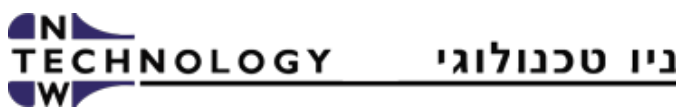




Annual Meeting 2018  
Tel-Aviv University, Feb. 28

The Plenary lecture  
By Prof. Richard Tsien



NBT  
New Biotechnology Ltd.

Program for the ISPP 2018 Meeting : Feb 28th, 2018 in Tel-Aviv University

- 08:15 - 09:00 Registration and coffee
- 09:00 - 10:20 **Morning Sessions 1**
- 10:20 - 10:40 Coffee break
- 10:40 - 12:00 **Morning Sessions 2**
- 12:00 - 13:00 Plenary Lecture (Auditorium 003)
- Prof. Richard W. Tsien**  
**"Signaling from synapse to nucleus in health and brain disease"**
- 13:00 - 14:00 Poster Session (Even posters presenting) and Lunch Break
- 14:00 - 14:10 John Finberg, Updates on Recent Developments in Animal Experimentation Ethics in Israel (Auditorium 003)
- 14:10 - 15:10 Young Investigators Presentations,  
A Session in Memory of Prof Haim Garty (Auditorium 003)
- 15:10 - 15:30 Business meeting (Auditorium 003)
- 15:10 - 16:00 Poster Session (Odd posters presenting) and Coffee break
- 16:00 - 17:20 **Afternoon Sessions**
- 17:20 Concluding Remarks and awarding the winners of poster and student lecture competitions (Auditorium 003)

## 9:00-10:20 Morning Sessions 1

Session A: (Hall-A) auditorium 003

**Chair: Ehud Ohana (BGU) and Raz Palty (Technion)**

### **"Membrane Transport Mechanisms and Physiology"**

09:00-09:20 **Yossi Tam** (HUJI) Modulating Glucose Transport by the Endocannabinoid/CB1 Receptor System

09:20-09:40 **Oded Lewinson** (Technion) A working enzyme: a movie in HD

09:40-10:00 **Tomer Shlomi** (TAU) Cancer cellular metabolism at a spatio-temporal resolution

10:00-10:20 **Ehud Ohana** (BGU) Systemic succinate homeostasis and local succinate signaling control blood pressure and protect against calcium oxalate kidney stones

Session B: (Hall- B) auditorium 110

**Chair: Yoram Eztion (BGU) and Yael Yaniv (Technion)**

**In collaboration with the Israeli Section of the ISHR**

### **"Frontiers in Cardiac Research"**

09:00-09:20 **Eldad Tzachor** (WIS) Novel strategies for cardiac regeneration

09:20-09:40 **Edith Hochhauser** (Rabin Medical Center) TLR4 Activation in Heart Following Ischemic or LPS Damage and in patients undergoing CABG

09:40-10:00 **Joachim Behar** (Technion) Aged -associated pacemaker function

10:00-10:20 **Jonathan Leor** (Sheba Medical Center) Targeting cardiac inflammation and regeneration

## 10:40-12:00 Morning Sessions 2

Session C: (Hall-A) auditorium 003

Chair: **Yoni Haitin (TAU) and Reuven Wiener (HUJI)**

### **"Structural Biology"**

10:40-11:00 **Moshe Giladi (TAU)** Allosteric Regulation of Sodium-Calcium Exchangers: Insights From Hybrid Structural Biology Studies

11:00-11:20 **Yoni Haitin (TAU)** Structure and Oligomerization of Chloride Intracellular Channels(?) - A Developing Story...

11:20-11:40 **Yarden Opatowsky (BIU)** Slit-Robo in the Crosshairs, A Structural Study

11:40-12:00 **Reuven Wiener (HUJI)** Molecular Mechanisms of Protein Modification by UFM1

Session D:(Hall-C) auditorium 106

Chair: **Shai Berlin (Technion)**

### **"Optogenetics: Remote Control Tools in Biological Systems"**

10:40-11:00 **Pierre Paoletti (PSL, Paris)** Illuminating glutamate receptor structure and function

11:00-11:20 **Amit Gruber (Technion)** Optogenetic Control of Human Induced Pluripotent Stem Cell Derived Cardiac Tissues

11:20-11:40 **Ilan Lampl (WIS)** Optogenetic studies of correlation and decorrelation mechanisms in the barrel cortex

11:40-12:00 **Shai Berlin (Technion)** Two-photon compatible light-gated NMDARs

**13:00-14:00 ISPP POSTER SESSION (Even Page #-Board #)**

Session E: (Hall-B) auditorium 110

Chair: **Rena Yarom - Young Investigator Competition**

**In collaboration with the Israeli Section of the ISHR**

- 10:40-10:55 **La-Paz Levin-Kotler (102)** Overexpression of the longevity gene **SIRT6** in myeloid cells reduced left ventricular hypertrophy after myocardial infraction
- 10:55-11:10 **Idit Goldfracht (116)** Functional comparison of engineered atrial and ventricular heart tissues derived from human pluripotent stem cells
- 11:10-11:25 **Michael Mutlak (114)** Extracellular regulated kinases protects against maladaptive cardiac hypertrophy following chronic pressure overload
- 11:25-11:40 **Wesam Mulla (118)** QT interval and ventricular refractory period measurements indicate absence of typical rate-dependence in conscious freely moving rodents
- 11:40-11:55 **Binyamin Eisen (106)** Functional abnormalities in induced pluripotent stem cell-derived cardiomyocytes generated from a Duchenne muscular dystrophy patient and a female manifesting carrier
- 11:55-12:10 **Roy Kalfon (115)** The role of dual loss-of-function mutations in the bZIP-repressor proteins, ATF3 and JDP2, in cardiac remodeling and hypertrophy

**12:00-14:00 ISHR POSTER SESSION**

### 14:10-15:10 ISPP Young Investigators Presentations.

Session F: (Hall-A) auditorium 003

14:10-14:25 **Limor Cohen** Blockage of AP-1 transcriptional complex sensitizes esophageal and head and neck cancers to PI3K $\alpha$  inhibition

14:25-14:40 **Reut Zaguri** A detailed analysis of the first human loss of function mutation in TRPV1

14:40-14:55 **Omer Barkai** A new spice to inflammatory soup: platelet-derived growth factor activates peripheral pain-related neurons by inhibiting Kv7/m-type potassium channels

14:55-15:10 **Ayalon Reis** identification of the first Kv4.1 specific scorpion toxins

**15:10-16:00 ISPP POSTER SESSION (Odd Page #-Board #)**

## 14:00-16:00 MIDDAY Session

### In collaboration with the Israeli Section of the ISHR

Session G: (Hall- B) auditorium 110

Chair: "Back to the Future"

- 14:00-14:10 **Guy Douvdevany (127)** Transcripts half-life regulatory function in cardiomyocytes
- 14:10-14:24 **Hadar klapper-Goldstein (128)** Atrial fibrillation substrate analysis  
in conscious freely moving rats with hyperaldosteronism
- 14:24-14:36 **Maayan Waldman (105)** PARP-1 inhibition attenuates oxidative stress and hypertension in diabetic mice
- 14:36-14:48 **Zachary Petrover (107)** Autophagy Guided Interventions to Modify the Phenotype of Danon Disease
- 14:48-15:00 **Limor Arbel-Ganon (109)** Mechano signal transduction by calcium and phosphorylation in healthy and dysfunctional heart pacemaker tissue
- 15:00-15:12 **Yair E. Lewis (119)** A working model of sarcomere maintenance in cardiomyocytes
- 15:12-15:24 **Yuval Shemer (123)** Investigating LMNA-related DCM using patients' induced pluripotent stem cells-derived cardiomyocytes

**15:40-16:00 Summary, ISHR annual meeting and announcement of YIA winners**

## 16:00-17:20 Afternoon Sessions

Session H: (Hall- A) auditorium 003

Chair: **Israel Sekler (BGU)**

### **" Neuronal Signaling and Physiology"**

**16:00-16:20 Michal Herhsfinkel (BGU)** The role of a zinc receptor, ZnR/GPR39 in neuronal function

**16:20-16:40 Daphne Atlas (HUJI)** The signaling role of calcium channels in triggering excitation-transcription coupling

**16:40-17:00 Jonathan Lezmy (TAU)** Biphasic homeostatic plasticity in hippocampal neurons triggered by M-current imbalance

**17:00-17:20 Ilya Fleidervish (BGU)** Stroke-associated spreading depolarization impairs high frequency encoding and alters axonal Na<sup>+</sup> dynamics in cortical pyramidal neurons

Session I: (Hall-C) auditorium 106

Chair: **Udi Qimron (TAU) and Ayal Hendel (BIU)**

### **"The CRISPR-CAS system"**

**16:00-16:20 Oren Parnas (HUJI)** Dissecting Molecular Circuits with Scalable Single-Cell RNA Profiling of Pooled Genetic CRISPR Screens

**16:20-16:40 Diego Jaitin (WIS)** Dissecting Immune Circuits by Linking CRISPR-Pooled Screens with Single-Cell RNA-Seq

**16:40-17:00 Adi Barzel (TAU)** Therapeutic gene targeting without nucleases

**17:00-17:20 Ayal Hendel (BIU)** A CRISPR Approach to Precision Medicine



## **Abstracts for Poster Session**

## **STRUCTURE-FUNCTION ANALYSIS OF Hv1- A VOLTAGE GATED PROTON CHANNEL**

Milit Marom David<sup>1,2</sup>, Emelia Melvin<sup>1</sup>, Francesco Tombola<sup>3</sup> and Yoni Haitin<sup>1,2</sup>

<sup>1</sup> *Department of physiology and pharmacology, Tel Aviv University, Tel Aviv, Israel*

<sup>2</sup> *Sagol School of Neuroscience*

<sup>3</sup> *Department of Physiology and Biophysics, University of California, Irvine, CA 92697, USA*

Correspondence: milit.marom@gmail.com

Voltage gated cation channels play crucial roles in a multitude of cellular functions. Canonically, these channels contain six transmembrane helices, divided into the voltage sensor (S1-S4) and pore (S5-p-S6) domains. However, voltage gated proton channels (Hv1) are unique members of this superfamily since they harbor only four transmembrane helices and are lacking a ‘conventional’ pore domain. Here, we focus on deciphering the molecular properties of human Hv1 and its coccolithophore *Emiliania huxleyi* homologue (Eh-Hv1). While the human Hv1 plays important roles in immune, sperm maturation, and  $\beta$ -lymphocyte cells, the structural and cellular properties of Eh-Hv1 are still being unraveled. Coccolithophores are unicellular phytoplankton, producing calcium carbonate plates termed coccoliths, which have an important role as oceanic carbon sink. CO<sub>2</sub> emissions are predicted to influence calcifying organisms by altering ocean water pH and changing the saturation state of calcium carbonate. During calcification, H<sup>+</sup> ions are formed and Eh-Hv1 participates in pH homeostasis. Thus, unravelling the properties of Hv1 structure and function may facilitate our knowledge on important molecular processes in human cells and in cancer biology in addition to deepen our understanding on coccolithophores’ crucial ecological roles. Human and Eh-Hv1 share similar electrophysiological properties and homologous structures. Interestingly, Eh-Hv1 contains a unique elongated extracellular loop, which is predicted to be globular. One remarkable feature of this loop is the large number of histidines which may suggest that this domain is involved in pH sensing or metal binding. In this work we aim to determine the atomic resolution structure of Hv1 channels. Hitherto we successfully expressed and purified the full-length coccolithophore channel from *S. cerevisiae* and the truncated human channel from bacteria. We obtained initial crystals of the human channel and currently continue with optimizing the purification protocol and establishing a liposome reconstitution system for functional channel analyses.

