Annual Symposium of the Doctoral School of Molecular Medicine (Academic Year 2017-2018)

University of Debrecen
September 6, 2018

Program

Date: September 6, 2018

Location: F003-004 Lecture Hall, Life Science Building, University of Debrecen

8:25 Arrival (Coffee, cakes)

8:40 Prof. János Szöllősi: Welcome address

Session 1
Chair: Prof. János Szöllősi
Head of the "Membrane biophysical questions and research methods" doctoral program

8:45 Azzam Aladdin (2nd year PhD student)
CHARACTERIZATION OF THE ROLES OF TG2 AND DRP1 INTERPLAY IN HUNTINGTON'S DISEASE MODEL
Supervisor: Krisztina Tar, Róbert Király

9:00 Shahrzad Alimohammadi (3rd year PhD student)
HUMAN MONOCYTE-DERIVED LANGERHANS CELLS EXPRESS MULTIPLE PROINFLAMMATORY RECEPTORS LINKED TO PRURITUS
Supervisor: Tamás Bíró Attila, Gábor Szöllősi

9:15 Tamás Czirják (3rd year PhD student)
EXAMINATION OF THE MOLECULAR BACKGROUND OF THE DECREASED MUSCLE PERFORMANCE
Supervisor: Dr. Dienes Beatrix

9:30 Eliza Guti (1st year PhD student)
GENETIC ENGINEERING BY USING OF CRISPR-CAS9 SYSTEM
Supervisor: Zsuzsanna Polgár

9:45 Tímea Hajdu (3rd year PhD student)
FUNCTIONAL ANALYSIS OF FLUORESCENTLY LABELLED ANTIBODIES
Supervisor: Péter Nagy

Break (15 min) Coffee and cakes

Session 2
Chair: Prof. Ferenc Erdődi
Department of Medical Chemistry, UD

10:15 Balázs Kelemen (3rd year PhD student)
PRURICEPTIVE ROLE OF TRPM3
Supervisor: Dr. Tóth István Balázs

10:30 Ádám Kenesei (1st year PhD student)
STUDYING INTERLEUKIN-15 TRANS-PRESENTATION BY FLUORESCENCE MICROSCOPY
Supervisor: György Vámosi

10:45 Lukas Lau (visiting scientist)
COMBINING IMAGING FCCS WITH ALTERNATING LASER EXCITAITON
Supervisor: Katalin Tóth

11:00 Evelin Major (2nd year PhD student)
INVESTIGATION OF THE ROLE OF SMOOTHELIN-LIKE PROTEIN 1 IN HYPERTHYROIDISM
Supervisor: Beáta Lontay

11:15 Noémi Miltner (3rd year PhD student)
ASSESSMENT OF THE ANTI-INFLAMMATORY EFFECTS OF BCP, CBD AND SEMI-SYNTHETIC PHYTOCANNABINOIDS IN IN VITRO HUMAN AND MOUSE INFLAMMATORY MODELS
Supervisor: Prof.Dr.Tamás Bíró, Dr.Johanna Mihály

Break (15 min) Coffee and cakes

Session 3
Chair: Dr. Tamás Bányász
Department of Physiology, UD

11:45 Marianna Nagy (3rd year PhD student)
INVESTIGATION OF THE CHANGES OF REGIONAL HEMODYNAMIC PARAMETERS INDUCED BRAIN NEURAL ACTIVITY
Supervisor: Miklós Emri

12:00 Roland Veress (3rd year PhD student)
INTERSPECIES DIFFERENCES IN THE LATE SODIUM CURRENT MEASURED DURING THE VENTRICULAR ACTION POTENTIAL
Supervisor: Dr. Balázs Horváth

12:15 Anita Vladár (1st year PhD student)
THE ROLE OF EPIDERMAL TRP CHANNELS IN ITCH SIGNALING
Supervisor: István Balázs Tóth

12:30 Florina Zákány (predoc student)
CHARACTERIZING THE EFFECTS OF DIFFERENT MEMBRANE LIPIDS ON THE GATING OF KV1.3 ION CHANNELS USING VOLTAGE-CLAMP FLUOROMETRY TECHNIQUE
Supervisor: György Panyi

