ANNUAL SYMPOSIUM OF THE DOCTORAL SCHOOL OF MOLECULAR MEDICINE, UNIVERSITY OF DEBRECEN

AUGUST 30, 2012

Annual Symposium of the Doctoral School of Molecular Medicine

F.008-009 Lecture Hall, Life Science Building, University of Debrecen

August 30, 2012

Structure of the Doctoral School of Molecular Medicine

Head of the Doctoral School	Secretary of the Doctoral School
László Csernoch	**Andrea Dóczy-Bodnár**
Head of the Department of Physiology, Medical and Health Science Center, University of Debrecen	Department of Biophysics and Cell Biology, Medical and Health Science Center, University of Debrecen

Doctoral Programs

	Head	Core members	Members
I. Cell and molecular biology of signal transduction	**László Virág**	Csilla Csortos Viktor Dombrádi Ferenc Erdődi Pál Gergely	Péter Bay Éva Bakó Ilona Farkas Zsigmond Fehér András Guttman György Haskó Csaba Hegedűs Endre Kókai Beáta Lontay András Penyige Gábor Somogyi Éva Szabó György Vargha Róza Zákány
II. Membrane biophysical questions and research methods	**János Szöllősi**	Sándor Damjanovich Zoltán Krasznai Teréz Márián László Mátyus Péter Nagy György Panyi	László Balkay László Bene Andrea Dóczy-Bodnár Miklós Emri Zsolt Fazekas Péter Hajdú Attila Jenei Zoárd Krasznai György Vámosi Zoltán Varga
III. Physiology and neurobiology	**László Csernoch**	Tamás Bányász Tamás Bíró László Galuska László Kovács János Magyar Zoltán Rusznák Géza Szűcs	Ervin Berényi Gabriella Czifra Julianna Cseri István Jóna István Nagy Balázs Pál Sándor Sárközi András Szatmári Norbert Szentandrássy Péter Szentesi

Registered PhD students of the Doctoral School (2nd semester, 2012)

Doctoral program	Name	Supervisor(s)
I. Cell and molecular biology of signal transduction	Anita Boratkó, 3rd year	Csilla Csortos
	Éva Kerekes, 3rd year	Viktor Dombrádi
	Petra Lakatos, 3rd year	László Virág
	Adrienn Sipos, 3rd year	Beáta Lontay
	Csilla Somogyi, 3rd year	Róza Zákány
	Ákos Szekrényes, 3rd year	András Guttman
	Éva Katona, 2nd year	Róza Zákány
	Zoltán Kónya, 2nd year	Ferenc Erdődi
	Roland Takács, 2nd year	Róza Zákány
	Edina Bodnár, 1st year	Éva Szabó
	Márta Kerékgyártó, 1st year	András Guttman
	Gábor Törő, 1st year	György Haskó
	Csaba Váradi, 1st year	András Guttman
II. Membrane biophysical questions and research methods	Attila Forgács, 3rd year	László Bene
	Imre Lajtos, 3rd year	László Balkay
	Orsolya Szilágyi, 3rd year	Péter Hajdú
	István Csomós, 2nd year	Andrea Dóczy-Bodnár
	Brigitta Domján, 2nd year	Zoltán Krasznai
	Tamás Kovács, 2nd year	Péter Nagy
	István Hajdú, 1st year	György Vámosi
	Áron Krizsán, 1st year	László Balkay
III. Physiology and neurobiology	Lídia Ambrus, 3rd year	Tamás Bíró
	Nikolett Geyer, 3rd year	László Csernoch
	Erika Lisztes, 3rd year	Tamás Bíró
	Zsuzsanna Nagy, 3rd year	Gabriella Czifra
	Ferenc Ruzsnavszky, 3rd year	János Magyar
	Dóra Bodnár, 2nd year	Péter Szentesi
	Kornél Kistamás, 2nd year	János Magyar
	Levente Lánczi, 2nd year	Ervin Berényi
	Bence Hegyi, 1st year	Norbert Szentandrássy
	Gábor Máté, 1st year	László Galuska
	Adrienn Tóth, 1st year	László Csernoch

Pre-degree certificates
(June 1, 2011 – May 31, 2012)

Doctoral program	Name	Supervisor(s)
I. Cell and molecular biology of signal transduction	Bálint Bécsi András Kovács Stefan Mittermayr Magdolna Szántó	Ferenc Erdődi András Guttman András Guttman Bay Péter
II. Membrane biophysical questions and research methods	Ádám Bartók Dilip Shrestha Tímea Váradi Julianna Volkó	Zoltán Varga Attila Jenei/János Szöllősi Péter Nagy György Vámosi/László Damjanovich
III. Physiology and neurobiology	Dénes Nagy Attila Oláh Zoltán Palicz Olga Ruzsnavszky László Szabó Bernadett Szűcs	Géza Szűcs Tamás Bíró Péter Szentesi László Csernoch Géza Szűcs Ervin Berényi

PhD dissertations defended in last year (June 1, 2011 – June 30, 2012)

Tímea Váradi (supervisor: Péter Nagy)
Investigation of Receptor-Oriented Cancer Therapy in Human Tumors Overexpressing ErbB Proteins

Magdona Szántó (supervisor: Péter Bay)
Role of Poly(ADP-ribose) Polymerase (PARP)-2 in Mitochondrial Metabolism and in Doxorubicin-induced Vascular Damage

Lívia Kosztka (supervisor: Zoltán Rusznák)
Mitochondrial Expression of TASK-3 Channels in Human Malignant Melanoma Cultures and their role in Cell Survival and Proliferation