## CRYSTAL STRUCTURE AND SOLUTION PROPERTIES OF CLIC6: IMPLICATIONS FOR MEMBRANE ASSOCIATION

Alisa Ferofontov<sup>1</sup>, Roi Strulovich<sup>1</sup>, Milit Marom<sup>1</sup>, Moshe Giladi<sup>1</sup>, and Yoni Haitin<sup>1†</sup>

<sup>1</sup> Department of Physiology and Pharmacology, Sackler School of Medicine, Tel-Aviv University, Ramat Aviv, Tel-Aviv 6997801, Israel

alisaf@mail.tau.ac.il

Chloride intracellular channels (CLICs) are a family of unique metamorphic proteins, that were suggested to adopt both soluble and membrane-associated forms. Moreover, following membrane interaction *in vitro*, members of this family were shown to mediate ion conduction, implying of a multimerization process taking place upon membrane insertion. Nevertheless, while consisting of six members which are expressed throughout the human body, the physiological significance of the CLIC family remains unknown. Here, we present a 1.8 Å resolution crystal structure of the mouse CLIC6 (mCLIC6). The structure reveals a globular monomeric conformational arrangement and shows a high degree of structural conservation with other CLICs. Small angle X-ray scattering (SAXS) analysis of mCLIC6 in solution demonstrated that the overall solution structure is similar to the crystallographic conformation. Strikingly, further analysis of the SAXS data using ensemble optimization method unveiled additional more elongate conformations, elucidating high structural plasticity as an inherent property of the protein. Next, we employed differential scanning fluorimetry, using the SYPRO Orange thermal shift assay (TSA), which demonstrated that oxidative conditions induce an increase in mCLIC6 hydrophobicity. Further, while CLICs are considered as ion channels, in order to form a conductive pore they must undergo an obligatory multimerization process. Cross-linking experiment showed that an addition of liposomes resulted in a slight oligomerization of CLIC6. Strikingly, the oligomerization was enhanced in the presence of oxidative conditions. Finally, we propose a model in which CLICs oligomerization and membrane insertion is a multistep process. First, environmental changes such as an elevation in oxidative pressure increase the frequency of extensive tertiary changes, ‘priming’ CLICs for interaction with the membrane. Next, the ‘primed’ subunits interact with the membrane, forming oligomeric interactions which are maintained and stabilized by the presence of lipids. Together, these results provide mechanistic insights into the metamorphic nature of CLIC proteins.

## THE UNIQUE ROLE OF G $\gamma$ SUBUNIT IN ACTIVATION OF THE G PROTEIN-GATED CHANNEL GIRK1

Galit Tabak, Tal Keren Raifman, Nathan Dascal

*Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University.*

Email: [galitabak@gmail.com](mailto:galitabak@gmail.com)

G protein-sensitive inwardly rectifying potassium (GIRK) channels mediate inhibitory effects of neurotransmitters acting *via* G protein-coupled receptors. Following receptor activation and dissociation of the G $\alpha\beta\gamma$  heterotrimer, G $\beta\gamma$  subunits bind to GIRK's cytosolic domain, promoting channel opening. The main activating component of GIRK channels is G $\beta$ . G $\gamma$  is not considered important for GIRK activation, although one work challenged this perspective [1]. We found that expression of G $\gamma$  in *Xenopus* oocytes activates GIRK1\* (a homotetrameric GIRK1<sub>F137S</sub> channel), enhancing both basal and agonist-evoked currents ( $I_{\text{basal}}$  and  $I_{\text{evoked}}$ ). YFP-tagged G $\gamma$  activated GIRK1\* better than untagged G $\gamma$ . YFP-G $\gamma$  activated GIRK1/3 and GIRK1/4 to variable extent, but, importantly, not the homotetrameric GIRK2. The presence of the unique, long distal C-terminus of GIRK1 (dCT) was necessary but not sufficient for GIRK1\* activation by G $\gamma$ . Expression of G $\gamma$  did not change plasma membrane levels of GIRK1\*, thus G $\gamma$  acted by affecting the gating of the channel. Activation by G $\gamma$  depended on the presence of endogenous G $\beta$ . Interestingly, coexpression of low doses of G $\beta$ 's RNA enhanced the effect of G $\gamma$  on  $I_{\text{basal}}$  but high doses reduced it and suppressed  $I_{\text{evoked}}$ , indicating that excess G $\beta$  intercepts both free G $\gamma$  and possibly G $\alpha$  and obstructs their action(s). The observed phenomena are in line with the hypothesis that dCT of GIRK1 subunit is part of a "lock" mechanism that reduces channel's open probability, helping to keep it closed at rest [2]. We propose that G $\gamma$  relieves the inhibitory constraint imposed by the "lock", helping G $\beta$  to activate the GIRK channels.

1. Kawano et al. *FEBS Lett* **463**, 355-359 (1999).
2. Rubinstein et al. *J Physiol* **587**, 3473-3491 (2009).

## **ANKYRIN REPEAT DOMAIN AFFECTS TRPV1 CHANNEL GATING: A STRUCTURE-FUNCTION STUDY**

Rachel Zaguri, Ben Katz, Alexander Binshtok and Baruch Minke

*Department of Medical Neurobiology, Faculty of Medicine and the Edmond and Lily Safra Center for Brain Sciences (ELSC), Hebrew University, Jerusalem, Israel*

rachel.segura@mail.huji.ac.il

TRPV1 is a polymodal channel that can be activated directly by heat ( $>42^{\circ}\text{C}$ ) and by several molecules such as endovanilloids, protons ( $\text{pH} < 5.9$ ) and different toxins such as capsaicin (the pungent ingredient of chili pepper), resiniferatoxin (RTX) or the tarantula toxin (DkTx). The channel is also activated indirectly by various pro-inflammatory factors such as downstream effectors of receptor-mediated signaling cascade. At the structural level, TRPV1 consists of six ankyrin-repeats located at the N-terminus, followed by six transmembrane segments with a pore-forming region and a highly conserved C-terminus TRP domain.

The ankyrin-repeat domain was shown to bind chemical modulators which regulate channel activity. However, the mechanism by which chemical modulators affect channel activity is unknown. In an attempt to understand the role of the ankyrin-repeat domain of TRPV1, we performed multiple sequence alignment among members of the TRP channel subfamilies, searching for conserved amino acids. Using the inducible T-REX- HEK293 expression system and  $\text{Ca}^{2+}$  imaging experiments, we found two mutations in Asparagine residues located at position 286 and 331 in the ankyrin-repeat domain that render the TRPV1 channel inactive. This loss of function was not due to the instability or mislocalization of the protein. Moreover, the mutated channels were not rescued by mutations causing constitutive activity of the channel. Using the solved atomic structure of the ankyrin-repeat domain, we found that these mutations interrupt a network of hydrogen bonds, which are probably essential for channel activation. Our results raise the question of how single point mutations located in a remote region of the protein, the ankyrin-repeat domain, affect TRPV1 channel gating.

## **IDENTIFICATION OF THE FIRST Kv4.1 SPECIFIC SCORPION TOXINS**

Ayalon Reis and Noam Zilberberg,

*Department of Life Sciences Ben-Gurion University of the Negev, Beer-Sheva, Israel.*

Email: [reisa@post.bgu.ac.il](mailto:reisa@post.bgu.ac.il)

Scorpion venoms are a rich source of bioactive peptides. Most of these peptides are small (23-78 amino acids-long), well packed by several disulfide bridges and effect on channel function in excitable and non-excitable cells. The venom of each scorpion is a complex mixture of dozens or even hundreds of mainly neurotoxic peptides, interacting specifically with various ionic channels.

$\alpha$ KTx15 scorpion toxins are a family of ~35 amino acid-long peptides that block channels of the Kv4 group as well as hERG channels. We have performed a transcriptome analysis of the venom gland of the Israeli scorpion *Buthacus leptochelys leptochelys* (Bll). Using reverse genetics, we have studied the two Bll representatives of the  $\alpha$ KTx15 family. Toxins were cloned, expressed in a yeast expression system and their activity was assessed using the two-electrode voltage clamp technique. Both toxins were found to be selective blockers of Kv4.1 channels, as they did not affect either Kv4.2 nor Kv4.3 or each of the other 20 human potassium channels tested. These toxins will help us in understanding the interaction between scorpion toxins and Kv4 channels and enable the study of the physiological roles of Kv4.1 channels. In addition, as Kv4.1 channels were found to be highly expressed in certain cancer types and their activity was found to be essential for their proliferation, specific Kv4.1 blockers might serve as promising leads for the development of anti-cancer drugs.

## **THE ROLE OF MgSO<sub>4</sub> AS AN NMDA RECEPTOR BLOCKER IN THE PROTECTION AGAINST CNS-OXYGEN TOXICITY IN RATS**

Yehuda-Matan Danino<sup>1</sup>, Liav Leiboviz<sup>2</sup>, Valentina Borgil<sup>2</sup>, Mirit Eynan<sup>1</sup>, Rivka Efrat<sup>2</sup>, Adi Biram<sup>1</sup> and Ben Aviner<sup>1</sup>

*1Israel Naval Medical Institute, Haifa, Israel. 2Technology Division Test, Evaluation & QA Unit, Materials & Chemistry Department, Tel-Hashomer, Ramat-Gan, Israel.*

*e-mail address for correspondence: [yehuda015@gmail.com](mailto:yehuda015@gmail.com).*

**Introduction:** Combat divers, using closed-circuit breathing apparatus, run the risk of developing central nervous system oxygen toxicity (CNS-OT). This may lead to seizures underwater and eventually drowning. Evidence have accumulated to suggest that CNS-OT is related to increased production of reactive oxygen and nitrogen species. Hyperactivation of N-Methyl-D-Aspartate receptors (NMDARs) contribute to the production of these reactive molecules as well. Mg<sup>2+</sup> is a natural blocker of NMDARs in both the peripheral and central nervous system. Magnesium Sulfate (MgSO<sub>4</sub>) is an accepted treatment for prevention of pre-eclampsia related seizures by blocking NMDARs in skeletal muscles. However, it has also been suggested that MgSO<sub>4</sub> affects NMDAR activation in the CNS. A previous study in our laboratory demonstrated that MgSO<sub>4</sub> significantly prolongs latency to electroencephalo-graphic (EEG) manifestation of CNS-OT in rats. MgSO<sub>4</sub> may therefore provide protection against CNS-OT by blocking NMDARs. Electrophysiological studies in rat brain slices showed postsynaptic excitability of NMDAR at hyperbaric pressure (HP), due to reduction in Mg<sup>2+</sup> voltage depended inhibition efficiency of the NMDAR.

**Purpose:** Examining the protective effect of MgSO<sub>4</sub> against CNS-OT via its role as an NMDAR blocker under HP, using EEG-telemetry system in rats.

**Results:** EEG signals and seizures were recorded in living-rats by a telemetry system under HP. Relative to control group, the latencies to CNS-OT significantly increased in a MgSO<sub>4</sub> treated group and significantly decreased in a Mg<sup>2+</sup>-deficient group.

**Conclusions:** Our data suggests that MgSO<sub>4</sub> may be used as a protective agent against CNS-OT and that NMDARs are probably involved in this mechanism.