12:30-13:30 Buffet Lunch

ABSTRACTS

Azzam Aladdin (2nd year PhD student)
Department of Medical Chemistry

CHARACTERIZATION OF THE ROLES OF TG2 AND DRP1 INTERPLAY IN HUNTINGTON'S DISEASE MODEL

Huntington's disease (HD) is a neurodegenerative disease caused by the expansion of polyglutamine (polyQ) repeats in the huntingtin protein (Htt) resulting in increased physical and mental disabilities. Current pharmacological treatments of HD do not prevent or slow down the progression of this devastating disease. Many studies report mitochondrial dysfunction in various tissues and animal models for HD suggesting that mitochondrial homeostasis is impaired. In addition, higher level of transglutaminase type2 (TG2) comparing to related controls play role of the development of the disease. We opt to focus on describing the altered mitochondrial dynamics and activity, particularly focusing on Drp1. We established a model using patients' derived fibroblasts with different polyQ repeats. We characterized mitochondrial morphology, bioenergetics, then measured lactate dehydrogenase (LDH) release in HD models. We found that mitochondria morphology is altered to tubular ring-shape like structure in HD patient cells, in addition to down-regulation of Drp1 at both, protein and gene expression levels.

We have started to set up an immortalization protocol of HD patients' derived primary fibroblasts by overexpressing hTERT and Bmi1. In parallel, we also established a trans-differentiation protocol from patients' derived fibroblast to neurons. The characterization of neuron like cells is ongoing including the detection of special neuronal markers at both, gene expression and protein levels. We characterize the neurite outgrowth and branching and we also investigate mitochondrial integrity in neurons derived from HD patients' and healthy fibroblasts.

HD patient neurons exhibit morphological abnormalities, thus providing a unique model to study HD neuropathology in the context of patient genotype. The model allows us to perform confocal microscopy studies to determine the localization and aggregate formation of mutant huntingtin.

This study is ongoing and the more general and ambitious objective is to study the dynamics of HD-related pathobiology and to have a reliable model which can be a useful tool for validation of therapeutic targets.

Supervisor: Krisztina Tar, Róbert Király

Shahrzad Alimohammadi (3rd year PhD student)
Department of Immunology

HUMAN MONOCYTE-DERIVED LANGERHANS CELLS EXPRESS MULTIPLE PROINFLAMMATORY RECEPTORS LINKED TO PRURITUS

Langerhans cells (LCs) are the only professional antigen presenting cell of the human epidermis under steady state conditions. Their role in various types of skin inflammatory conditions has been most extensively researched in mouse models, yet there is a brevity of information regarding their exact role in human skin. Most research focused on LCs uses primary cells isolated from the epidermis, which are activated as a consequence of the procedure.
The possible role of LCs in the most prevalent skin symptom, pruritus has not been investigated to date. In our current study we utilized monocyte-derived LCs as a model of LCs that have not undergone maturation to investigate the expression of receptors that have been implicated in pruritic signaling in both neuronal and non-neural elements of the skin. Of the investigated receptors LCs express histamine receptors HRH1, HRH4, toll-like receptor 3 (TLR3), Transient receptor potential vanilloid (TRPV) 1, TRPV2 and TRPV4. Treating LCs with agonists to TLR3, TRPV1 and TRPV4 (polyinosinic:polycytidylic acid, capsaicin and GSK101679A, respectively) resulted in the upregulation of CD86 expression, as well as that of CXCL8, CCL20, CCR7, all of which are potent T-cell activators. Interestingly all three activators also increased the expression of TLR3, which could conceivably prime LCs to be more easily activated by subsequent inflammatory stimuli.
Overall our results highlight the possible role of novel proinflammatory pathways in LCs, and hints at the possible role of these antigen presenting cells in the development of pruritus.