Tibor Docsa (supervisor: Pál Gergely)
Studying of Antihyperglycaemic Agents on the Inhibition of Glycogen Phosphorylase

Csaba Ádám (supervisor: Viktor Dombrádi)
Characterization of Novel Protein Phosphatases in Drosophila species

Gábor Harmati (supervisor: János Magyar)
Effects of β-adrenergic Stimulation on Delayed Rectifier Potassium Currents in Canine Ventricular Cardiomyocytes

István Czikora (supervisor: Csilla Csortos)
The Role of TIMAP in the Regulation of Protein Phosphatase 1 and Endothelial Barrier Function

Attila Brunyánszki (supervisor: Péter Bay)
Role of poly(ADP-ribose) polymerase in the Regulation of Transcription

Balázs Csóka (supervisor: György Haskó)
Role of A2A Adenosine Receptors in Regulating Sepsis

Program of the Symposium

09:30	**Arrival** (coffe, cake)
09:55	**Welcome address –** László Csernoch
10:00-10:45	**Invited Lecture** *Chair* **Ferenc Erdődi**
	Grant Hennig *Department of Physiology & Cell Biology, School of Medicine, University of Nevada* Visualizing the activity of pacemaker networks in the gastrointestinal tract using Ca^{2+} imaging: from cell to organ
10:45-11:00	**Coffee break**
11:00-12:00	**Section I.** *Chairs* **János Szöllősi** **Ferenc Erdődi**
	Speakers
11:00-11:10	**Kornél Kistamás** 2nd year PhD student *supervisor: János Magyar* Role of ionic currents in β-adrenergic modulation of ventricular action potential
11:15-11:25	**Ferenc Ruzsnavszky** 3nd year PhD student *supervisor: János Magyar* The effects of chronic nifedipine treatment on potassium currents of canine heart
11:30-11:40	**Roland Takács** 2nd year PhD student *supervisor: Róza Zákány* Comparative examination of different murine *in vitro* chondrogenic differentiation models
11:45-11:55	**Lídia Ambrus** 3rd year PhD student

	supervisor: Tamás Bíró Effects of palmithoylethanolamine in biological processes of skin-derived cells
12:00-13:30	**Lunch break**
13:30-15:00	**Section II.** *Chairs* **László Csernoch** **György Panyi**
	Speakers
13:30-13:40	**Levente Lánczi** 2nd year PhD student *supervisor: Ervin Berényi* Magnetic resonance relaxometry and imaging of biological samples on Earth's magnetic field
13:45-13:55	**Éva Katona** 2nd year PhD student *supervisor: Róza Zákány* Investigation of hyaluronan induced migration in human melanoma cell lines
14:00-14:10	**Tamás Kovács** 2nd year PhD student *supervisor: Péter Nagy* The dipole potential influences the clustering of ErbB proteins
14:15-14:25	**Petra Lakatos** 3rd year PhD student *supervisor: László Virág* 3-aminobenzamide protects human keratinocytes from UVA- and UVB - induced cell death by a PARP independent mechanism
14:30-14:40	**Anita Boratkó** 3rd year PhD student *supervisor: Csilla Csortos* Adaptor proteins in the endothelium
14:45-15:00	**Adrienn Sipos** 3rd year PhD student *supervisor: Beáta Lontay* The localization and function of myosin phosphatase in the nucleus of HepG2 cells

15:00-15:15	**Coffee break**
15:15-16:45	**Section III.**
	Chairs
	László Virág
	Tamás Bíró
	Speakers
15:15-15:25	**Éva Kerekes** 3rd year PhD student
	supervisor: Viktor Dombrádi
	Searching for the function(s) of the CG9238 gene in Drosophila
15:30-15:40	**Zoltán Kónya** 2nd year PhD student
	supervisor: Ferenc Erdődi
	Inhibition of protein phosphatase-1 and -2A by hexa-hydroxy-diphenoyl-tannin derivatives
15:45-15:50	**Ákos Szekrényes** 3rd year PhD student
	supervisor: András Guttman
	High throughput analysis of therapeutic and diagnostic monoclonal antibodies by multicapillary SDS gel electrophoresis in conjunction with covalent fluorescent labeling
16:00-16:10	**Brigitta Domján** 2nd year PhD student
	supervisor: Zoltán Krasznai
	Direct and acute effect of RF exposure on the function of cardiac sodium (Nav1.5) ion channel
16:15-16:25	**Dóra Bodnár** 2nd year PhD student
	supervisor: Péter Szentesi
	Modified EC coupling in myostatin deficient ($MSTN^{-/-}$) mice
16:30-16:40	**Nikolett Geyer** 3rd year PhD student
	supervisor: László Csernoch
	Store-operated Ca^{2+} entry in myostatin deficient mice
16:45	**Conclusion** – László Virág

Abstracts

Invited speaker

Visualizing the activity of pacemaker networks in the gastrointestinal tract using $Ca2^{+}$ imaging: from cell to organ

Grant Hennig

Department of Physiology & Cell Biology, School of Medicine, University of Nevada

