[3] Kelemen, E., & Fenton, A. A. (2010). Dynamic grouping of hippocampal neural activity during cognitive control of two spatial frames. PLoS Biology, 8(6), e1000403.

EFFECTS OF CLOMIPRAMINE AND RISPERIDONE ON LEARNING ANF FLEXIBILITY IN AN ANIMAL MODEL OF OBSESSIVE-COMPULSIVE DISORDER

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Chronic sensitization of dopamine D2/D3 receptors by agonist quinpirole (QNP) induces compulsive checking behaviour in rats, which is considered an animal model of obsessive-compulsive disorder (OCD). Previous study revealed deficit in cognitive flexibility in QNP sensitized rats (1). This thesis focused on determining if this cognitive flexibility deficit is ameliorated by co-administration of clomipramine (CMI), risperidone (RIS) or combination of both (CMI+RIS) to QNP treatment. Aversively motivated active place avoidance task on a Carousel maze with reversal was used. The number of entrances into a to-be-avoided shock sector was evaluated as measure of performance. Six treatment groups were used: control group, QNP group, CMI group, QNP/CMI combination, QNP/RIS combination and QNP/CMI/RIS combination. Surprisingly, when compared alone, significantly worse acquisition was observed for QNP group compared to control group. However, similarly to previous study, QNP group had a worse performance in a first reversal session compared to control group. When all groups were compared, only QNP/CMI group had worse initial learning compared to control group. In reversal learning, only QNP treated group had a significantly more entrances than control group in first reversal session. Results suggest that co-treatment with CIM reduces overall learning, while co-treatment with RIS or CMI combined with RIS improves reversal learning.

[1] Hatalova, H., Radostova, D., Pistikova, A., Vales, K., & Stuchlik, A. (2014). Spatial reversal learning in chronically sensitized rats and in undrugged sensitized rats with dopamine D2-like receptor agonist quinpirole. *Frontiers in behavioral neuroscience, 8*.

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THE EFFECT OF MK-801 ON A COGNITIVE FLEXIBILITY IN GRADUALLY IMPEDING VARIANTS OF THE SPATIAL REVERSAL TASK ON THE ROTATING ARENA (CAROUSEL)

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Cognitive flexibility allows animals to dynamically adapt their behavior to environmental changes. Flexibility impairments are important symptoms of schizophrenia. We tested flexibility in a rat model of psychosis using acute treatment with MK–801, a non-comeptitive NMDA receptor antagonist. The Carousel is a slowly rotating circular platform where rats have to avoid an unmarked place entrance of which is punished by a mild foot shock. Rotation of the arena separates cues in two sets, forming incongruent "reference frames": room frame (RF) – defined by distal cues in the room; and arena frame (AF) – defined by the rotating arena surface (e.g. scent and self-motion cues). The first phase of the experiment was acquisition – rats were trained to avoid sector fixed in one position in one of the reference frames during five daily sessions. In the second phase, sector position or reference frame was changed. MK-801 was applied in the second phase, 30 min before each session. There were three groups of rats in each variant (receiving doses of 0.05 and 0.10 mg/kg).

Four experiments differed in a complexity of the change. In Variant A sector was defined in RF during acquisition. In the second phase it remained in the same position, but the direction of arena rotation was opposite. This measured motor skills and was not cognitively demanding. There was no difference between MK-801 groups and controls. In Variant B, rats were first trained to avoid sector in RF and then the sector was switched to the opposite side of the arena, yet in the same reference frame. This requires simple reversal learning. The group with higher dose of MK-801 tended to be impaired. In Variants C and D sector position changed from one reference frame to another (AF → RF and RF → AF, respectively). This kind of change resembled extradimensional set shift and presents a demand for cognitive flexibility. Rats treated with 0.10 mg/kg of MK–801 were significantly impaired.

MK-801 administration did not affect ability to learn and solve simpler cognitive tasks, but other variants were sensitive enough to distinguish subtle impairment of cognitive flexibility. Such more complex versions of contingency changes may be more sensitive and accurate tests of cognitive impairment appearing in early stages of psychiatric diseases.