## **A DETAILED ANALYSIS OF THE FIRST HUMAN LOSS OF FUNCTION MUTATION IN TRPV1**

Tal Brandwine<sup>1</sup>, Ben Katz<sup>1</sup>, Rachel Zaguri<sup>1</sup>, Shaya Lev<sup>1</sup>, Isaiah T. Arkin<sup>2</sup>, Alexander Binshtok<sup>1</sup> and Baruch Minke<sup>1</sup>

<sup>1</sup>*Department of Medical Neurobiology, Faculty of Medicine and the Edmond and Lily Safra Center for Brain Sciences (ELSC), Hebrew University, Jerusalem, Israel.*

<sup>2</sup>*Department of Biological Chemistry, The Institute of Life Sciences, Faculty of Sciences Hebrew University, Jerusalem, Israel.*

[Tal.brandwine@mail.huji.ac.il](mailto:Tal.brandwine@mail.huji.ac.il)

The Transient Receptor Potential Vanilloid 1 (TRPV1) channel is one of the most researched and targeted protein due to its role in mediating pain and itch. It is expressed in a subset of nociceptive neurons and is activated by variety of noxious stimuli. Typical stimuli include heat above 42°C, acid, proinflammatory mediators and natural irritants such as capsaicin, the pungent ingredient of hot chili pepper. Here, we examined an 8-year-old patient, who consumes hot chili pepper without any taste aversion or tearing. Whole exome sequencing revealed a single nucleotide alteration, which causes a novel missense mutation in a conserved amino acid residue at the N'-terminal of TRPV1 channel. To examine the effects of the mutation on the TRPV1 function, we generated inducible stable cell lines expressing the native wild-type channel (hTRPV1<sup>WT</sup>) or the TRPV1 mutant channel (hTRPV1<sup>mut</sup>). Application of the known activators of TRPV1: capsaicin, low pH, heat and the tarantula DkTx toxin, resulted in robust hTRPV1<sup>WT</sup> activation but had no effect on expressed hTRPV1<sup>mut</sup> channel. Biochemical analysis revealed proper tetrameric assembly and plasma membrane localization of hTRPV1<sup>mut</sup>, suggesting that this is the first identified complete loss-of-function TRPV1 patient. To reveal the effect of lack of TRPV1 channels activity in humans, we performed series of clinical examination. General physical examination found that the patient fits the normal growing curves with no apparent health issues. Taste examination revealed normal sensitivity to four taste modalities; sweet, sour, bitter and salty but total insensitivity to capsaicin. Quantitative sensory testing revealed aberration in thermal detection such that heat and noxious heat detection threshold of the patient were elevated compared to control group. This study, is expected to promote our understanding on the participation of TRPV1 in human pain physiology and help develop new specific and effective TRPV1 antagonists to treat pain.



## **MEMBRANE CHOLESTEROL IS ESSENTIAL FOR THE ACTIVATION OF TRP CHANNELS IN *DROSOPHILA* PHOTORECEPTOR CELLS**

Rita Gutorov, Ben Katz, Maximilian Peters, Baruch Minke

*Department of Medical Neurobiology, Faculty of Medicine, Institute for Medical Research Israel Canada (IMRIC), Edmond and Lily Safra Center for Brain Sciences (ELSC), Hebrew University, Jerusalem, Israel*

Rita.gutorov@mail.huji.ac.il

Light activation of *Drosophila* photoreceptors leads to the opening of two classes of Ca<sup>2+</sup> permeable channels, the transient receptor potential (TRP) and the TRP-like (TRPL), via a G-protein-coupled PLC signaling cascade. Cholesterol is an essential component of the cell membrane, shown by many studies to modulate ion channel activity. Accordingly, application of the specific cholesterol sequestering agent methyl- $\beta$ -cyclodextrin (M $\beta$ CD) was shown to cause cholesterol depletion and modulate ion channel activity of Kir, BK and TRPV channels by interrupting specific sterol-protein interactions or indirectly by changing the lipid environment. Therefore, we asked whether the *Drosophila* TRP channel activity is affected by modulating the plasma membrane levels of cholesterol/ergosterol. Using whole-cell voltage-clamp measurements, we examined the effect on TRP channel activity of cholesterol/ergosterol depletion. Application of 10mM M $\beta$ CD reduced the sensitivity to light as reflected by reducing the amplitude of the macroscopic light response. In depth analysis at the single photon response level (quantum bump), which sum to produce the macroscopic light response, revealed that 10mM M $\beta$ CD reduced both the mean amplitude and frequency of the quantum bumps. The reduced sensitivity to light was accompanied by a slowdown of response kinetics including increased latency and time-to-peak of the macroscopic light response. To examine whether the effect of cholesterol/ergosterol sequestration operates at the TRP channel level, we analyzed the response of the photoreceptors to metabolic stress using cyanide (CCCP). Application of CCCP is known to induce constitutive activation of the channels, as manifested by enhanced TRP current fluctuations in a light independent manner. Application of CCCP in the presence of M $\beta$ CD reduced the amplitude of TRP current fluctuations but did not affect the latency of channel opening, supporting the notion that cholesterol operates at the channel level. This study thus provides insight to the still unknown mechanism of TRP channel gating.

## MONOTERPENES ARE POWERFUL $K_{2P}$ CHANNELS REGULATORS

Eden Arazi and Noam Zilberberg

*Department of Life Sciences and the Zlotowski Center for Neuroscience Ben-Gurion University of the Negev, Beer-Sheva, Israel*

email: [edenaraz@post.bgu.ac.il](mailto:edenaraz@post.bgu.ac.il)

$K_{2P}$  potassium channels display constant conductance in the physiological membrane potential range and their activity is highly regulated by both chemical and physical modulators.  $K_{2P}$  channels' activity affects numerous physiological processes such as cardiac function, pain perception, depression, neuroprotection and cancer development. Terpenes are a large family of compounds, mostly produced in plants. Several monoterpenes were found to affect the activity of a variety of ion channels. To date, the effect of terpenes on  $K_{2P}$  channels has not been studied.

Our goal is to investigate the effect of terpenes on human  $K_{2P}$  channels and to elucidate their mechanism of action. Human  $K_{2P}$  channels were expressed in *Xenopus* oocytes and currents were measured using the two-electrode voltage clamp technique. Channels' activity found to be highly affected by monoterpenes.  $K_{2P2.1}$  and other mechanosensitive  $K_{2P}$  channels currents were enhanced several-folds by carvacrol, thymol and menthol. Eugenol decreases  $K_{2P2.1}$  currents by two folds. The effect of monoterpenes was not limited to the known stretch-activated channel  $K_{2P}$  channels, as  $K_{2P5.1}$  and  $K_{2P18.1}$  currents were also elevated by up to 15-fold using carvacrol. Cinnamaldehyde was found to have a dual effect as it elevated  $K_{2P5.1}$  currents but decreased  $K_{2P18.1}$  currents. The influence on  $K_{2P2.1}$  currents was found to be mediated by a cytoplasmic carboxyl tail domain. This domain is partially composed of three positively charged amino acids and differs from the domains previously identified as important for PIP2 or the arachidonic acid activation.

Our results demonstrate, for the first time, that terpenes are powerful modulators of  $K_{2P}$  potassium channels and that the mechanism of action involves the carboxyl-terminus regulatory domain.

## **REGULATION OF SUCCINATE TRANSPORT AND HOMEOSTASIS**

Ahlan Kamaysi<sup>1</sup>, Moran Fremder<sup>1</sup>, Hadar Eini Rider<sup>1</sup>, Moran Fremder<sup>1</sup> and Ehud Ohana<sup>1</sup>

*Department of Clinical Biochemistry and Pharmacology, the Faculty of Health Sciences, Ben Gurion University of the Negev, 8410501 Beer Sheva, Israel*

Succinate is a tricarboxylic acid cycle intermediate that is gaining attention as a universal metabolic marker of ischemia and a pivotal signaling molecule. High-glucose-induced elevation and secretion of intracellular succinate stimulates the succinate-sensing receptor (GPR91), a Gq-coupled receptor that is expressed in several tissues, most notably, in kidney epithelia and in blood vessels. Importantly, since succinate is a charged molecule, succinate transport across membranes is mediated by transport proteins. The major succinate transporters are NaDC-1 and the organic anion transporters (OAT1 and 3) expressed on the apical and basolateral membranes of epithelia, respectively. However the regulation of transepithelial succinate transport via these transporters and how they maintain succinate homeostasis is not well understood. We have previously reported that NaDC-1 forms a complex with slc26a6, a Cl<sup>-</sup>/oxalate and Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchanger, to regulate succinate transport through interaction. Therefore, we hypothesize that succinate homeostasis is regulated by crosstalk between G-coupled receptors signaling and succinate transport via apical NaDC-1-slc26a6 complex and basolateral OATs. We first asked how the function of the apical and basolateral succinate transport proteins is regulated by the ubiquitous intracellular 'master regulator' protein, IRBIT. To address this question, we utilized electrophysiological measurements to monitor succinate transport following IRBIT expression. Furthermore, we have utilized biochemical assays and fluorescent imaging to study the interaction of the succinate transporters with IRBIT in live cells. Our results indicate that IRBIT regulates both apical and basolateral succinate transporters. Moreover, our results indicate that stimulation of the succinate receptor GPR91 increases IRBIT interaction with NaDC-1 attesting for IRBIT mediated regulation of succinate transport via receptor stimulation.

## **INTRACELLULAR REGULATION OF THE *slc26a9* Cl<sup>-</sup> CHANNEL AND INHIBITION BY SALICYLIC ACID**

Shireen Anbtawi<sup>1</sup>, Hadar Eini Rider<sup>1</sup> and Ehud Ohana<sup>1</sup>

*Department of Clinical Biochemistry and Pharmacology, the Faculty of Health Sciences, Ben Gurion University of the Negev, 8410501 Beer Sheva, Israel*

Cl<sup>-</sup> is the major anion in most living cells, which maintain the concentration of intracellular Cl<sup>-</sup> (Cl<sup>-</sup><sub>in</sub>) above electrochemical equilibrium. Importantly, aberrant Cl<sup>-</sup>-dependent fluid and ion secretion is associated with many high morbidity, high mortality diseases such as hypertension, cystic fibrosis (CF), pancreatitis, and more. One of the major chloride channels that have been recently characterized is SLC26A9. However, functional and regulatory aspects of *slc26a9* Cl<sup>-</sup> transport activity are still poorly understood.

Interest in the SLC26A9 Cl<sup>-</sup> channel dramatically increased when a link between CF and SLC26A9 was established by genome wide association study with marked upregulation of SLC26A9 in CF. Moreover, *slc26a9* regulates arterial blood pressure since measurements in *slc26a9*<sup>-/-</sup> mice indicate that they are hypertensive. Other members of the *slc26* family of transporters as well as the Cl<sup>-</sup> channel CFTR have been shown to be regulated by the 'master regulator' protein, IRBIT.

Here, we show that IRBIT plays a major role in *slc26a9* regulation. Moreover, we show that *Slc26a9*- mediated Cl<sup>-</sup> transport is inhibited by salicylic acid in a dose dependent manner.

Our findings identify *slc26a9* regulation pathway as a potential therapeutic target for future treatment of Cl<sup>-</sup> channel pathologies.