Examining the activity of isolated cells allows their intrinsic capabilities to be characterized, but rarely describes how these cells function in their organ of origin. To better understand how various manipulations of cellular proteins (e.g. ion channels) affects whole organ behavior, we utilized low power Ca^{2+} imaging of the pacemaker (Interstitial Cell of Cajal: ICC) network and smooth muscle (SM) contractile activity in the gut. ICCs are connected to the SM syncitium via gap junctions which allows depolarization in ICC to spread to and depolarize surrounding SM, inducing contraction. Inhibiting gap junctions with 18β-glycyrrhetinic acid uncoupled ICC within the network, resulting in asynchronous Ca^{2+} waves in individual ICCs. ICC also contain mibefradil-sensitive Ca^{2+} channels (t-type) that are involved in the propagation of the slow wave wavefront between ICC. Blocking this channel slowed the velocity of the wavefront, but did not affect the other characteristics of the Ca^{2+} transient. Various drugs that interfere with Ca^{2+} handling and/or mitochondrial function altered or abolished Ca^{2+} transients in individual ICC resulting in abnormal slow wave generation and propagation. We have also examined how coupling of pacemaker activity and motor responses are highly variable in the gut (unlike the heart). Dysrhythmic gastric motor behavior is observed in half of all stomachs studied, consisting of ectopic pacing sites, alternating "skipping" behaviors and incomplete propagation of activity. If the frequency of pacemaker activity is too high, SM is unable to respond to every slow wave, and reverts to a non 1:1 electro-mechanical coupling ratio. Low power Ca^{2+} imaging allows the activity of individual cells and the behavior of networks to be studied simultaneously, allowing the "physiological" function of various cell types to be studied in context of the whole organ.

Section I

Role of ionic currents in β-adrenergic modulation of ventricular action potential

Kornél Kistamás, 2nd year PhD student

Department of Physiology

supervisor: János Magyar

Introduction: Although isoproterenol (ISO) is known to activate several ionic currents in mammalian myocardium, their relative contribution to the ISO-induced changes in action potential morphology is not well explored. Therefore, the effects of ISO on action potential configuration, L-type Ca^{2+} current ($I_{Ca,L}$), slow delayed rectifier K^+ current (I_{Ks}) and fast delayed rectifier K^+ current (I_{Kr}) were studied.

Methods: Canine left ventricular myocytes were isolated by enzymatic dispersion. Action potentials were recorded with conventional sharp microelectrodes, ionic currents were measured using conventional and action potential voltage clamp techniques.

Results: ISO (10-100 nM) caused significant and reversible shortening of action potential duration accompanied by elevation of the plateau potential at half duration of the action potential. Similar results were observed when ISO was applied after pretreatment with the I_{Kr} blocker E-4031 (1 μM). In the presence of the I_{Ks} blocker HMR1556 (1 μM) action potentials were significantly lengthened by ISO in spite of the pronounced plateau elevation. Both ISO-induced changes were prevented by pretreatment with nisoldipine ($I_{Ca,L}$ blocker, 5 μM). Action potential voltage clamp experiments revealed a prominent slowly inactivating $I_{Ca,L}$ followed by a rise in I_{Ks}. Similar ISO-induced responses were seen in the presence of 1 μM E-4031, while the ISO-induced activation of I_{Ks} was abolished by 1 μM HMR1556. Conventional voltage clamp experiments revealed that ISO increased I_{Ks}, I_{Kr} and $I_{Ca,L}$ to 420±4, 133±1 and 340±13 % of their baseline values, respectively, with the concomitant EC_{50} values of 14.5±1.1, 13.7±2.5 and 15.3±3.5 nM.

Conclusions: The results suggest that the ISO-induced activation of I_{Ks} - but not I_{Kr} - may be responsible for the observed shortening of action potentials in canine ventricular cells. The similar EC_{50} values estimated for I_{Ks}, I_{Kr} and $I_{Ca,L}$ may indicate a common single mechanism responsible for the ISO-induced activation of these currents.

The effects of chronic nifedipine treatment on potassium currents of canine heart

Ferenc Ruzsnavszky, 3rd year PhD student

Department of Physiology

Supervisor: János Magyar

Introduction: Calcium channel blockers are frequently used to treat hypertension but little known about the long-term cardiac effects of these drugs. The specific aim of our work was to study the effects of the chronic calcium channel antagonist treatment on the ionic currents of canine ventricular cells.

*Methods:*1 tabl/day „Adalat GITS 60" retard pills were administered to adult dogs for a month. This pill releases the L-type calcium channel antagonist nifedipine evenly. The ionic currents of the isolated canine ventricular cells were measured with whole cell patch-clamp technique at 37 ºC.

Results: Chronic nifedipine treatment significantly increased the L-type calcium current ($I_{Ca\text{-}L}$) density. The amplitude of the $I_{Ca\text{-}L}$ increased from 6.8±0.5 pA/pF to 8.8±0.9 pA/pF in control and nifedipine treated animals, respectively. The chronic nifedipine treatment also shifted the voltage-dependence of the steady-state inactivation of the $I_{Ca\text{-}L}$ towards the positive membrane potentials. The chronic nifedipine treatment failed to alter the amplitude of transient outward potassium current (I_{to}) and the rapid component of the delayed rectifier potassium current (I_{Kr}). At membrane potentials negative to -85 mV the amplitude of inward rectifier potassium current (I_{K1}) of nifedipine treated animals was significantly smaller than that of the control. At -115 mV membrane potential -40.8±5.5 pA/pF and -20.7±2.8 pA/pF were measured in control and in nifedipine treated animals, respectively.

Conclusions: This study and our previous results show that the chronic nifedipine treatment increases the L-type calcium current density and alters the calcium homeostasis of ventricular cells. These changes have minor effects on the electrophysiological properties of potassium currents.