Supervisor: Tamás Bíró, Attila Gábor Szöllősi

Tamás Czirják (3rd year PhD student)
Department of Physiology

EXAMINATION OF THE MOLECULAR BACKGROUND OF THE DECREASED MUSCLE PERFORMANCE

The muscle contraction starts with the excitation contraction coupling, where the membrane of the skeletal muscle depolarises and an action potential is generated. This stimulus reaches the transversal tubule, where the voltage sensor L-type Ca2+ channel (Cav1.1) changes its conformation by which the ryanodine-receptor (RyR) will open on the surface of the sarcoplasmic reticulum (SR). The resulting synchronous Ca2+ releases from the SR lead to the muscle contraction. In the Cav1.1ΔE29 mouse the embrionic isoform of the Cav1.1 is expressed, where it acts as a Ca2+ channel, but its role as a voltage sensor is also retained. Moreover there are spontaneousCa2+ events can be observed on isolated muscle fibers. We also examined alterations of gene expressions where we found a small decrease in the level of Ca2+-binding calsequestrin protein in the Cav1.1ΔE29 animal.
Spontaneous events in the Cav1.1ΔE29 mouse were characterized based on their intensity profiles, distribution and their pharmacological response. Three main groups were separated: sparks are short (46,0±1,5 ms), embers are long events (473±14 ms) and there were, so called, complex events.
In aging, decreased physical activity and reduced muscle mass (sarcopenia) lead to impaired muscle force and increased fatigability. One potential cause could be the modified calcium homeostasis.Our result show that training and selenium as an essential trace element have a significant impact on muscle functions. In different groups (control, Cmpt, selenium diet, trained) of aged (20 months) animals the changes in intracellular calcium concentration and the rate of sarcoplasmic reticulum (SR) calcium release were calculated on enzymatically isolated intact flexor digitorum brevis muscle fibers, using Fura-2 AM. The results show that training helps to maintain the normal calcium homeostasis. We also found beneficial effects of selenium consumption both on in vivo and in vitro muscle functions and, in addition, on calcium homeostasis in aged mice. Moreover, the RyR1 expression was also declined with aging, which was reversed by longterm training.

Supervisor: Beatrix Dienes

Eliza Guti (1st year PhD student)
Department of Medical Chemistry

GENETIC ENGINEERING BY USING OF CRISPR-CAS9 SYSTEM

Background: CRISPR-Cas9 technology is a technique that has revolutionized the sequence-specific gene editing. This system has two major components: the Cas9 nuclease and the sequence-specific guide RNA (gRNA). Once a specific DNA region is matched by the gRNA carrying Cas9, the target DNA is cleaved with a double stranded break so it could be used for gene editing. Our research group examines the role of PARP1 in PARylation for years. Poly-ADP ribose polymerase 1 (PARP1) is one of the key enzyme which takes part of this phenomenon. A wide scale of proteins can be targets of PARylation. These acceptors play role in DNA repair, metabolism, gene expression or differentiation as well. The PARP1 can be activated by means of PARylation or phosphorylation. In the second case, following its activation, the enzymes could regulate the cell differentiation. However, it is still questionable whether the role of the PARP1 regulator in cell differentiation is possible, after it has been activated by PARylation. Therefore, we would to examine the role of PARylation in NMNAT1, PARP1 and PARG knock out (KO) human induced stem (hiPS) cells.
Methods: PARP1 specific gRNAs were designed by in silico. The annealing of single stranded gRNAs were performed by PCR. The px458 expression vector which contains the sequence of Cas9, was digested with the BpiI restriction enzymes to linearize the vector for ligation. Supercompetent E. coli cells were transformed with this vector. After the plasmid isolation and purification, HEK cells, as models were transfected and sorted to generate PARP1 knock out cell lines. The analysing of PARP1 gene expression was examined by qPCR, while the PARP1 protein synthesis were followed up by Western blot.
Results: The PARP1 gene expression of transfected and sorted HEK cells shown significantly lower values compared to controls which results were verified by Western blot as well.
Conclusion: Based on the results of qPCR and Western blot we can conclude that our CRISPR-Cas9 construct is suitable to generate functional PARP1 KO cell lines.