## **KINETICS OF PERSISTENT Na<sup>+</sup> CURRENT IN AXON INITIAL SEGMENT OF LAYER 5 PYRAMIDAL NEURONS**

Shvartsman A., Khrapunsky Y. and Fleidervish I.A.

*Department of Physiology and Cell Biology, Faculty of Health Sciences and Zlotowski Center for Neuroscience, Ben-Gurion University, Beer-Sheva, 84015, Israel*

**Background:** In addition to the well-described, fast-inactivating component of the Na<sup>+</sup> current, neocortical neurons also exhibit a slowly inactivating, persistent Na<sup>+</sup> current ( $I_{NaP}$ ), that plays a role in determining AP threshold and in synaptic integration. By imaging Na<sup>+</sup> influx during slow voltage ramps, we previously showed that, at functionally critical subthreshold voltages, most of the  $I_{NaP}$  is generated in the axon initial segment (AIS). We now measured the AIS Na<sup>+</sup> influx during the depolarizing and hyperpolarizing voltage ramps of variable speed and amplitude in order to characterize the  $I_{NaP}$  kinetics.

**Results:** The voltage dependence of the AIS Na<sup>+</sup> flux was shifted in hyperpolarizing direction by about 15 mV as compared to the somatic  $I_{NaP}$ . The conductance- $V_m$  relationship for the axonal  $I_{NaP}$  derived from the Na<sup>+</sup> flux measurements was bell-shaped with peak at about -43 mV suggesting that the axonal  $I_{NaP}$  is generated predominantly by “window conductance”, as predicted by the Hodgkin Huxley formalism. Moreover, uncontrolled axonal spikes were frequently observed during hyperpolarizing voltage ramps, indicating the presence of wide window of voltages between the steady state activation and inactivation curves for the axonal Na<sup>+</sup> channels. The slow inactivation kinetics of the AIS  $I_{NaP}$  assessed by delivering depolarizing voltage steps of varying amplitude and duration before the ramp, were not significantly different from those of somatic Na<sup>+</sup> channels.

**Conclusions:** Kinetic properties of the persistent Na<sup>+</sup> conductance in the axon differ significantly from those in other neuronal compartments. The quantitative characterization of the axonal  $I_{NaP}$  would be helpful for modeling of neuronal synaptic integration and spike generation.

This research was supported by the Israel Science Foundation (grant No. 1302/14)

## **PHOTOACTIVATABLE FLUORESCENT ACTIVITY PROBES FOR STUDYING NEURONS**

Hussien Wessal<sup>1</sup>, Shai Berlin<sup>1</sup>

*The Ruth and Bruce Rappaport faculty of medicine, Technion Israel Institute of Technology*

Genetically encoded red Ca<sup>2+</sup> indicators (red GECIs) are fluorescent optical reporters that increase in fluorescence when bound to Ca<sup>2+</sup>. However, the major limitation of red GECIs is their absorption of blue light, giving rise to Ca<sup>2+</sup>- independent increases in fluorescence (i.e., produce artifact). Therefore, it is challenging to combine with the latter with optogenetic tools, such as ChR2, that also absorb blue-light. Here we explore the photoactivation properties of red GECIs in hope to eliminate the Ca<sup>2+</sup>-independent increase in fluorescence. To do this, we tested the effect of different mutations on the fluorescence of the red GECI – R-GECO 1.2. Interestingly, we find two mutations that cancel the artifact, of which only one variant remains Ca<sup>2+</sup>- responsive. Also, we find that several mutations strengthen the effect of blue light, (i.e., increase in fluorescence) and further render the reporter irreversibly photoactivatable, whilst maintain their ability to sense Ca<sup>2+</sup>. Taken together, we present new variants of R-GECO 1.2 with variable features such as artifact-free Ca<sup>2+</sup> sensing and photoactivatability.

## **MULTI-MODE MRI AND OPTICAL IMAGING OF SELECT NEURAL POPULATIONS WITH ENRICHED LOCALIZATION OF HYBRID MAGNETO-FLUORESCENT AGENTS, FOR EARLY DIAGNOSIS OF BRAIN DISORDERS-MAGNIFISCENT**

Olszakier S1, Berlin S1

*Medicine, Technion – Israel Institute of Technology, Haifa, Israel*

There are increasing efforts to address large-scale brain imaging, with cellular specificity and resolution in the living brain. Specifically, a major challenge is to characterize and track specific neuronal populations, over time, during the development of brain disorders or diseases. Medical imaging techniques are particularly useful for diagnosing diseases, with MRI as the leading strategy. However, MRI is limited by its spatial and temporal resolutions, contrast, as well as lacks cellular specificity. Here we present a novel approach that enables MRI to image several specific cellular populations concurrently, with high contrasts, for prolonged durations; at the single cell level. In this work, we use a novel chemogenetic strategy to develop MAGNIFISCENT, a patent-pending approach. The method consists of the synthesis of novel magnetic and fluorescent agents that can be targeted to label defined cells with the use of genetic tools. With this approach, we intend to study pathologic changes occurring in the brain by progressively tracking distinct neuronal populations, at the mesoscale by MRI, during the development of neuropsychiatric and degenerative diseases in animal models.

## COMPETITION OF CALCIFIED CALMODULIN N-LOBE AND PIP<sub>2</sub> TO A LQT MUTATION SITE IN Kv7.1 CHANNEL

Bernard Attali<sup>a</sup>, William S. Tobelaim<sup>a</sup>, Meidan Dvir<sup>a</sup>, Guy Lebel<sup>b</sup>, Meng Cui<sup>d</sup>, Tal Buki<sup>b</sup>, Asher Peretz<sup>a</sup>, Milit Marom<sup>c</sup>, Yoni Haitin<sup>c</sup>, Diomedes E. Logothetis<sup>d</sup>, Joel A. Hirsch<sup>b</sup>

<sup>a</sup>*Department of Physiology & Pharmacology, Sackler Faculty of Medicine and Sagol School of Neurosciences,*

<sup>b</sup>*Department of Biochemistry & Molecular Biology, George S. Wise Faculty of Life Sciences, Institute of Structural Biology,* <sup>c</sup>*Laboratory of structural physiology, Department of Physiology & Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel and* <sup>d</sup>*Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, VA 23298, USA*

Kv7.1 and KCNE1 protein co-assembly forms the slow potassium current  $I_{KS}$  that repolarizes the cardiac action potential. The physiological importance of the  $I_{KS}$  channel is underscored by the existence of mutations in human Kv7.1 and KCNE1 genes, which cause cardiac arrhythmias such as the long QT syndrome (LQT) and atrial fibrillation. The proximal Kv7.1 C-terminus (Kv7.1-CT) binds calmodulin (CaM) and phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>), but the role of CaM in channel function is still unclear and its possible interaction with PIP<sub>2</sub> is unknown. Our recent crystallographic study showed that CaM embraces helices A and B with the apo C-lobe and calcified N-lobe, respectively. Here we reveal the competition of PIP<sub>2</sub> and the calcified CaM N-lobe to a previously unidentified site in Kv7.1 helix B, also known to harbor a LQT mutation. Protein pulldown, molecular docking, molecular dynamics simulations and patch-clamp recordings indicate that residues K526 and K527 in Kv7.1 helix B form a critical site where CaM competes with PIP<sub>2</sub> to stabilize the channel open state. Data indicate that both PIP<sub>2</sub> and Ca<sup>2+</sup>-CaM perform the same function on  $I_{KS}$  channel gating by producing a left-shift in the voltage dependence of activation. The LQT mutant K526E revealed a severely impaired channel function with a right-shift in the voltage dependence of activation, a reduced current density and insensitivity to gating modulation by Ca<sup>2+</sup>-CaM. The results suggest that following receptor-mediated PIP<sub>2</sub> depletion and increased cytosolic Ca<sup>2+</sup>, calcified CaM N-lobe interacts with helix B in place of PIP<sub>2</sub>, to limit excessive  $I_{KS}$  current inhibition.



## **CALCIUM BINDING TO THE TURRET REGION CONTROLS INACTIVATION GATING OF A VOLTAGE-GATED K<sup>+</sup> CHANNEL**

Asher Peretz<sup>1</sup>, William Tobelaim<sup>1</sup>, Daniel Yakubovitch<sup>1</sup>, Yoav Paas<sup>2</sup> and Bernard Attali<sup>1\*</sup>

<sup>1</sup>*Department of Physiology and Pharmacology, Sackler Medical School, Tel Aviv University, Tel Aviv 69978 Israel*

<sup>2</sup>*The Mina and Everard Goodman Faculty of Life Sciences Bar-Ilan University, Ramat-Gan 52900 Israel*

Inactivation is an intrinsic property of numerous voltage-gated K<sup>+</sup> (Kv) channels and can occur by N-type or/and C-type mechanisms. While fast N-type inactivation involves the inner pore occlusion by N-terminal peptide domains of  $\alpha$  and  $\beta$  subunits, C-type inactivation is suggested to involve structural rearrangements in the outer pore leading to a loss of K<sup>+</sup> coordination sites in the selectivity filter. In Kv7.1 channels, inactivation is invisible macroscopically and does not exhibit the hallmarks of N- and C-type mechanisms. However, Kv7.1 inactivation is revealed by hooked tail currents, which reflect the recovery from an inactivation state. Here, we show that removal of external Ca<sup>2+</sup> produces a striking voltage-dependent macroscopic inactivation of Kv7.1 channels. Increasing external Ca<sup>2+</sup> suppresses macroscopic inactivation with an EC<sub>50</sub> of 1.5  $\mu$ M. While Sr<sup>2+</sup> and Cd<sup>2+</sup> mimic the effects of Ca<sup>2+</sup>, other divalent cations like Mg<sup>2+</sup> and Mn<sup>2+</sup> are ineffective. Elevating external K<sup>+</sup> concentration (50 mM) does not prevent macroscopic inactivation evoked in Ca<sup>2+</sup>-free external solutions. Experimental data and kinetic modeling indicate that KCNQ1 channels exhibit two distinct inactivation states. Mutagenesis studies and molecular modeling suggest that external Ca<sup>2+</sup> ions are coordinated, at least by two glutamate residues E295 and E284 located at the outer pore in the turret domain. Our results reveal a new mechanism whereby external Ca<sup>2+</sup> exquisitely controls inactivation gating of a Kv channel via a discrete pore turret region.