Comparative examination of different murine in vitro chondrogenic differentiation models

Roland Takács, 2nd year PhD student

Department of Anatomy, Histology and Embryology

Supervisor: Róza Zákány

Introduction: One of the main murine models to conduct research on cartilage differentiation is the embryonic mesenchymal cell line, C3H10T. Under high density (HD) culturing conditions, with the addition or the overexpression of the growth factor BMP2 chondrogenesis can be induced. Another possible method is establishment of primary HD cultures from chondrogenic cells isolated from limb buds of mouse embryos. Here we attempt comparing the differentiation processes occuring in the two different models including results from non-overexpressing C3H10T cultures as a control for the treated cells.

Objectives: We aimed to optimise the culturing conditions of the BMP2-expressing cells to induce chondrogenic differentiation. Simultaneously, we attempted to develop our own procedure for the establishment of mouse primary HD limb bud cultures. Finally, by identifying and targeting certain markers, we investigated the process of differentiation taking place in the different models.

Methods: We carried out our gene expression studies by the means of RT-PCR reactions, using primers designed on sequences available in the NCBI database. Dimethyl methylene blue, Alizarin Red and Oil Red O stainings were applied to visualize differentiation in various mesenchymal-derived directions.

Results: In the BMP2-overexpressing model appearance of metachromatic extracellular matrix takes approximately 8 days, while in the case of the primary cultures the same process occurs at an increased pace. We confirmed the differentiation of these cultures in chondrogenic, osteogenic and adipogenic directions. Overall, the results strongly suggest the primary HD cultures are more focused in the chondrogenic direction, than the HD cultures derived from the cell lines.

Conclusions: The results propose primary HD cultures as a favourable alternative to the C3H10T cell line; metachromatic extracellular matrix is deposited at a faster rate, expression levels of osteogenic and adipogenic markers is depressed compared to the other models involved in the experiments.

Support: OTKA CNK 80709, Mec-9/2011, TÁMOP-4.2.2/B-10/1-2010-0024

Effects of palmithoylethanolamine in biological processes of skin-derived cells

Lídia Ambrus, 3rd year PhD student

Department of Physiology

supervisor: Tamás Bíró

Intensive research activities of the last decade have unambiguously confirmed that different members of the endocannabinoid system (ECS) are present in the human skin and play an important role in the regulation of various skin-related functions. The potential therapeutic opportunities of the ECS are currently being studied by a number of research groups worldwide. According to the recent findings, the "novel" endocannabinoid palmithoylethanolamine (PEA) showed remarkable adjuvant effects in the treatment of atopic dermatitis (AD; ATOPA Study), which is (among others) characterized by sebaceous hypofunction and cutaneous inflammation. However, mechanisms, through which PEA exerts its beneficial effects needs to be revealed; therefore, in our current experiments, we aimed at investigating the cellular effects of PEA on various skin-derived cells.

First, using SZ95 human immortalized sebocytes, we showed that, similar to the previously investigated "classical" endocannabinoids, PEA (without compromising the viability; MTT) was also able to increase the sebaceous lipid synthesis, albeit, with a much lower efficiency (Nile Red). By using both immortalized and primer normal human epidermal keratinocytes (NHEK), we also showed that viability of the cells was not significantly influenced by 24-hr treatments with up to 20 µM PEA (MTT). However, in the case of immortalized keratinocytes, a minor increase was observed in the ratio of necrotic cells ($DilC_1(5)$-SYTOX Green). Next we showed that PEA (applied at the above non-cytotoxic concentrations) significantly reduced the expression of various pro-inflammatory cytokines (interleukin [IL]-1α, IL1β, IL6 and IL8) in immortalized keratinocytes. Of further importance, PEA was also able to fully suppress the pro-inflammatory action of the Toll-like receptor-4 activator lipopolysaccharide on the IL6 production of NHEKs (RT-qPCR).

Taken together, our results argue for that the clinically observed beneficial effects of PEA in AD might be mediated via direct effects on keratinocytes and sebocytes. Hence, systematic mapping of the related signaling pathway(s) could highlight novel therapeutic targets in the treatment of AD.

Section II

Magnetic resonance relaxometry and imaging of biological samples on Earth's magnetic field

Levente Lánczi, 2nd year PhD student
Department of Biomedical Laboratory and Imaging Science
supervisor: Ervin Berényi

Introduction: Magnetic resonance imaging (MRI) is the most developing field of medical imaging. It still holds plenty of possible applications, thanks to its basic principles. In medical imaging and research high magnetic field devices are in use and under development. Another way to uncover what happens inside the human body or a bottle of water during an MRI examination is performing studies on low magnetic field.
Thanks to its advantages (eg. accessibility, feasibility, price) we performed examinations on an Earth field MRI (efMRI) device (Terranova efMRI, Magritek Inc., New Zealand) at our department. Terranova is available to perform both relaxometry and imaging (such as T1, T2, diffusion), similar to high field devices. Our main goal was to standardize experiments on Terranova and introduce into education.
Subjects and methods: We investigated the following solutions in different concentrations: NaCl, KCl, CaCl2, MnCl2, Mohr's Salt, glucose. In addition, we measured on both high field (GE Signa Excite 1.5 T) and Earth field some natural contrast agents, used routinely during abdominal MRI scans: pineapple juice, blackberry juice, blueberry juice, home-made pineapple concentrate, milk. We performed T1, T2 and diffusion experiments, 5 times in a row and 5 times, separately. We monitored solutions' temperature during experiments.
Results: Relaxation times correlated on 1.5 T and Earth field. Blackberry juice had the shortest relaxation times on both fields. Solutions' relaxation times were altering depending on concentration. Slight changes in temperature did not affect relaxation times.
Conclusion: Pineapple or blackberry juice, moreover milk can be used as a negative contrast agent during MRI experiments. Solution concentration affects relaxation times, characteristic for the solutions. Experiments on Terranova efMRI system are predecessors for contrast agent development and finding new biomarkers that can be applied on high field medical devices as well.