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MODEL OF FOCAL CORTICAL ISCHEMIA AND ITS PARAMETRIZATION

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Work objectives: The aim of the diploma thesis was to apply modified model of focal cortical brain ischemia induced by phototrombosis and subsequently determinate its parameters.
Methods: Intravenous application of Rose Bengal dye was followed by continual illumination of green laser beam over the left sensorimotocortex for 10 minutes. Following illumination, the dye is activated and produces singlet oxygen that damages components of endothelial cell membranes with subsequent platelet aggregation and thrombin formation, which eventually determines the interruption of local blood flow. This approach, initially proposed by Rosenblum and El-Sabban in 1977, was later improved by Watson in 1985 in rat brain. For histological evaluation of ischemic brain damage, animals were overdosed with urethane and transcardially perfused.
Results: Histological examination of brain showed significant ischemic damage of cerebral cortex in all experimental animals. Lesion was located in left hemisphere and its extent varied through the grey matter. Size of lesion, its localization and depth has shown only a small variability between animals.
Key words: photothrombosis, ischemia, rat, blood brain barrier, neurovascular unit, epilepsy

MORPHOLOGICAL CHANGES OF THE HIPPOCAMPUS IN TETANUS TOXIN MODEL OF TEMPORAL LOBE EPILEPSY

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Temporal lobe epilepsy is the most common form of epilepsy and hippocampal sclerosis represents the main underlying structural abnormity. Approximately 20% of TLE cases are non-lesional due to absence of any obvious epileptogenic lesion and tetanus toxin model is traditionally considered as a model of non-lesional temporal lobe epilepsy. The main aim of this study was to evaluate the presence of the cell damage and to determine its spatiotemporal profile. Tetanus toxin was stereotaxically injected into CA3 subregion of dorsal hippocampus in adult male Wistar rats. Brain tissue was extracted 4, 8 and 16 days following the surgery. Postfixed brains were sectioned to 50 μm slices and labeled using Nissl's and FluoroJade B staining (FJB). Hippocampal sclerosis was present only in animals from D16 group, however, it was localized mainly in contralateral CA1 area. Additional finding was decreased Nissl's staining in contralateral hippocampus which corresponded with the presence of FJB positive neurons. In animals from group D8, we have identified presence of FJB positive neurons predominantly in ipsilateral hippocampus. In D4 animals, cellular degeneration was absent. To examine the non-lesional nature of tetanus toxin model, we have performed blind study, when Nissl's staining were reviewed independently by two experience of neuropathologists. Results suggest that from the perspective of classical neuropathology the tetanus toxin model can be still classified as a non-lesional. This study brings new findings on morphological properties of tetanus toxin model. Neuronal loss is present in specific hippocampal subregions. The cell loss has specific time dependent spatial patter. Qualitatively this pattern differs from morphological findings observed in lesional models of temporal lobe epilepsy. Finally, presence of microscopic cell loss must be always considered when this model is used for experimental purposes.

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THE ROLE OF CRMP2 ISOFORMS IN ALZHEIMER'S DISEASE

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Collapsin response mediator protein 2 (CRMP2) is a microtubule associated protein in the nervous system downstream of Semaphorin-3 signaling pathway and a mediator of growth cone collapse. There are two splice variants of CRMP2, CRMP2A and CRMP2B, which differ in their localization and function, both strongly depending on their phosphorylation state. Hyperphosphorylation of CRMP2 at the Cdk5 and GSK3 target sites is an early event of pathogenesis in brains of Alzheimer's disease (AD) patients and is likewise observed in AD mouse models. In both cases, it co-localizes with neurofibrillary tangles of hyperphosphorylated tau. In AD, proteolytic cleavage of Cdk5 regulatory subunit p35 produces p25 fragment leading to malfunctioning of the kinase. Resulting mislocalization and hyperactivation of Cdk5 is partially responsible for hyperphosphorylation of both CRMP2 and tau. Recently, we demonstrated that CRMP2A is a substrate of prolyl isomerase Pin1 and that Pin1 stabilizes CRMP2A in distal axons. Pin1 binds to target proteins that have been phosphorylated on Ser/Thr-Pro motif and switches between cis and trans conformations, therefore affecting protein functions. Pin1 knock-out mice develop age-dependent tau and Aβ pathologies and neuronal degeneration and loss, suggesting neuroprotecitve role of Pin1 regulation of protein conformation.