## PROTEIN KINASE A DIRECTLY INTERACTS WITH L-TYPE VOLTAGE-GATED CALCIUM CHANNEL

Vladimir Tsemakhovich<sup>1</sup>, Mariam Ashkar<sup>1,2</sup>, Moshe Katz<sup>1,2</sup>, Maike Schulz<sup>3</sup>, Enno Klussmann<sup>3</sup>, Anouar Belkacemi<sup>4</sup>, Veit Flockerzi<sup>4</sup>, Debi Ranjan Tripathi<sup>5</sup>, Joel Hirsch<sup>5</sup>, Shimrit Oz<sup>2</sup>, Nathan Dascal<sup>1</sup>

<sup>1</sup>*Sackler School of Medicine, Tel Aviv University, Israel;* <sup>2</sup>*Heart Institute, Sheba Medical Center, Israel;* <sup>3</sup>*Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germany;* <sup>4</sup>*Pharmakologie & Toxikologie, Universität des Saarlandes, Homburg, Germany;* <sup>5</sup>*Faculty of Life Sciences, Tel Aviv University, Israel*

Vladimir Tsemakhovich [tsemakh@post.tau.ac.il](mailto:tsemakh@post.tau.ac.il)

L-type voltage dependent Ca<sup>2+</sup> channels (L-VDCCs; Ca<sub>v</sub>1.2), crucial in cardiovascular physiology and pathology, are modulated via activation of G-protein-coupled receptors and protein kinases A (PKA) and C (PKC). The mechanism by which cardiac L-VDCCs are regulated by PKA is unclear and controversial. So far none of the known PKA phosphorylation sites of the main  $\alpha_{1C}$  subunit of Ca<sub>v</sub>1.2 has been confirmed as being crucial for PKA regulation. We aim to discover how PKA regulates L-VDCC. We have previously shown that the N-terminus (NT) of  $\alpha_{1C}$  is essential for functional PKA effect on  $\alpha_{1C}$  expressed in *Xenopus* oocytes. We now show that mutation of individual NT amino acids that do not comprise PKA phosphorylation sites abolishes PKA regulation. Therefore, we hypothesized a non-catalytic effect of PKA. To test this hypothesis we performed pull down experiments with purified proteins to determine the interaction between PKA catalytic subunit (PKA-CS), CT and NT of  $\alpha_{1C}$ . We observed that PKA-CS directly binds two specific C terminus (CT) segments, CTD1 and CTD2. Moreover, preliminary results indicate a direct interaction between CTD1 and CTD2 segments and specific NT segments. There might also be a direct interaction between the NT and the CT. The role of PKA-CS in affecting this binding remains to be studied. We hypothesize that direct binding of PKA-CS to  $\alpha_{1C}$  through the C-terminal domain, and possibly though N-terminal domain, plays a pivotal role in the L-VDCCs activation, and that the interaction between the N-terminus and PKA-CS might also be important.

## THE PHOSPHORYLATION STATE OF THE *DROSOPHILA* TRP CHANNEL MODULATES THE FREQUENCY RESPONSE TO OSCILLATING LIGHT *IN VIVO*

Elisheva Rhodes-Mordov<sup>1</sup>, Olaf Voolstra<sup>2</sup>, Ben Katz<sup>1</sup>, Jonas-Peter Bartels<sup>2</sup>, Claudia Oberegelsbacher<sup>2</sup>, Susanne Katharina Schotthofer<sup>2</sup>, Bushra Yasin<sup>1</sup>, Hanan Tzadok<sup>1</sup>, Armin Huber<sup>2</sup>, and Baruch Minke<sup>1</sup>

<sup>1</sup> Department of Medical Neurobiology, Faculty of Medicine and the Edmond and Lily Safra Center for Brain Sciences, Hebrew University, Jerusalem 91120, Israel

<sup>2</sup> Department of Biosensorics, Institute of Physiology, University of Hohenheim, 70599 Stuttgart, Germany

[elisheva.rhodes@mail.huji.ac.il](mailto:elisheva.rhodes@mail.huji.ac.il)

*Drosophila* photoreceptors respond to oscillating light of high frequency (~100 Hz), relative to human rods (~30 Hz) and cones (~50 Hz). The detected maximal frequency is modulated by the light rearing conditions, thus enabling high sensitivity to light under dark rearing conditions (by temporal summation) and high temporal resolution under light rearing conditions. However, the molecular basis for this adaptive process is unclear. Here, we report that dephosphorylation of the light-activated transient receptor potential (TRP) ion channel at S936 is a fast, graded, light-dependent, and Ca<sup>2+</sup>-dependent process. Electroretinogram measurements of the frequency response to oscillating lights *in vivo* revealed that dark-reared flies expressing wild-type TRP exhibited a detection limit of oscillating light at relatively low frequencies, which was shifted to higher frequencies upon light adaptation. Strikingly, preventing phosphorylation of the S936-TRP site by alanine substitution in transgenic *Drosophila* (*trp*<sup>S936A</sup>) abolished the difference in frequency response between dark-adapted and light-adapted flies, resulting in high-frequency response also in dark-adapted flies. In contrast, inserting a phosphomimetic mutation by substituting the S936-TRP site to aspartic acid (*trp*<sup>S936D</sup>) set the frequency response of light-adapted flies to low frequencies typical of dark-adapted flies. Together, this study indicates that TRP channel dephosphorylation is a regulatory process that affects the detection limit of oscillating light according to the light rearing condition, thus adjusting dynamic processing of visual information under varying light conditions.

## ACTIVE EXTRUSION OF CELLULAR $Mn^{2+}$ POWERED BY A $Ca^{2+}$ DRIVEN EXCHANGE VIA ZnT10

Moshe Levi<sup>1\*</sup>, Nadav Elkoshi<sup>1\*</sup>, Shiran Zucker Berber<sup>2</sup>, Eitan Hoch<sup>1</sup>, Raz Zarivatch<sup>2</sup>, Michal Hershfinkel<sup>1</sup> and Israel Sekler<sup>1</sup>

<sup>1</sup>*Dept. of physiology and cell biology*, <sup>2</sup>*Dept. of Life sciences Ben-Gurion university*

*moshl@post.bgu.ac.il, elkoshi@post.bgu.ac.il* \* Both authors contributed equally to this work.

Manganese ( $Mn^{2+}$ ) is extruded from the cell by the metal transporter ZnT10. Loss of expression, by autosomal mutations of this transporter, leads to hypermagnesemia in multiple organs. Here we show that ZnT10 is exploiting the trans-membranal  $Ca^{2+}$  inward gradient for active cellular exchange of  $Mn^{2+}$  based on the following findings; Application of  $Ca^{2+}$  free solution to ZnT10 expressing cells triggered an influx of  $Mn^{2+}$ . Reintroduction of  $Ca^{2+}$  lead to cellular  $Mn^{2+}$  extrusion against an inward  $Mn^{2+}$  gradient. The cellular transport of  $Mn^{2+}$  by ZnT10 was coupled to a reciprocal movement of  $Ca^{2+}$ . In contrast to other ZnT members, ZnT10 did not mediate  $Zn^{2+}$  transport but replacing a single Asn (ZnT10 N43 residue) with His (ZnT10 N43H) at the tetrahedral metal transport site was sufficient to reconstituted  $Zn^{2+}$  transport albeit at slower rate than the  $Mn^{2+}$  transport of WT ZnT10. Remarkably, ZnT10 N43T mutation converted the  $Mn^{2+}/Ca^{2+}$  exchange to an uncoupled channel mode, permeable to both  $Ca^{2+}$  and  $Mn^{2+}$ . The result of this study identifies the first transporter that utilizes the  $Ca^{2+}$  gradient for active counter ion exchange. It further shows a remarkable versatility in metal selectivity and mode of transport controlled by the tetrahedral metal transport site of ZnT proteins.

## **STROKE-ASSOCIATED SPREADING DEPOLARIZATION IMPAIRS HIGH FREQUENCY ENCODING AND ALTERS AXONAL Na<sup>+</sup> DYNAMICS IN CORTICAL PYRAMIDAL NEURONS**

Stoler O, Revah O, Neef A, Gutnick MJ, Fleidervish IA

*Department of Physiology and Cell Biology & Zlotowski Center for Neuroscience, Ben-Gurion University  
Koret School of Veterinary Medicine, Hebrew University of Jerusalem  
Max Planck Institute for Dynamics and Self Organization, Göttingen, Germany*

**Background:** The core of ischemic stroke is surrounded by the wider penumbra region where large percentage of neurons survive. However, little is known about the functionality of the surviving neurons and their ability to communicate coherently. Here, we report that spreading depolarizing waves (SDW), similar to those observed in the ischemic penumbra, critically damage the encoding capabilities of cortical neurons.

**Results:** We found that the ability to track high-frequency (> 100 Hz) changes in input is lost in the neurons subjected to two brief hypoxic episodes, each associated with generation of a transient SDW. We also found that, following the SDWs, the survived cortical neurons suffered from proteolysis of the axon initial segment (AIS) cytoskeleton, suggesting that the structural organization of the AIS may contribute to the ability of the axon to track high-frequency inputs. High-speed fluorescence imaging of Na<sup>+</sup>-sensitive indicator, SBFI, revealed that the peak amplitudes of the single spike elicited Na<sup>+</sup> transients in the AIS of the post-SDW neurons were smaller. Their decay times, however, were longer than in the control cells, indicating that the diffusion-mediated clearance of Na<sup>+</sup> ions is significantly decelerated. Both structural and functional SDW-associated derangements require activation of high calcium-dependent protease, calpain, since they were prevented when we blocked the SDW generation by using a synaptic blockers cocktail or by bath application of calpain inhibitor, CI-3.

**Conclusion:** Our results highlight new type of neuronal functional injury which may have great implications on the ability of surviving cortical neurons to communicate coherently in the first hours following stroke.

This research was supported by the Israel Science Foundation (grant No. 1302/14)

## **PLASTIC ADAPTIVE CHANGES TRIGGERED BY CHRONIC M-CHANNEL ACTIVATION IN HIPPOCAMPAL NEURONS**

Lia Shalom, Jonathan Lezmy, Maya Lipinsky, Eliav Tikochinsky and Bernard Attali

*Department of Physiology & Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv 69978, Israel*

Neurons employ powerful mechanisms to stabilize firing rates in response to perturbations of network activity, a process known as homeostatic plasticity. Structural reorganization of the axon initial segment (AIS) is one of the adaptive processes used to homeostatically stabilize neuronal excitability; however, the specific mechanisms remain unknown. In our lab, we triggered AIS plasticity in hippocampal neurons by altering the activity of M-channels, which fine tune neuron excitability and are prominently localized in the AIS. Recently, we found that sustained M-channel inhibition lead to adaptive changes that reduce the induced hyperexcitability. Is the homeostatic compensation bidirectional? Does chronic hypo-excitability also lead to compensations that will reduce the perturbation? In this study, we altered M-channel activity via chronic exposure of cultured hippocampal neurons to the M-channel opener Retigabine (RGB) and examined the homeostatic changes occurring in the intrinsic neuronal excitability, notably those of the AIS as well as those involving synaptic compensations. During sustained M-channel activation, a homeostatic adaptation occurs. Prolonged RGB exposure of four hours produces homeostatic compensations of intrinsic neuronal excitability (RMP and rate of evoked spike discharge after 4h); however, after 4h RGB, the threshold current remains higher yet, similar to the value obtained for acute treatment. Only following prolonged RGB exposures (24 and 48 hours), the homeostatic process leads the threshold current values to return to those of untreated neurons. The protracted M-channel activation produces homeostatic changes in synaptic scaling. After 24h and 48h of RGB treatment the spontaneous firing rate is readjusted and not significantly different from that of untreated neurons. Similarly to the sustained RGB treatment, prolonged exposures to Baclofen also leads to homeostatic compensations. At 4 hours of Baclofen exposure, the spontaneous firing rate still decreases, while at longer exposures (24 and 48 hours), it returns to values similar to those of untreated neurons.

## **M-CHANNEL-INDUCED PLASTICITY IN DORSAL ROOT GANGLIA SENSORY NEURONS**

Dana Rachminov, Asher Peretz and Bernard Attali

*Department of Physiology & Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv 69978, Israel*

**Background:** The voltage-gated K<sup>+</sup> channel subunits Kv7.2 and Kv7.3 encode the M-current, which is important for controlling neuronal excitability in both central and peripheral sensory neurons. In sensory neurons, TRPV1 activation causes an inward flow of cations, thereby depolarizing the membrane potential to a threshold that enables action potential generation and propagation along the peripheral nociceptive fibers of dorsal root ganglia (DRG) sensory neurons. Recent studies showed that there is a physical and functional interaction between TRPV1 and Kv7.2/3 channels. Here we investigated the plasticity of rat DRG neurons triggered by M-channel modulation.