Investigation of hyaluronan induced migration in human melanoma cell lines

Éva Katona, 2nd year PhD student

Department of Anatomy, Histology and Embryology

supervisor: Róza Zákány

In spreading of melanoma, the extracellular matrix component hyaluronic acid (HA) shows an increased production. HA through its receptors (e.g. CD44 and RHAMM) stimulates specific signaling pathways, such as the proliferation and migration of the tumor cells. In our studies we used two melanoma cell lines with different malignancy: WM35 is an invasive, but not metastasizing cell line, while HT168 is an aggressive metastasizing melanoma cell line.

In our experiments both intracellular and extracellular HA were detected. Out of the three isoforms of hyaluronic acid synthases (HAS) we identified the mRNA and protein expression of HAS2 and HAS3. In WM35 cells the dominant isoform is the HAS2 responsible for the production of higher molecular weight (~1000 kDa range) HA, while in HT168 cells the HAS3 is the dominant which synthetizes the lower molecular weight (~100 kDa range) HA. Neither mRNA nor protein expression of HAS1 was found in the cells. Migration studies were performed in Boyden chamber, in the presence of different molecular weight HA (1600 kDa and 300-800 kDa). We observed that HT168 cells have a higher migratory potential than WM35 cells and their migration level was significantly higher in the presence of the lower molecular weight HA. Out of the hyaladherins we examined RHAMM and CD44. RHAMM shows stronger protein expression in HT168 cells than in WM35 cells while CD44 has similar expression pattern in both cell lines. In the regulation of RHAMM ERK1/2 plays role; after the application of ERK1/2 inhibitor PD098059 the migratory potential, HS production and also RHAMM protein expression increased in both cell lines. Cyclosporin A, the pharmacological inhibitor of the Ca-calmodulin dependent PP2B, has different effects on the migration of different malignancy melanoma cells and decreased the protein expression of the HAS enzymes and HA receptors. These results suggest that different molecular mass HA plays different role in the proliferation and migration of melanoma cells through the regulation of HA synthesis and binding via protein kinases and phosphatases- ERK and PP2B.

Our work was supported by grant OTKA-CNK80709.

The dipole potential influences the clustering of ErbB proteins

Tamás Kovács, 2nd year PhD student
Department of Biophysics and Cell Biology
supervisor: Péter Nagy

Background: ErbB proteins are receptor tyrosine-kinase molecules often involved in malignant processes. Homo- and heteroassociation between ErbB receptors lead to activation of transmembrane signaling which can play a significant role in the pathogenesis of tumors. The role of the extracellular domain is the best characterized in regulating receptor clustering but the transmembrane domain has also been shown to play a role. The dipole potential is a relatively large positive potential barrier at the membrane midplane created by inward-pointing molecular dipoles at the interfacial planes and it might have a role in influencing the functions of transmembrane domains of receptors by modifying their associations.
Methods: 6-ketocholestanol and phloretin were used to increase and decrease, respectively, the dipole potential. Their effect was evaluated by the dipole potential sensitive fluorescent dye, di-8-ANEPPS, by fluorescence microscopy. The homoassociation of ErbB2, ErbB1 and their heteroassociation in serum-starved and EGF-stimulated SKBR-3, JIMT-1 and A431 cells were measured by flow cytometric fluorescence resonance energy transfer.
Results: The dipole potential was successfully increased by 6-ketocholestanol and decreased by phloretin in SKBR-3, JIMT-1 and CHO cell lines. An increased dipole potential resulted in a significant increase in ErbB2-ErbB2 homoassociation both in starved and EGF stimulated samples while decreasing the dipole potential had the opposite effect. Ketocholestanol also increased ErbB1-ErbB2 heteroassociation in EGF stimulated JIMT-1 and ErbB1 homoassociation in stimulated A431 cells. Phloretin had no significant effect.
Conclusions: The dipole potential may play an important role in controlling the homo- and heteroassociation of transmembrane receptors. Intentional or accidental modification of the dipole potential by drugs might result in modified signal transduction processes.

3-aminobenzamide protects human keratinocytes from UVA- and UVB - induced cell death by a PARP independent mechanism

Petra Lakatos, 3rd year PhD student

Department of Medical Chemistry

supervisor: László Virág

Poly(ADP-ribosylation) is a posttranslational protein modification catalyzed by poly(ADP-ribose) polymerase (PARP) enzymes. The best known member of the family is PARP-1 that is activated mostly by DNA damage. UV radiation in sunlight is divided into three regions depending on wavelength: UV-C (200-280 nm), UV-B (280-320 nm), UV-A (320-400 nm). Both UV-B, and to a lesser extent, UV-A radiation are responsible for various skin disorders including photoaging and skin cancer. UV radiation alters cellular function, generates radical oxygen species (ROS), alters signalling events and causes DNA damage. UVA mainly generates free radicals by effecting membrane lipids and polymers in the cytoplasm. In our previous experiments we showed that the PARP inhibitor 3-aminobenzamide (3-AB) provided significant protection from UVB induced cell death (based on the measurement of apoptotic and necrotic parameters). However, the more specific PARP inhibitor PJ34 failed to protect cells from the UVB induced cell death. In order to investigate further the role of 3-AB we studied its effect in UVA irradiated cells as well. Two cellular models were applied: primary human keratinocytes were irradiated with UVA in the absence or presence of the photosensitizing agent enoxacin (ENX). We found that UVA irradiation resulted in PARP activation as indicated by poly(ADP-ribose) (PAR) formation with ENX treatment causing more severe DNA damage as indicated by higher amount of PAR formed after UVA irradiation. The potent and specific PARP inhibitor PJ34 proved to be a photosensitizing agent when cells were exposed to UVA irradiation. In the presence of photosensitizing agent ENX, PJ34 further increased UVA induced cell death aggravating both apoptosis (caspase activity and DNA fragmentation) and necrotic death (plasma membrane injury was assessed by LDH release). In contrast, the weak first generation PARP inhibitor 3-aminobenzamide (3AB) protected cells from both apoptotic and necrotic death suggesting that the effects of 3-AB are not specific and are not due to PARP inhibition.