Supervisor: Zsuzsanna Polgár

Tímea Hajdu (3rd year PhD student)
Department of Biophysics and Cell Biology

FUNCTIONAL ANALYSIS OF FLUORESCENTLY LABELLED ANTIBODIES

The degree of labelling (DoL) of antibodies has an important role in quantitative fluorescence measurements. Previously our workgroup found that the affinity of the epitope binding domain decreases as a function of the labelling ratio (Szabó Á. et al, 2017).
Our objective was to investigate the function of different domains of the antibody depending on the DoL.
For our experiments we chose trastuzumab (Herceptin) that is widely used for treating breast malignancies. It binds HER2 (ErbB2) receptor. We used trastuzumab conjugated with Alexa-546 and Alexa-647 dyes. SKBR-3 (human breast carcinoma) and THP-1 (human monocytic leukemia) cell lines were used for the experiments. The labelling ratios of trastuzumab varied from 0.61 to 2.94 for Alexa-546, and between 0.83 to 3.47 for Alexa-647.
Quantum Simply Cellular beads were used to investigate the function of the whole antibody as they contain Fc specific capture antibodies on their surface whose epitopes are randomly distributed in the Fc region. The function of the epitope binding region was estimated by measuring trastuzumab binding to SKBR-3 cells. We found that there is no difference between the antibody binding of cells and beads and this result led us to investigate the function of further antibody domains. Since the CH1 domain and the region between the CH2 and CH3 domains bind Protein-G and –A, respectively, we used Protein-G and Protein-A -coated beads to assess the functional sensitivity of these domains to fluorescence labelling.
The function of the region between the CH2 and CH3 domains was investigated by Fc receptor binding assays as well. THP-1 cells were pre-treated with interferon for 48 hours that increases the expression of FcR twofold. We found that the DoL influences Protein-A, Protein-G and Fc receptor binding to an extent comparable to its effect on epitope binding.
As a conclusion we can state that the presence of the fluorescent dye affects the function of the antibody in a global manner; as not only the function of the epitope binding domain weakens as a function of the DoL but the Fc domain and its several regions also tend to be sensitive to the fluorescent labelling.

Supervisor: Péter Nagy

Balázs Kelemen (3rd year PhD student)
Department of Physiology

PRURICEPTIVE ROLE OF TRPM3

Pruritus is one of the most widespread symptom in dermatology. Typically, during the molecular transduction of itch, the activation of metabotropic receptors on the pruriceptive nerve endings stimulated either by exogenous pruritogenic molecules or endogenous signals, leads to the activation or sensitization of an ion channel crucial in the consequent depolarization of the neuronal membrane. Recent research indicated that these ion channels mostly belong to the transient receptor potential (TRP) family of ion channels and show significant overlap with those involved in nociception (TRPV1, TRPA1). TRPM3 was recently identified as a novel thermosensitive channel contributing to nociception. However the role of TRPM3 in the development of pruritus has not been studied yet. Therefore, in the current project we aim at investigating the potential role of TRPM3 in the transduction of pruritus.
We investigated the itch inducing effect of various pruritogenic substances on wild type and TRPM3-/- C57/Bl6 mice, in the “cheek model” paradigm, which allows to differentiate the nociceptive and pruriceptive behavioural responses. During this paradigm, pruritogenic substances diluted into 10µl vehicle (PBS+7%TWEEN-80) were injected subcutaneously into the cheek of mouse and reactive behaviour were recorded and analysed quantitatively
We investigated the pruritogen histamine and the algogen capsaicin induced itch and pain on n=8-13 wt and TRPM3-/- mice, compared with vehicle treatment. To characterise the behavioural responses we determined the “itch ratio” as a novel parameter. Our results clearly indicate, that the “cheek model” as it was previously described in the literature, is perfectly suitable to differentiate between pain and itch related behavioural responses. After a subcutaneous injection with the test compounds into the cheek of the mouse. Our findings argue for the involvement of TRPM3 only in certain pain, but not itch responses: histamine and serotonin evoked similar scratching behaviour on both TRPM3+/+ and TRPM3-/- animals; whereas TRPM3 activator Pregnenolone Sulphate evoked only pain related responses which were abolished in TRPM3-/- animals.