**Results:** Following prolonged inhibition or activation of M-channels by a blocker (XE991) or an opener (retigabine), respectively, the intrinsic excitability parameters measured by DRG current injection such as rheobase current, input resistance, resting membrane potential and firing rate of spike discharge were compared to those of DRG treated acutely with the same drugs. While acute application (3 min) of the M-channel blocker (10 μM XE991) induced DRG hyperexcitability, extended exposure caused striking attenuation of neuronal excitability, reaching steady hypoexcitability by 24 hours. Reciprocally, acute application (3 min) of the M-channel opener (10 μM retigabine) elicited decreased DRG excitability, while prolonged exposure produced a prominent hyperexcitability already attained by 4 hours only.

**Conclusions:** Our results indicate that chronic exposure of DRG neurons to M-channel modulators leads to significant compensatory processes. The homeostatic changes are bidirectional but not entirely symmetric in terms of kinetics, since the compensatory hyperexcitability occurring after chronic M-channel activation is achieved slightly faster. This study shows that like in central neurons, homeostatic plasticity can also operate in sensory DRG neurons in front bidirectional perturbations.

## **M-CURRENT INHIBITION RAPIDLY INDUCES A UNIQUE CK2-DEPENDENT PLASTICITY OF THE AXON INITIAL SEGMENT**

Jonathan Lezmy<sup>a\*</sup>, Maya Lipinsky<sup>a</sup>, Yana Khrapunsky<sup>b</sup>, Eti Patrich<sup>a</sup>, Lia Shalom<sup>a</sup>, Asher Peretz<sup>a</sup>, Ilya Fleidervish<sup>b</sup> and Bernard Attali<sup>a</sup>

<sup>a</sup>*Department of Physiology & Pharmacology, Sackler Faculty of Medicine and Sagol School of Neurosciences, Tel Aviv 69978* and <sup>b</sup>*Department of Physiology and Cell Biology and Zlotowski Center for Neuroscience, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel*

\*email for correspondence: [jonatha8@mail.tau.ac.il](mailto:jonatha8@mail.tau.ac.il)

Alterations in synaptic input, persisting for hours to days, elicit homeostatic plastic changes in the axon initial segment (AIS), which is pivotal for spike generation. Here we triggered in hippocampal pyramidal neurons of both primary cultures and slices a unique form of AIS plasticity by selectively targeting M-type K<sup>+</sup> channels, which prominently localize to the AIS and are essential for tuning neuronal excitability. While acute M-current inhibition via cholinergic activation or direct channel block made neurons more excitable, minutes to hours of sustained M-current depression resulted in a gradual reduction in intrinsic excitability. Dual soma–axon patch-clamp recordings combined with axonal Na<sup>+</sup> imaging and immunocytochemistry revealed that these compensatory alterations were associated with a distal shift of the spike trigger zone and distal relocation of FGF14, Na<sup>+</sup> and Kv7 channels but not Ankyrin G. The concomitant distal redistribution of FGF14 together with Nav and Kv7 segments along the AIS suggests that these channels relocate as a structural and functional unit. These fast homeostatic changes were independent of L-type Ca<sup>2+</sup> channel activity but were contingent on the crucial AIS protein, protein kinase CK2. Using compartmental simulations, we examined the effects of varying the AIS position relative to the soma and found that AIS distal relocation of both Nav and Kv7 channels elicited a decrease in neuronal excitability. Thus, alterations in M-channel activity rapidly trigger unique AIS plasticity to stabilize network excitability.



## ACUTE EFFECTS OF SUMOATION ON CORTICAL NEURONAL EXCITABILITY

Kotler O. and Fleidervish I.A.

*Department of Physiology and Cell Biology, Faculty of Health Sciences and Zlotowski Center for Neuroscience, Ben-Gurion University, Beer-Sheva, 84015, Israel*

**Background:** The activity of proteins can be modulated by covalent binding of molecules to specific sites in that protein; Small Ubiquitin-like Modifier (SUMO) is one such modulator. SUMO has been shown to modify the activity of the K<sub>2</sub>P, K<sub>v</sub> and Nav channels and thereby change the activity of cultured neuron. Here, using current clamp, whole cell recording from L5 cortical pyramidal neurons in murine brain slices, we examined the effects of SUMOation on excitable properties of their membrane. Exogenous SUMO1 (1 nM) or catalytic domain of deSUMOylating enzyme, SENP1 (1 nM), were applied intracellularly via the somatic pipette.

**Results:** We found that changes in SUMOation levels had little effect on passive neuronal properties including resting potential, apparent input resistance and membrane time constant. By contrast, SUMOation rapidly affected the parameters of individual spike generation as well as the frequency of repetitive firing at a given current input. An examination of the  $dV/dt-V_m$  relationship of somatic action potentials elicited by brief current pulses revealed a leftward shift in threshold, slowing of the somatic phase of the spike upstroke and slowing of spike repolarization following SUMO application, whereas application of SENP had opposite effect. Neuronal gain was significantly increased by SUMO and decreased when applying the SENP enzyme. These changes are consistent with hypothesis that, in cortical neurons, SUMO directly affects kinetics of voltage-gated Na<sup>+</sup> and K<sup>+</sup> channels.

**Conclusion:** We show that activation of the SUMOation cascade could elicit a rapid neuromodulatory response in cortical pyramidal neurons. These functional changes could be a "missing" element in understanding of neuronal dysfunction in stroke and epilepsy.

This research was supported by the Israel Science Foundation (grant No. 1302/14)

## **PRO-NEUROGENIC EFFECT OF STATIC MAGNETIC FIELD IN RAT BRAIN**

Moriya Ben Yakir-Blumkin<sup>1</sup>, Yelena Loboda<sup>1</sup>, Levi Schächter<sup>2</sup>, John P.M. Finberg<sup>1</sup>.

<sup>1</sup>*Neuroscience Department, Rappaport Faculty of Medicine, Technion*

<sup>2</sup>*Department of Electrical Engineering, Technion, Haifa, Israel.*

[moriya.blumkin@gmail.com](mailto:moriya.blumkin@gmail.com)

In previous work in our laboratory we have observed an increase in proliferation of neuroblast cells of primary cultures of neonatal rat cerebral cortex caused by exposure to weak static magnetic fields (SMF) of the order of 50 Gauss (G). In order to determine whether in vivo exposure of rat brain to similar strength SMF enhances neurogenesis in the intact animal we implanted small (3mm diameter) titanium-coated magnetic discs onto the external surface of the skull at bregma. Control animals were implanted with a non-magnetic disc of the same size. The magnetic field strength at the area of the sub-ventricular zone was 20-50 G. EdU (50 mg/kg) was injected i.p. 2 X daily on days 7,8,14,15 and 1X daily on days 9,16 and 21 after magnet implantation. Animals were euthanized 1 h after EdU injection on day 21. Exposure to SMF caused a 59 % increase in numbers of newly-generated EdU-positive cells in SVZ. Numbers of DCX-positive cells in SVZ was not increased by SMF exposure, but the number of EdU and DCX double-labelled cells in the same area was increased by 66%. The expression of EdU- and DCX-positive cells in a defined area of neo-cortex from bregma -1.50 mm to -2.12 mm was also increased by SMF exposure. These results may have implications for use of SMF exposure in a variety of CNS disorders including head trauma and Alzheimer's disease.

## **NEUROPROTECTIVE AND BEHAVIORAL EFFECTS OF WEAK STATIC MAGNETIC FIELDS IN THE RAT.**

Uzan-Gueta Ravit\*, Loboda Yelena\*, Schachter, Levi<sup>#</sup>., and Finberg John\*

*\*Department of Neurobiology, Rappaport Faculty of Medicine, <sup>#</sup>Faculty of Electrical Engineering, Technion, Haifa, Israel.*

Corresponding email:ravituza@gmail.com

There is an increasing evidence of magnetic fields (MF's) effect on biological tissues as a non-invasive and innovative medical treatment for different disease conditions. In a previous study from our lab, (Ben-Yakir Blumkin et al. 2014), it was found that exposure of primary cortical neurons to static magnetic field (SMF) causes a reduction in etoposide-induced apoptosis. Accordingly, we explored SMF's neuroprotective effect on the neurodegenerative effect of 6-hydroxydopamine (6OHDA) in vivo, by implanting a small (3mm X 0.7mm) titanium-coated magnetic disc on the cranium above bregma. In addition, since Transcranial magnetic stimulation (TMS) is commonly used for the treatment of depression, and alterations in memory and increased anxiety are observed in ageing, we studied SMF's age related and time course related effects on rat behavior, using young (2 month) , middle age (12 month) and aged rats (24 month). Moreover, SMFs' effect on hippocampal neurogenesis was studied using the thymidine analog, Ethynyl deoxyuridine (Edu).Our study demonstrated that 1 week of SMF caused an anxiolytic effect in elderly rats as observed in the elevated plus maze (EPM) tests. In addition, young rats exposed to SMF showed increased levels of Edu labeled cells in the hippocampus compared to controls, however, no significant effect on neurogenesis levels were detected. We also found that SMF exposure protected the dopaminergic cells in substantia nigra pars compacta (SNc) from 6OHDA-induced neurodegeneration preserving cell number at normal levels.

The results indicate that 1 week of SMF exposure exerts age dependent effects on anxiety related parameters, and a neuroprotective effect on dopaminergic neurons of SNc. In addition, SMF increases hippocampal cell proliferation in young rats' with no effect on neurogenesis. In general, the study indicates a possibility of using SMF for treatment of neurodegeneration and anxiety in humans.

## **A NEW SPICE TO INFLAMMATORY SOUP: PLATELET-DERIVED GROWTH FACTOR ACTIVATES PERIPHERAL PAIN-RELATED NEURONS BY INHIBITING Kv7Kv7/M-TYPE POTASSIUM CHANNELS**

Barkai Omer<sup>1,2</sup>, Goldstein Robert<sup>1,2</sup>, H, Lev Shaya<sup>1,2</sup>, Binshtok AM Alexander<sup>1,2</sup>

<sup>1</sup> Edmond and Lilly Safra Center for Brain Sciences <sup>2</sup> Dept. of Medical Neurobiology, IMRIC Faculty of Medicine, The Hebrew University of Jerusalem

Omer.barkai@mail.huji.ac.il

When a tissue is injured, a “soup” of proinflammatory mediators is released into the site of injury, producing sensitization of peripheral pain-related neurons (nociceptors), hence leading to inflammatory pain. Among these factors is Platelet-Derived Growth Factor (PDGF), which is released after tissue injury and has been shown to be involved in inflammation and wound healing. Here we hypothesize that PDGF, in addition to its proinflammatory effects on cell migration, differentiation and proliferation in wound healing, also affects nociceptive excitability, thus leading to inflammatory pain. We examined this hypothesis using electrophysiological recordings from nociceptive neurons, behavior tests and computational modeling. We show that application of PDGF on acutely dissociated rat nociceptive neurons in culture leads to a substantial membrane depolarization, followed by a barrage of action potential firing. Furthermore, subcutaneous injection of PDGF leads to pain hypersensitivity in rats. We explored the underlying mechanism of PDGF-induced hyperexcitability and demonstrated that application of PDGF leads to blockade of potassium Kv7/M-current in nociceptors. We demonstrated that blockade of the Kv7/M-current is sufficient to induce nociceptive activation. Moreover, blockade of the PDGF receptor (PDGFR) with Imatinib, a clinically used PDGFR inhibitor, leads to a significant reduction of inflammatory pain, suggesting that the PDGF-Kv7/M-current pathway plays a pivotal role in development of inflammatory pain. Therefore, our results suggest that blockade of PDGFR with Imatinib may be clinically used to attenuate inflammatory pain.