Adaptor proteins in the endothelium

Anita Boratkó, 3rd year PhD student

Department of Medical Chemistry

supervisor: Csilla Csortos

TIMAP (TGF-β inhibited membrane-associated protein) protein has been considered as a member of the MYPT-family as the regulatory subunits of protein phosphatase 1 (PP1) based on its structural features. Its structure contains five ankyrin repeats, a PP1c binding motif, a nuclear localization signal (NLS) and a C-terminal prenylation motif that mediates its association to the plasma membrane. TIMAP is the most abundant in endothelial cells (EC), yet little is known about its exact function and its interacting partners. Recently, specific interaction was demonstrated in our laboratory between TIMAP and PP1cδ; and the effect of TIMAP phosphorylation on this interaction was also characterized. From studies made on TIMAP depleted EC, it was concluded that TIMAP has a regulatory effect on the EC barrier function via PKA-mediated ERM phosphorylation levels.

In our present study we found that RACK1 (receptor for activated protein kinase C) interacts with TIMAP in EC. The interaction between RACK1 and TIMAP was recognized by LC-MS/MS and was confirmed by immunoprecipitation (IP) and by pull down assay. Further IPs performed with mammalian wild type and mutant TIMAP recombinants verified that although PP1cδ is present in the complex, it is due to the strong interaction between PP1c and TIMAP, but no direct binding of PP1c to RACK1 was detected. Several structural mutants were created to map the domains involved in the RACK1-TIMAP interaction. The NLS region of TIMAP and the first four WD repeats of RACK1 were identified as critical regions for the interaction. Activation of the cAMP/PKA pathway by forskolin weakened the interaction; TIMAP, but not RACK1, translocated to the membrane. However, mRNA and protein level of TIMAP increased greatly in the RACK1 depleted EC, membrane localization of TIMAP was lost. We hypothesize that the prenylation of TIMAP is made by farnesyl transferase 1 (FT1) and the interaction between FT1 and TIMAP requires RACK1.

This work was supported by grant CNK80709.

The localization and function of myosin phosphatase in the nucleus of HepG2 cells

Adrienn Sipos, 3rd year PhD student

Department of Medical Chemistry

supervisor: Beáta Lontay

The myosin phosphatase (PP1M) holoenzyme is a serine/threonine specific protein phosphatase. It consists of a 38 kDa type 1 protein phosphatase catalytic subunit (PP1c), a 130/133 kDa MYPT (myosin phosphatase targeting subunit) and 20 kDa subunits with unknown function. PP1M regulates contractility through the dephosphorylation of myosin light chain. Apart from the myosin, which is a classical cytoskeletal substrate of PP1M, other non-muscle substrates have also been identified. It draws the attention to the complex function of PP1M in different tissues and cellular processes. MYPT was found to be localized not only in the cytosol and cytoskeleton but in the nucleuses of ***rat aortic smooth muscle*** cells, primary cultures of neuronal cells as well as of human hepatocarcinoma (HepG2) cells. Our goal is to investigate the nuclear functions of PP1M by determining the subnuclear localization and the interacting proteins of MYPT. Subnuclear fractions of HepG2 cells were analysed by Western blotting and by protein phoshatase enzyme activity assays in the presence of specific PP1 inhibitors such as okadaic acid and tautomycin. The dominant nuclear protein phosphatase was found to be the PP1 in the nuclear fractions. Flag-MYPT pull down assays were carried out using nuclear fractions of HepG2 cells and the proteins were identified by mass spectrometry. Numerous potential nuclear MYPT1-interacting proteins were identified such as histone 1, splicing factor proteins, possible enzyme regulators of PP1M and members of the methylosome complex, f. i. the protein arginine methyltransferase 5 (PRMT5). PP1cδ was detected from the nuclear pull down eluate and showed nuclear colocalization with MYPT1 suggesting that the delta and not the alpha/gamma isoform is the member of the holoenzyme in the nucleus. MYPT1 showed colocalization with histone 1 and presented distinct localization in the spliceosomes by confocal microscopy suggesting that PP1M may play a role in mRNA splicing. We plan to investigate the physiological role of PP1M in the nuclear dephosphorylation processes related to the regulation of transcription, RNA splicing and the functions of the methylosome complex.