Supervisor: István Balázs Tóth

Ádám Kenesei (1st year PhD student)
Department of Biophysics and Cell Biology

STUDYING INTERLEUKIN-15 TRANS-PRESENTATION BY FLUORESCENCE MICROSCOPY

Interleukin-15 (IL-15) cytokine growth factor is a member of type I cytokines playing a pivotal role in the regulation of the immune system. The main sites of its synthesis are dendritic cells and monocytes. The IL-15 receptor (IL-15R) consists of three subunits, IL-15Rα, IL-2/15Rβ and the common γc chain (also used by IL-2R, IL-4R, IL-7R, IL-9R and IL-21R). The IL-15R principally uses trans-presentation for signal transduction. During this process a professional antigen presenting cell (e.g. dendritic cells) or epithelial cell expresses IL-15 already bound to the IL-15Rα subunit and presents the cytokine to the βγc heterodimer complex located on T- or natural killer cells.
To study the mechanism of trans-presentation our work group created a model system. A B-cell (Raji) and a T-cell (Jurkat) forms a stable immunological synapse if we treat the B-cells with Staphylococcus Enterotoxin E (SEE) antigen. The SEE bounds to the major histocompatibility complex class II (MHC II) on the B-cell and to the T-cell receptor (TCR) on the T-cell, thus forming an immunological synapse between the two cells. We created different cell lines with viral transduction: a Raji cell line expressing IL-15Rα tagged with mCherry, and a Jurkat cell line expressing untagged IL-2/15Rβ. We have directly shown the formation of the IL-15R complex during trans-presentation by Förster resonance energy transfer (FRET) between the IL-15Rα and the IL-2/15Rβ subunits. We also study whether the MHC II-TCR complex is necessary or at least a facilitating factor for trans-presentation to occur. We will also investigate the fate of the IL-15Rα subunit following trans-presentation. It has been proposed that the extracellular domain of the IL-15Rα chain is internalized with the βγc complex by the T cell. By using confocal microscopy we can detect the translocation of said domain, and follow its path in the T cell.

Supervisor: György Vámosi

Lukas Lau (visiting scientist)
Biophysics of Macromolecules, German Cancer Research Center

COMBINING IMAGING FCCS WITH ALTERNATING LASER EXCITAITON

The majority of transport and interaction in the cellular context is carried out via passive transport, i.e., diffusion-mediated processes. Fluorescence (Cross) Correlation Spectroscopy (FC(C)S) is a very efficient method to examine transport dynamics and molecular binding in this context, whereas Förster Resonance Energy Transfer (FRET) is used to measure distances between fluorophores on a nanometer scale. The latter gives valuable information about conformational diversity and proximity in molecular interactions. We seek to combine the two methods in a spatially resolved manner by alternating laser excitation in imaging FCCS. This can be achieved by a Single Plane Illumination Microscopy (SPIM) setup, in which a two-channel EMCCD camera gives sufficient time resolution for FCCS on macromolecules. Extending the setup with alternating excitation will eliminate cross-talk in FCCS and allow assessing information about FRET from the same data set. For low cross-correlations - which can be observed in many biological applications- this is crucial to distinguish true positive from false positive results due to cross-talk. Hence, we expect a considerable improvement in data reliability. The RAR-RXR system will be the first target to be examined with this method.