## **PAIN AT ITS SOURCE: SIGNAL TRANSDUCTION AND PROPAGATION AT THE NOCICEPTIVE PERIPHERAL TERMINALS**

Robert H. Goldstein<sup>1,2\*</sup>, B. Katz<sup>1,2</sup>, Lev S.<sup>1,2</sup>, A.M. Binshtok<sup>1,2</sup>

<sup>1</sup> *Department of Medical Neurobiology, Institute of Medical research Israel-Canada, The Hebrew University Faculty of Medicine;* <sup>2</sup> *The Edmond and Lily Safra Center for Brain Sciences, The Hebrew University*

\* [inrobe.goldste@mail.huji.ac.il](mailto:inrobe.goldste@mail.huji.ac.il)

Noxious signals are detected by terminals of nociceptive fibers situated among the keratinocytes and epithelial cells. These minute structures possess the functional elements for detecting, transmitting and modulating pain-related signals, thus being key structures for pain sensation, in normal and pathological conditions. However, little is known about the physiology of terminals mainly due to their miniature size and location. Hence, basic questions such as where is the location of action potential initiation and does this location undergo plastic changes to effect terminal excitability, are still unanswered. Here we have implemented fast optical recording from mice nociceptive terminals *in vivo* enabling us for the first time to directly study the activity terminals and distal axons in the most relevant conditions. We have examined changes in intra-terminal  $\text{Ca}^{2+}$  following electrical stimulation or focal application of capsaicin, using the genetically encoded  $\text{Ca}^{2+}$  indicator GCaMP6s. We found that blockade of Na(v)s did not affect the  $\text{Ca}^{2+}$  signals in the terminal tips, however,  $\sim 20\mu\text{m}$  away from the tip,  $\text{Ca}^{2+}$  signals are partially dependent on Na(v)s. At  $\sim 30\mu\text{m}$  from the tip the  $\text{Ca}^{2+}$  signal was fully and reversibly abolished by Na(v) blockade, suggesting that the spike initiation zone (SIZ) is located at this area of the nociceptive axon. Importantly, the SIZ moved closer to the terminal tip when we pharmacologically mimicked inflammatory conditions. These plastic changes in the location of SIZ could underlie inflammatory-induced increase in pain.

## **THE TASTE OF KCl – WHAT A DIFFERENCE A SUGAR MAKES**

Natalie Ben Abu<sup>1</sup>, Masha Y. Niv<sup>1</sup>

*<sup>1</sup>The Institute of Biochemistry, Food Science and Nutrition Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel*

corresponding e-mail: natalie.benabu@mail.huji.ac.il

Dramatic increase in NaCl consumption lead to sodium intake beyond health guidelines. KCl substitution helps reduce sodium intake but results in a bitter-metallic off-taste. Two disaccharides, trehalose and sucrose, were tested in order to untangle the chemical (increase in effective concentraion of KCl due to sugar addition) from the sensory effects. The bitter-metallic taste of KCl was reduced by these sugars, saltiness was enhanced or unaltered. The perceived sweetness of sugar, regardless of its type and concentration, was an important factor in KCl taste modulation. Though KCl was previously shown to increase the chemical activity of trehalose but not of sucrose, we found that it suppressed the perceived sweetness of both sugars. Therefore, sensory integration was the dominant factor in the tested KCl-sugar combinations.

## **THE ROLE OF ZnR/GPR39 IN REGULATION OF H<sup>+</sup> EFFLUX IN SALIVARY GLAND DUCT CELLS**

Moran Melamed, Michal Hershinkel

*Department of Physiology, Cell Biology, Faculty of Health Sciences,  
Ben-Gurion University of the Negev, Beer-Sheva, Israel.*

Human tissues and fluids contain large concentrations of zinc, which fulfills crucial roles in cell functionality, growth, proliferation and survival. Zinc may also be found as a free ion, Zn<sup>2+</sup>, in organelles. For example, Zn<sup>2+</sup> is selectively concentrated in secretory granules of the salivary glands. In the salivary duct cell line, HSY, extracellular Zn<sup>2+</sup> activates a Zn<sup>2+</sup> sensing receptor, ZnR/GPR39, which activates downstream Ca<sup>2+</sup> signaling. The ZnR/GPR39 has been shown to modulate ion transport in colon epithelial cells. Since zinc deficiency is associated with impaired salivary secretion, we asked if ZnR/GPR39 also modulates ion transport in HSY cells. Cellular pH imaging studies, using NH<sub>4</sub><sup>+</sup> as a surrogate to K<sup>+</sup>, suggest that ZnR/GPR39 signaling leads to enhanced K<sup>+</sup> transport. We initially asked if ZnR/GPR39 upregulates K<sup>+</sup>-dependent Cl<sup>-</sup> (KCC) transport, reflected by NH<sub>4</sub><sup>+</sup> enhanced transport and thereby acidification. Pharmacological inhibitors of KCC did not attenuate the acidification, indicating that KCC is not mediating the ion transport in HSY cells. We then determined the role of the K<sup>+</sup>/Na<sup>+</sup> ATPase pump, using its inhibitor Ouabain, and saw that the ZnR/GPR39-dependent ion transport is reduced. Based on my results, we suggest that ZnR/GPR39 is upregulating the K<sup>+</sup>/Na<sup>+</sup> ATPase pump, which may regulate salivary duct secretory activity that is important for regulation of salivary contents.

## ZINC REGULATES KCC ACTIVITY VIA ZNR/GPR39 IN BREAST CANCER CELLS

Moumita Chakraborty, Hila Asraf, Michal Hershinkel

*Department of Physiology, Cell Biology, Faculty of Health Sciences,  
Ben-Gurion University of the Negev, Beer-Sheva, Israel.*

**Back ground:** Breast cancer is one of the most common and devastating diseases affecting women. A hallmark of breast cancer malignant cells involves aberrant signaling patterns and changes in  $Zn^{2+}$  accumulation and distribution. The Gq-coupled receptor, ZnR/GPR39, is activated by extracellular  $Zn^{2+}$  and regulates major pathways linked to cell proliferation and survival. KCC is a family of proteins that mediate  $Cl^-$  and  $K^+$  cotransport and was associated with cell proliferation, migration and invasion of cancer cells. We hypothesize that  $Zn^{2+}$ , via its sensing receptor ZnR/GPR39, regulates KCC function in breast cancer cells and thereby cell growth. Here we investigate the mechanism linking ZnR/GPR39 and KCC activity.

**Results:** We show  $Zn^{2+}$  dependent  $Ca^{2+}$  release, a hallmark of GPR39 activity, in ER negative BT20 and MDA-MB-453 breast cancer cells. Moreover,  $Zn^{2+}$  upregulates K/Cl transport, likely mediated by KCC/NKCC, in BT20 and MDA-MB-453 cells.

**Conclusions:** Based on these results, we conclude that extracellular  $Zn^{2+}$  triggers release of intracellular  $Ca^{2+}$  in BT20 and MDA-MB-453 cells which may act via ZnR/GPR39. Extracellular  $Zn^{2+}$  is also affecting the KCC/NKCC activity in both the cell lines. Thus ZnR/GPR39 signaling may contribute to cell proliferation and invasion of cancer cells.



## **BLOCKAGE OF AP-1 TRANSCRIPTIONAL COMPLEX SENSITIZE ESOPHAGEAL AND HEAD AND NECK CANCERS TO PI3K $\alpha$ INHIBITION**

Limor Cohen, Mai Bdarni, Noa Balaban, Manu Prasad, Ben-Zion Yehoshua, Moshe Elkabets

*The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Beer-Sheva, Israel*

Esophageal and head and neck squamous cell carcinoma (ESCC and HNSCC) are among the common and lethal cancers with low survival rates. Phosphoinositide-3-kinase (PI3K) pathway is often hyper activated in HNSCC and ESCC, leading to tumor cell proliferation and survival. Inhibition of the pathway using isoform specific inhibitor of the PI3K -BYL719, have shown clinical activity in patients bearing PIK3CA mutations or amplification. Unfortunately, the efficacy of the drug was limited by the emergence of resistance, that was accompanied by overexpression of the receptor tyrosine kinase AXL, The molecular mechanisms underlying AXL overexpression in HNSCC and ESCC remained elusive. Here we show that AXL expression is regulated by the AP-1 transcriptional complex involving the transcription factors c-JUN and c-FOS. We show that resistance to BYL719 is associated with upregulation of the transcription factor c-JUN concomitantly with an increase of AXL expression. This c-JUN\AXL correlation was also demonstrated across HNSCC and ESCC cells lines, and patients tumor samples. Silencing of c-JUN or c-FOS expression leads to a downregulation of AXL expression, and re-sensitization to BYL719 in-vitro and in-vivo. Importantly, blocking of c-JUN-N-terminal kinase (JNK) using SP600125 showed a synergistic anti-tumor activity when combined with BYL719 in-vitro, and superior anti-tumor activity in vivo, in cell line derived and also patient-derived xenograft models. These results support the rational for combined inhibition of JNK\c-JUN\AXL axis and PI3K in HNSCC and ESCC patients.

## **REDUCTION IN ENDOGENOUS CARDIAC STEROIDS PROTECTS THE BRAIN FROM OXIDATIVE STRESS IN MANIA**

Anastasia Hodes<sup>a\*</sup>, Haim Rosen<sup>b</sup> Tzuri Lifschytz<sup>c</sup>, Hagit Cohen-Ben Ami<sup>a</sup> and David Lichtstein<sup>a</sup>

<sup>a</sup>*Department of Medical Neurobiology and* <sup>b</sup>*Department of Microbiology and Molecular Genetics, Institute for Medical Research Israel-Canada, The Hebrew University-Hadassah Medical School, Jerusalem, Israel;*

<sup>c</sup>*Department of Psychiatry, Hadassah-Hebrew University Medical Center, Jerusalem, Israel;*

\*Correspondence to: anastasi.singalevich@mail.huji.ac.il

**Objectives:** Bipolar disorder (BD) is a severe and chronic mental illness characterized by episodes of mania and depression. Numerous studies have implicated the involvement of endogenous cardiac steroids (CS), and their receptor, Na<sup>+</sup>, K<sup>+</sup> -ATPase, in BD. We have previously demonstrated that a reduction in brain CS protects against manic-like behavior in an amphetamine (AMPH)-induced mania model in mice. The aim of the present study was to examine the role of brain oxidative stress in the CS-induced behavioral effects.

**Methods:** AMPH-induced hyperactivity, assessed in the open-field test, served as a model for manic-like behavior in mice. A reduction in brain CS was obtained by specific and sensitive anti-ouabain antibodies injected into the lateral ventricle (ICV). The level of oxidative stress was tested in the hippocampus and frontal cortex of the animals. We measured the activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), as well as the levels of antioxidant non-protein thiols (NPSH) and oxidative damage biomarkers thiobarbituric acid reactive substances (TBARS) and protein carbonyl (PC).