Section III

Searching for the function(s) of the CG9238 gene in *Drosophila*

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R5 is a subunit of mammalian protein phosphatase 1 (PP1) that regulates glycogen metabolism. We initiated a bioinformatics search in the *Drosophila* genome for the ortholog(s) of R5 and identified the *CG9238* gene as a good candidate. Based on sequence analysis we predicted a PP1 binding motif and a glycogen binding domain in the primary structure of *Drosophila CG9238* protein (R5h). To verify the predictions, at first we performed a yeast two-hybrid screening and we confirmed that all isoforms of *Drosophila* PP1 catalytic subunit interacted with R5h. Then we placed the cDNA of *CG9238*a into the pGEX-5x-1 plasmid, thereafter we expressed and purified the recombinant protein. In sedimentation experiment the recombinant R5h was able to bind glycogen. In addition we found that the recombinant R5h inhibited the activity of the PP1. Our *in vitro* data suggest that R5h is the glycogen binding subunit of PP1 in *Drosophila*.

Based on the gene structure, two mRNAs can be transcribed from *CG9238*. With RT-PCR we detected both the shorter and longer mRNA in embryos and in adult females, but only the longer mRNA was detected in larvae, pupas and adult males. Our results are in correlation with the available EST data. To reveal the function of R5h, we generated a deletion mutant by excision the P-element from the *CG9238* gene's 5' non-coding region of EY10816 strain. The lack of the longer transcript and a drastic reduction of the shorter mRNA level were proven by RT-PCR. However, we found no notable differences in either the glycogen content, or the glycogen synthetase and phosphorylase enzyme activities between the wild type and the deletion mutant strains. Consequently, our data do not support the role of R5h in glycogen metabolism.

To investigate the function of R5h further we used a collection of hypomorphic *CG9238* alleles (P-element insertions, deletions, and RNAi strains) that exhibited different levels of gene expression, and measured the lifespan of the fruitflies. We found that RNAi strains with moderately reduced expression lived longer, while EY10816/81 with the lowest mRNA level lived shorter than the wild type. Thus the functions of CG9238 are more complex than expected.

Inhibition of protein phosphatase-1 and -2A by hexa-hydroxy-diphenoyl-tannin derivatives

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Protein phosphorylation mediates many cellular processes and its level is determined by the activity ratio of protein kinase and phosphatase enzymes. Regulation of protein phosphatase-1 (PP1) and -2A (PP2A) are the center of interest since they dephosphorylate more than 90 % of the total phosphoserine/threonine in cellular proteins. Our previous studies demonstrated that gallic acid and catechin derivatives, such as penta-O-galloyl-β-D-glucose, (PGG) and epigallocatechin-gallate, (EGCG), had differential inhibitory effects on PP1c and PP2Ac catalytic subunits, and they also affected cancer cell survival. Our aims were to study the structural background of this phosphatase inhibition by related compounds and its correlations with the biological effects. The phosphatase inhibitory and the cell death inducing effects of hexa-hydroxy-diphenoyl-tannin derivatives (tellimagrandin 1, praecoxin B, mahtabin A, pedunculagin, 1,2-Di-O-Galloyl-4,6-HHDP-β-D-glucose) were examined. These compounds include more rigid structural elements compared to that of PGG. PP1c and PP2Ac purified from rabbit skeletal muscle, or PP1c isoforms (PP1cα and PP1cβ) expressed and purified from *E. coli* were assayed. Tellimagrandin 1 proved to be a more effective (IC50=0,1-0,22 µM) inhibitor of PP1c than PGG (IC50=0,26-0,4 µM) and either praecoxin B (IC50=0,45-1 µM), mahtabin A (IC50=0,13-0,42 µM), pedunculagin (IC50=2,54-4 µM) or 1,2-Di-O-Galloyl-4,6-HHDP-β-D-glycosid (IC50=1,31-1,39 µM). PP2Ac inhibition occurred at ~100-fold higher concentrations of these compounds compared to PP1c. Tellimagrandin 1 induced cell death of HeLa cells in the range of 5-25 µM concentrations, pedunculagin and 1,2-Di-O-Galloyl-4,6-HHDP-β-D-glycosid were effective only in higher concentrations (50-100 µM), while praecoxin B and mahtabin A were without effect even at 100 µM. Our results suggest that certain structural rigidity in tannins may increase inhibitory potency on PP1 and the selectivity between PP1 and PP2A. Further studies will be focused on NMR-STD experiments and molecular dynamics simulations to gain further details of the structural background in phosphatase inhibition.

High throughput analysis of therapeutic and diagnostic monoclonal antibodies by multicapillary SDS gel electrophoresis in conjunction with covalent fluorescent labeling

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Capillary gel electrophoresis (CGE) in the presence of sodium dodecyl sulfate (SDS) is a well-established and widely used protein analysis technique in the biotechnology industry, and increasingly becoming the method of choice that meets the requirements of the standards of International Conference of Harmonization (ICH). Automated single channel capillary electrophoresis systems are usually equipped with UV absorbance and/or laser induced fluorescent (LIF) detection options offering general applicability and high detection sensitivity, respectively; however, with limited throughput. This shortcoming is addressed by the use of multicapillary gel electrophoresis (mCGE) systems with LED induced fluorescent detection (LED-IF), also featuring automation and excellent detection sensitivity, thus widely applicable to rapid and large scale analysis of biotherapeutics, especially monoclonal antibodies (mAb). The methodology we report in this paper is readily applicable for rapid purity assessment and subunit characterization of IgG molecules including detection of non-glycosylated heavy chains (NGHC) and separation of possible subunit variations such as truncated light chains (Pre-LC) or alternative splice variants. Covalent fluorophore derivatization and the mCGE analysis of the labeled IgG samples with multi-capillary gel electrophoresis are thoroughly described. Reducing and non-reducing conditions were both applied with and without peptide N-glycosidase F mediated deglycosylation.