Supervisor: Katalin Tóth

Evelin Major (2nd year PhD student)
Department of Medical Chemistry

INVESTIGATION OF THE ROLE OF SMOOTHELIN-LIKE PROTEIN 1 IN HYPERTHYROIDISM

Hyperthyroidism is one of the most common endocrine diseases, however, the molecular mechanism behind its symptoms such as muscle pain, muscle weakness and reduction of muscle mass has not been described yet. Experiments with mice show that elevated thyroid hormone (TH) concentration induce fiber type switch in skeletal muscle (SKM). We hypothesize that there is another molecule beside the THs which can cause fiber type switch, namely the smoothelin-like protein 1 (SMTNL1). Upon its phosphorylation by protein kinase A (PKA) it translocates into the nucleus and serves as a transcriptional cofactor. According to our previous data smtnl1-/- knockout mice had increased myosin heavy chain 2A isoform (MyHC2A) levels. Human Phospho-Kinase Array was applied to compare SKM samples of euthyroid and hyperthyroid patients. It showed significant changes in the phosphorylation levels of e.g. mTOR, AMPKα2 and STAT5a and b. These changes are in line with our microarray results in which AMPK, JAK/STAT and mTOR signaling pathways were activated or inhibited. Human SKM samples were analysed with Western blotting to investigate the effect of hyperthyroidism on the expression of markers of the different fiber types. The expression of MyHC2A was decreased while myosin heavy chain 2B (MyHC2B) expression was increased in hyperthyoid samples. After differentiation and SMTNL1 overexpression of C2C12 mouse myoblast cells, myotubules were treated with THs which resulted in an increase in the relative phosphorylation of SMTNL1 while the amount of it did not change significantly. Based on these observations, we suppose that under hyperthyroid conditions the predominantly oxidative 2A fibers transformed into more glycolytic 2B fibers and SMTNL1 plays a role in this process. Additionally, TH treatment hampered the expression of the thyroid receptor α (TRα) and the overexpression of SMTNL1 boosted this effect. To ensure that PKA is involved in these processes a PKA agonist was applied in combination with SMTNL1 overexpression and we experienced an even more pronounced decrease in TRα expression. These lines of evidence suggest a role of SMTNL1 in thyroid hormone signaling through PKA in SKM.

Supervisor: Beáta Lontay

Noémi Miltner (3rd year PhD student)
Department of Immunology

ASSESSMENT OF THE ANTI-INFLAMMATORY EFFECTS OF BCP, CBD AND SEMI-SYNTHETIC PHYTOCANNABINOIDS IN IN VITRO HUMAN AND MOUSE INFLAMMATORY MODELS

Cannabidiol (CBD) is the most abundant non-psychotropic phytocannabinoid present in the plant Cannabis sativa. Our group previously demonstrated that CBD and fluorinated CBD derivatives (F-CBDs) exert anti-inflammatory effect in human dermatitis models. In our current experiments, we aimed
a) at assessing the potential anti-inflammatory effect of β-caryophyllene (BCP) sesquiterpene in previously optimized in vitro epidermal keratinocyte models
b) at examining the anti-inflammatory effect of BCP, CBD and F-CBDs in a murine macrophage inflammation.model
Effects of BCP, CBD and semi-synthetic F-CBDs (HUF-101, HUF-103, HU-559a) on cell viability of HPV-KER, RAW 264.7 murine macrophages and RAW-Blue reporter cells was investigated by colorimetric MTT assay. Gene expression and protein release of pro-inflammatory cytokines were assessed by RT-qPCR and ELISA. SEAP activity measurement was performed by Quanti-Blue assay on RAW-Blue cells.
BCP did not reduce the viability of HPV-KER in any of the applied concentrations. 10 μM concentration of CBD and HUF-101 reduced the viability of RAW 264.7 cells after 24h, while the viability of RAW-Blue cell was reduced when 30 μM CBD and 300 μM BCP was applied for 24h, long term (72 h) cell viability however was not influenced.
a) Expressions of pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, IL-8, TNF-α) were significantly down-regulated upon the administration of BCP in most human keratinocyte models.
b) BCP and CBD decreased mRNA expression levels of pro-inflammatory cytokines in LPS-induced in vitro inflammatory mouse model however the F-CBDs exhibited significantly higher efficacy than CBD. SEAP activity was reduced by all applied concentrations of CBD but not BCP on RAW-Blue cells.
Our study provides the first evidence that BCP exerted anti-inflammatory actions on human epidermal HPV-keratinocytes and on RAW murine macrophages. Fluorinated phytocannabinoids proved to be more effective than CBD in an in vitro pro-inflammatory murine model. Therefore, these agents could be explored in future trials in the treatment of various inflammatory diseases.