In order to study the effect of hyperactivation of Cdk5 on CRMP2, we use transgenic mouse model expressing p25 fragment (Jackson Laboratories) in which two fold increase in Cdk5 activity is observed. Our preliminary data indicate, that elevated activity of Cdk5 in these mice results in increased phosphorylation of CRMP2A. Currently, we breed p25 transgenic mice with Pin1 knock-out mice. Combination of p25 hyperactivated Cdk5 and absence of Pin1 in the new mouse model could provide a better insight into functioning of these proteins and their role in CRMP2 metabolism and AD. In addition to Pin1 deficiency, we will analyze also the effect of combined Pin1 and Cdk5 upregulation *in vivo.* For this purpose, we will use *in utero* electroporation to overexpress Pin1 in p25 transgenic mice and characterize the effect on CRMP2A phosphorylation and localization *in vivo*. In order to better understand the effect of CRMP2A phosphorylation in neural development, we will use primary neuronal cultures isolated from CRMP2 knock-out mice, transfect them with CRMP2A or one of its mutant forms (either mimicking phosphorylation of CRMP2A at Ser27 or, in opposite, preventing the phosphorylation at this site) and evaluate their effect on the length of the axons, their branching or numbers of dendrites. Our experiments should further elucidate the role of CRMP2 in neurite outgrowth and AD pathogenesis.

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THE ROLE OF ATP-SYNTHASE INHIBITOR PROTEIN IF1 IN PANCREATIC BETA CELL PHYSIOLOGY

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Mitochondrial ATP synthase, complex V of the mitochondrial respiratory chain, catalyses the ATP synthesis from ADP and inorganic phosphate when coupled to transmembrane proton transport via F_O subunit. Besides its role in producing majority of cellular ATP, it regulates mitochondrial membrane potential, mitochondrial cristae structure, rate of ROS generation, ion homeostasis and other processes. ATP synthase is regulated by an endogenous protein, Inhibitory Factor 1 (IF1), which is a small (10 kDa) nuclear-encoded peptide. Up to now it was thought that the role of IF1 is to inhibit hydrolysis of ATP by the ATP synthase under conditions, when the proton gradient across the mitochondrial inner membrane is lost, such as during hypoxia/ischemia. Nonetheless, more recent data show that IF1 regulates also ATP synthesis and it was suggested that it might work as a switch between oxidative and glycolytic metabolism. Besides its role in the regulation of cellular ATP levels IF1 was proposed to induce oligomerization of ATP synthase (1).

In pancreatic beta cells, ATP level is a key parameter effecting insulin secretion. In order to address key questions of molecular regulations of ATP synthase, we will study the effect of inhibitor factor IF1 on ATP levels during glucose-stimulated insulin secretion in insulinoma INS1E cells, which serve as a study model of pancreatic beta cells. We have already established techniques of IF1 transient silencing in INS1E mediated by siRNAs. We aim to compare ATP levels, ATP synthase oligomerization, membrane potential, mitochondrial superoxide generation rates, and insulin release rates in these cells and corresponding negative controls. Next, we will create INS1E cell line with IF1 knock-out via CRISPR/Cas9 technology which will facilitate long-term studies. Besides determining the basic bioenergetics parameters we plan to visualize IF1 together with other proteins embedded in the inner mitochondrial membrane using high-resolution microscopy, namely, super-resolution dSTORM microscopy and STED microscopy.

[1] Faccenda D., Campanella M., International Journal of Cell Biology, 2012.

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