Direct and acute effect of RF exposure on the function of cardiac sodium (Nav1.5) ion channel

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The radiofrequency identification (RFID) technologies are used more frequently nowadays. However, there is just a little information about the short and long-term effects of artificial RF fields that add up in biological systems. In our work, we planned to investigate one of the possible targets of the acute impacts of RF radiation. Identifying the effects of EM radiation can give us a basis for understanding a number of biological influences that stem from altered excitability of cells. The main goal was to study ion channels taken part in regulating basic functions so that we chose the human cardiac voltage-gated sodium channel $Na_V1.5$ as a model to observe its behaviour under controlled RF exposure. We investigated the effects of a wide power range (0.001–10W) transmitted by RFID devices at 2.45 GHz. Sodium currents of transiently expressed $hNa_V1.5$ channels in *tsA*_201 cells exposed to RF waves were measured by patch clamp technique in whole-cell configuration at room temperature. Exposure only took for a few milliseconds during measurements to avoid thermal effects. Our results showed that RFID systems using the milliwatt power range could not influence the function of voltage-gated sodium channels during short-term exposure. However, we could demonstrate some direct effects, ie. RF radiation over the power range of 1 watt slows the inactivation kinetics of the $hNa_V1.5$ channel (normalised τ_{in} are 0.920±0.076, 1.010±0.114, 1.179±0.128 at power level of 2, 4, 10 W, respectively, N=6), increases the current amplitude and shifts the activation threshold to the more positive direction. We also observed that the different powers distinctly changed the kinetics of recovery from inactivation (normalised τ_r are 1.057±0.009, 0.968±0.040, 1.029±0.143, 0.590±0.080, 0.278±0.024 at power level of 0.1, 0.5, 1, 2 and 5 W, respectively, N=3). All effects were reversible. In conclusion, we reported that the EM radiation affects the voltage sensor of the voltage-gated sodium channels.

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Modified EC coupling in myostatin deficient ($MSTN^{-/-}$) mice

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Myostatin, a member of the transforming growth factor β superfamily has emerged as a potent negative regulator of skeletal muscle growth. It is strongly expressed in skeletal muscle and $Mstn^{-/-}$ mice have a great increase in muscle mass demonstrating that myostatin is a muscle-specific negative regulator of skeletal muscle size and it also regulates muscle mass in adult mice. Fiber type distribution is markedly shifted to the white glycolytic muscle fibers at the expense of red oxidative fibers. Total muscle fiber number is increased.

$MSTN^{-/-}$ mice display excessive muscle mass and this is associated with a profound loss of oxidative metabolic properties. In our studies we investigated the muscle performance during moderate intensity voluntary wheel running and in grip tests. In the latter the myostatin-deficient mice showed higher average force than the control C57/BL6 mice. In voluntary wheel running the control mice performed better in average and in maximal speed, in the total distance.

We measured the force and the fatigue of *musculus soleus* (SOL) and *extensor digitorum longus* (EDL) in both mice strains. The amplitude of single twitches in EDL and SOL is higher in the control strain.

Isometric force measurements were performed during repeated activation–relaxation cycles at a sarcomere length of 2.3 μm. pCa-force relations were determined to assess the Ca^{2+}-sensitivity of force production. This data does not show significant difference between the two mouse strains.

Changes in intracellular Ca^{2+} concentration on single fibers from *flexor digitorum brevis* muscle was measured with fluorescence dye FURA-2-AM. The resting intracellular calcium concentration was higher in $MSTN^{-/-}$ mice.

Our results from *in vitro* experiments do not support the findings from *in vivo* experiments.

Store- operated Ca^{2+} entry in myostatin deficient mice

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Store-operated Ca^{2+} entry (SOCE) is a Ca^{2+}-influx pathway present generally in all cell types, and it couples the calcium depletion of internal stores (in muscle: sarcoplasmatic reticulum, SR) to the activation of plasma membrane Ca^{2+}-permeable channels. The mechanism of SOCE has two key components recently identified: first, a sensor of calcium store depletion (STIM) and second, a channel that – when activated by the calcium sensor - facilitates calcium entry into the cell for store refilling (Orai).

Myostatin is a member of the transforming growth factor-beta (TGF-ß) superfamily and has been shown to be a negative regulator of skeletal muscle growth and mass. Mice lacking myostatin ($MSTN^{-/-}$) exhibit a remarkable hyper muscular phenotype due to both myofiber hypertrophy and hyperplasia. The tremendous increase in muscle fiber number is associated with increase in muscle mass and decrease in fat, however, negative side effects are observed in overall animal endurance. Our group's recent focus is to elucidate the role of STIM1 and Orai1 in control of $[Ca^{2+}]_{SR}$ and Ca^{2+}-release in skeletal muscle from wild type and myostatin deficient animals.

Western Blot analysis performed on whole muscle homogenates from FDB, EDL and soleus muscles from control and myostatin deficient mice suggested a significant reduction in the endogenous STIM and Orai levels in the latter, which implies perhaps less SOCE in these mice. To test this, first we used enzymatically dissociated FDB fibers, loaded with the Ca^{2+}-sensitive dye fluo-8AM. Following the application of a depleting cocktail to empty the SR, the activation of SOCE was monitored with a confocal laser scanning microscope (CLSM). These experiments suggested a significant reduction of SOCE activity in the knock out. Secondly, we measured SOCE on mechanically skinned EDL fibers and we found that the process is active in the $MSTN^{-/-}$ but is less powerful as compared to control.

To our knowledge, this work will be the first to employ myostatin deficient mice and assess a comparative study to characterize the Ca^{2+} homeostasis of these mice in light of SOCE.

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