Supervisor: Tamás Bíró, Johanna Mihály

Marianna Nagy (3rd year PhD student)
Department of Medical Imaging

INVESTIGATION OF THE CHANGES OF REGIONAL HEMODYNAMIC PARAMETERS INDUCED BRAIN NEURAL ACTIVITY

Objectives: For the examination of hemodynamic parameters resting state (rs) and task fMRI measurements was used. According to our prior knowledge two methods are available to determine these parameters from rsfMRI measurements: Dynamic Causal Modeling (DCM) and Blind Deconvolution (BLD). The first aim of this research was to statistically compare the hemodynamic parameters calculated by these methods. During the examination of the task fMRI data besides the hemodynamic parameters, we investigated the relationship between the motor areas and their connectivity changes in the healthy and affected hemisphere during passive leg movement in stroke patients.
Methods: Using the Human Connectome database, the rsfMRI data of 50 young right-handed adults (mean age: 28 years, SD: 3.26) were processed. For the analysis, we used the regions of the Default Mode Network (DMN). We calculated the hemodynamic parameters with DCM and BLD methods. For the task fMRI the data of 11 stroke patients were processed. The paradigm was based on passive leg movement on both the healthy and the paretic leg. The Group ICA Matlab Toolbox was used to emphasize the activated motor areas. Using DCM we evaluated the connectivity strengths and hemodynamic parameters of all stimulations of all patients.
Results: In the rsfMRI data the distribution of the parameters showed a negative binomial distribution, DCM provided minor variability. We received significantly different values for each parameter and for each region both methods. In the case of the task fMRI we have found that the connectivity strengths between ipsilesional SMA and M1 are significantly different in the healthy and the paretic sides. Our research has shown that hemodynamic parameters can be estimated with the Balloon model by DCM but the parameters do not change in stroke.
Conclusions: Further research is needed to answer the question of which method can be considered reliable to describe the hemodynamic response following spontaneous neural fluctuation. However our results shown, that in the case of task fMRI the DCM is a useful method to determine these parameters.

Supervisor: Miklós Emri

Roland Veress (3rd year PhD student)
Department of Physiology

INTERSPECIES DIFFERENCES IN THE LATE SODIUM CURRENT MEASURED DURING THE VENTRICULAR ACTION POTENTIAL

An increased late sodium current (INa,late) can bear pathophysiological role in several acquired heart diseases such as myocardial ischaemia and heart failure. The contribution of INa,late to the cardiac ventricular action potential (AP) has not been well characterized under physiological circumstances yet. Furthermore only sparse data are available on the differences in INa,late between species.
The aim of this work was to visualize and compare INa,late during canine, rabbit and guinea pig ventricular AP under close to physiological conditions.
Experiments were carried out on isolated cardiac myocytes obtained from left ventricles of adult dogs, rabbits or guinea pigs by enzymatic dispersion. The action potential voltage-clamp (APVC) method of the patch-clamp technique was used to visualize INa,late. Tetrodotoxin (TTX) was used as the inhibitor of INa,late.
The current density was proportionally increased to the increasing (1, 3, 10, 20 uM) TTX concentrations in canine cardiomyocytes.
The shape of the measured INa,late during the AP was opposite in the case of canine (decreasing during AP) cardiomyocytes compared to rabbit and guinea pig (increasing during AP) cells.
In canine ventricular cells under different command pulses (guinea pig, voltage ramp) the TTX sensitive current was decreasing (similar to the earlier showed canine results).
Pretreatment with 10 nM ATX-II resulted similar INa,late profiles in all three species.
The examined species show characteristic differences in their late sodium current. The rabbit and guinea pig INa,late is similar as they continuously get larger under the plateau phase and have their peak close to APD90. In contrast, the canine INa,late has about the same density until the middle of the plateau phase, and after that it gradually decreases. These differences must be due to channel expression or channel regulation, because if guinea pig AP was applied onto canine cells as command pulses, it failed to reproduce the INa,late seen in guinea pig cells. Interestingly though if the cells from different species were pretreated with ATX-II, the resulting INa,late trace was similar in all three species.

Supervisor: Balázs Horváth

Anita Vladár (1st year PhD student)
Department of Physiology

THE ROLE OF EPIDERMAL TRP CHANNELS IN ITCH SIGNALING

Transient receptor potential (TRP) channels are non-specific, mostly Ca2+ permeable cationic channels sensitive for changes in the physico-chemical environment. In sensory functions of the skin, multimodal thermosensitive members, TRPV1-4, TRPA1, TRPM3 and TRPM8 are of particular importance. They play important roles in sensing temperature, pain and itch in cutaneous sensory fibers. However, development of pruritus is strongly influenced by the intercellular communication between sensory neurons and cutaneous non-neuronal cells. TRP channels and their (patho)physiological roles were also described in epidermal keratinocytes and skin appendages. Here, we aimed at investigating the potential role of epidermal TRP channels in the development of pruritus.
The expression (RNASeq, Q-PCR) and function (measuring cytoplasmic Ca2 + concentration) of TRP channels potentially involved in pruritogenic signaling was investigated on primary normal human epidermal keratinocytes (NHEKs) isolated from surgical specimens.
Several sensory TRP channels were expressed by NHEKs. TRPV3 and TRPV4 were the dominant isoforms, their functionality was also verified in Ca2 + measurements. In addition, keratinocytes expressed a number of receptors that may play role in the development of pruritus or pruritic dermatological disorders. Next, we investigated whether TRP channels may be involved in the signaling of these potentially pruritogenous receptors. Therefore we treated the keratinocytes with activators of the pruriceptors and subsequently investigated the function and expression of TRP channels. We found, that pretreatment of NHEK cells with the TLR3 activator polyinosinic: polycythaidic acid (poly (I: C)) increased TRPV3 expression and the Ca2 + signals induced by TRPV3 activators but did not affect TRPV4 expression or activity. In our pilot measurements, some other pruritogenic IL-4, chloroquine and histamine were also found to stimulate TRPV3.
Our results suggest, that epidermal TRPV3 may play a role in the transduction of some pruritogenc signals. In our further studies we investigate the involvement of TRPV3 in mediator production of keratinocytes.

Supervisor: István Balázs Tóth

Florina Zákány (predoc student)
Department of Biophysics and Cell Biology

CHARACTERIZING THE EFFECTS OF DIFFERENT MEMBRANE LIPIDS ON THE GATING OF KV1.3 ION CHANNELS USING VOLTAGE-CLAMP FLUOROMETRY TECHNIQUE

Kv1.3 is built up by a voltage sensing (VSD) and a pore forming domain (PD). The movement of the VSD induced by depolarization is transmitted to the PD, and causes the opening of the channel. Membrane lipids can affect the gating of Kv1.3 through different non-specific and specific mechanisms. It has been shown that membrane cholesterol and ceramides have remarkable effects on the gating of Kv1.3 but it is not known whether these effects are mediated by actions through the VSD or the PD. Our aim was to investigate whether the major target of the action of cholesterol and C16 ceramide is on the VSD, PD or the coupling between these two domains. To test the specificity of the effect we carried out our measurements using different sterols (cholesterol, 7-dehydrocholesterol) and C16 ceramides (C16 ceramide, C16 glucosylceramide).
The RNA of Kv1.3 channel was injected into Xenopus laevis oocytes, current recordings were performed by VCF or for current noise analysis by patch-clamp. The main advantage of VCF technique is that simultaneously with current recordings we can monitor the movement of the VSD by labeling a cysteine residue on the S3-S4 extracellular linker with an MTS-TAMRA dye. Sterol loadings were performed by using sterol-MBCD (methyl-beta-cyclodextrine) complexes, while ceramides were dissolved directly into the medium.
During VCF measurements we determined the voltage dependence of activation and inactivation, the activation time constant based on current recordings, while the F-V curves were obtained from fluorescent signals in control and sterol- or ceramide-loaded cells. Sterol loadings resulted in a shift in voltage-dependence of activation and an elongated time course of activation, while we found no voltage shifts in F-V curves. In addition, sterol treatments led to significant current decreases. According to current noise analysis only the single channel conductance was decreased. The investigation of ceramides is in progress.
Our results suggest that sterols exert their effects by acting on the PD and/or the coupling mechanism, instead of influencing the voltage sensor.
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Supervisor: György Panyi

Support

Az orvos-, egészségtudományi- és gyógyszerészképzés tudományos műhelyeinek fejlesztése EFOP-3.6.3-VEKOP-16-2017-00009