**Results:** AMPH administration resulted in marked hyperactivity and increased oxidative stress, as manifested by increased SOD activity, decreased activities of CAT and GPx, reduced levels of NPSH and increased levels of TBARS and PC. The administration of anti-ouabain antibodies reduced the AMPH-induced hyperactivity, and protected against the concomitant oxidative stress in the brain by restoring the activity levels of the antioxidants and reducing the oxidative damage biomarkers.

**Conclusions:** Our results demonstrate that oxidative stress participates in the effects of endogenous CS on manic-like behavior induced by AMPH. These findings support the notion that CS and oxidative stress may be associated with the pathophysiology of mania and BD. We propose that reduction in brain CS level or inhibition of their activity are potential targets for drug development for BD.

## **AUGMENTATION OF OUABAIN-INDUCED INCREASE IN HEART CONTRACTILITY BY AKT INHIBITOR MK-2206**

<sup>1</sup>\*Nahum Buzaglo, <sup>2</sup>Haim Rosen, <sup>3</sup>Mordehai Golomb, <sup>3</sup>Ronen Beeri, <sup>1</sup>Hagit Cohen-Ben Ami, <sup>4</sup>Fattal Langane, <sup>4</sup>Sandrine Pierre and <sup>1</sup>David Lichtstein.

<sup>1</sup>*Department of Medical Neurobiology,* <sup>2</sup>*Department of Microbiology and Molecular Genetics, IMRIC, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.* <sup>3</sup>*The Heart Institute, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.* <sup>4</sup>*Marshall Institute for Interdisciplinary Research, Huntington, WV, USA.*

\*Correspondence to: [buzaglo@gmail.com](mailto:buzaglo@gmail.com)

Congestive heart failure (CHF) is a widespread disease that has an impact on health, worldwide. Despite advances in therapy, morbidity, and mortality due to CHF remain high. Advances in the understanding of the pathophysiological mechanisms of CHF and the development of new drugs for the treatment of the disease are of utmost importance. Cardiac steroids (CS) such as ouabain and digoxin increase the force of contraction of heart muscle and are used for the treatment of congestive heart failure (CHF). However, their small therapeutic window limits their use. It is well established that Na<sup>+</sup>, K<sup>+</sup>-ATPase inhibition mediates CS-induced increase in heart contractility. Recently, we showed also the involvement of intracellular signaling in CS-induced positive inotropy, in vivo and ex vivo, in a zebrafish model. The aim of the present study was to test the hypothesis that combined treatment with ouabain and Akt inhibitor (MK-2206) augments ouabain-induced inotropy in mammalian models. We demonstrate that the combined treatment led to an ouabain-induced increase in contractility at concentrations at which ouabain alone was ineffective. This was shown in three experimental systems: neonatal primary rat cardiomyocytes, a Langendorff preparation and an in vivo myocardial infarction induced by LAD-ligation. Furthermore, cell viability experiments revealed that this treatment protected primary cardiomyocytes from MK-2206 toxicity and in vivo reduced the size of scar tissue 10 days post- LAD-ligation. We propose that Akt activity imposes a constant inhibitory force on muscle contraction, which is attenuated by low concentrations of MK-2206, resulting in potentiation of the ouabain effect. This demonstration of the increase in the CS effect advocates the development of the combined treatment in CHF.

## FUNCTIONAL ABNORMALITIES IN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES GENERATED FROM A DUCHENNE MUSCULAR DYSTROPHY PATIENT AND A FEMALE MANIFESTING CARRIER

Binyamin Eisen, MSc,<sup>a</sup>; Ronen Ben Jehuda, MSc,<sup>a, b</sup>; Ashley J. Cuttitta, MSc,<sup>c</sup>; Lucy N. Mekies, MSc,<sup>a</sup>; Lubna Willi, MSc,<sup>a</sup>; Yuval Shemer, MSc,<sup>a</sup>; Irina Reiter, MSc,<sup>a</sup>; Ifat Abramovich, PhD,<sup>d</sup>; Dov Freimark, MD,<sup>e, f</sup>; Mihaela Gherghiceanu, MD, PhD,<sup>g</sup>; Lorenzo Monserrat, MD, PhD,<sup>h</sup>; Daniel E. Michele, PhD,<sup>c</sup>; Eyal Gottlieb, PhD,<sup>d</sup>; Michael Arad, MD,<sup>e, f</sup>; Ofer Binah, PhD,<sup>a</sup>

<sup>a</sup>Department of Physiology, Biophysics and Systems Biology, Ruth and Bruce Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel. <sup>b</sup>Department of Biotechnology, Technion – Israel Institute of Technology, Haifa, Israel. <sup>c</sup>Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan, USA. <sup>d</sup>Technion Integrated Cancer Center, Ruth and Bruce Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel. <sup>e</sup>Leviev Heart Center, Sheba Medical Center, Ramat Gan, Israel. <sup>f</sup>Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel. <sup>g</sup>Victor Babes National Institute of Pathology, Bucharest, Romania. <sup>h</sup>Health in Code, A Coruña, Spain.

Email: binyae@campus.technion.ac.il

### Background

Duchenne muscular dystrophy (DMD), an X-linked muscle degenerative fatal disease, is caused by dystrophin mutations. Dilated cardiomyopathy (DCM) is a major cause of morbidity and mortality in DMD patients.

### Objective

We hypothesized that DMD patients induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) manifest key aspects of the disease.

### Methods

We generated iPSC-CMs from a DMD male patient and a female manifesting carrier, and investigated their molecular and functional properties using NGS, patch clamp, RT-PCR, Ca<sup>2+</sup> imaging and metabolic indices.

### Results

NGS demonstrated DMD female iPSCs underwent X chromosome reactivation, and iPSC-CMs displayed mosaic X chromosome expression of the WT and mutated alleles. WT dystrophin allele levels were higher in female iPSC-CMs compared to control. DMD iPSC-CMs exhibited low automaticity, prolonged action potential duration, and presented delayed afterdepolarizations. Beat rate variability (BRV) analysis demonstrated DMD female iPSC-CMs displayed significantly increased BRV.

DMD iPSC-CMs displayed: (a) increased expression of hyperpolarization-activated cyclic nucleotide-gated channel – isoform 4 (HCN4); (b) increased L-type Ca<sup>2+</sup> current (I<sub>Ca,L</sub>) density; (c) increased expression of the Ca<sup>2+</sup> voltage-gated channel subunit alpha1 C (CACNA1C). Additionally, DMD male iPSC-CMs presented smaller pacemaker current (I<sub>f</sub>) density.

Under basal conditions, [Ca<sup>2+</sup>]<sub>i</sub> and contraction parameters were similar in DMD and control cardiomyocytes, but mutated iPSC-CMs manifested attenuated positive inotropic response to β-adrenergic stimulation with isoproterenol.

Seahorse XF analyzer demonstrated decreased oxidative phosphorylation accompanied by a correlated increase in glycolysis in DMD iPSC-CMs. Accordingly, mass spectrometry analysis showed a dramatic fall in phosphocreatine levels in DMD iPSC-CMs as well.

### Conclusions

DMD iPSC-CMs exhibit electrophysiological abnormalities, attenuated response to β-adrenergic stimulation, metabolic deficits and reduced energy stores. Importantly, mosaic dystrophin expression in DMD female iPSC-CMs contributes to the observed abnormalities. These features render this model as a valuable tool for investigating DCM in DMD.

## MUTATIONS IN GIRK4 CAUSE SPECIFIC CARDIOVASCULAR DISORDERS AND DISEASES BIOPHYSICAL AND CELLULAR MECHANISM

Reem Handklo, Boris Shalomov, Haritha P. Reddy, Amal Kanti Bera, Nathan Dascal<sup>1</sup>.

<sup>1</sup>Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University.

<sup>2</sup>Department of Biotechnology, Bhupat and Jyoti Mehta School of Biosciences Building, Indian Institute of Technology Madras.

Email: [reem.handklo@gmail.com](mailto:reem.handklo@gmail.com)

GIRK4 (*KCNJ5*) is one of four subunits of the G protein-gated potassium channels (GIRK1-4). GIRK4 subunits form homotetramers or heterotetramers with GIRK1, and mediate inhibitory transmission via G-protein coupled receptors. *KCNJ5* mutants are involved in primary aldosteronism (PA) and long Q-T (GIRK4<sub>G387R</sub>). A widely accepted mechanism for PA states caused by GIRK4 mutations in the selectivity filter is loss of selectivity to K<sup>+</sup>. Na<sup>+</sup> ions pass through the channel, causing depolarization and leading to opening of voltage gated Ca<sup>2+</sup> channels and eventually Ca<sup>2+</sup>-dependent aldosterone secretion. This mechanism later was extended to mutations in cytosolic N- and C-terminal domains far from the selectivity filter, GIRK4<sub>R52H</sub>, GIRK4<sub>E246K</sub>, and GIRK4<sub>G247R</sub>. Here, we studied the GIRK4<sub>R52H</sub>, GIRK4<sub>E246K</sub>, GIRK4<sub>G247R</sub> and GIRK4<sub>G387R</sub> mutants in *Xenopus laevis* oocytes. Surface expression of all GIRK1/4 mutants tested yielded similar or slightly reduced expression levels (for GIRK1/4<sub>E246K</sub>) compared to GIRK1/4<sub>WT</sub>. GIRK1/4<sub>G247R</sub> and GIRK1/4<sub>G387R</sub> had properties similar to GIRK1/4<sub>WT</sub>. However, GIRK1/4<sub>R52H</sub> and GIRK1/4<sub>E246K</sub> channels had significantly smaller whole-cell currents than GIRK1/4<sub>WT</sub>, suggesting impaired gating. Coexpression of Gβγ greatly increased the currents of both mutants, suggesting that Gβγ may “correct” a gating defect. Additionally, analysis of current-voltage curves revealed that all mutations tested did not affect the reversal potential or inward rectification of mutated GIRK1/4, in a wide range of K<sup>+</sup> concentrations. Single channel analysis showed lower than WT open probability (P<sub>o</sub>) of GIRK1/4<sub>R52H</sub> channels. Our results suggest that previous interpretations of the effects of mutations GIRK4<sub>R52H</sub> and GIRK4<sub>E246K</sub> might have been incorrect, and that these mutations impair channel’s gating and, in the case of GIRK1/4<sub>E246K</sub>, surface expression, but not selectivity or rectification. Overall, our results urge to reconsider the existing model of the mechanism responsible for aldosterone overproduction in patients with the out-of-pore mutations of *KCNJ5*, and prompt to look for alternative explanations.

## **Insight into the regulatory mechanisms and sympathetic involvement in the regulation of mesenteric blood flow**

Reshef Meshulam B.Sc. Farid Zreik, MD, Oren Shibolet, MD, Giris Jacob, MD, DSc

*Medicine F, Gastroenterology and imaging Unit at Tel Aviv Medical Center, and Faculty of Medicine, TAU, Tel Aviv*

**Introduction:** Mesenteric blood flow circulation is not yet well elucidated in human. We hypothesized that sympathetic nervous system has a cardinal role in controlling the splanchnic circulation during orthostatic stress and after meal. Particularly, alpha1-adrenoceptor has an important function in this setting, and it has differential function after meal.

**Methods:** First day study, twelve healthy participants (age  $28 \pm 1$  years, BMI  $23 \pm 0.2$  kg/m<sup>2</sup>) went through blood sampling (Glucose-insulin, catecholamine, renin-aldosterone), beat-to-beat hemodynamic monitoring, during rest supine and head up tilt (HUT). We assessed cardiac and vascular sympathetic tone and baroreflex sensitivity (spectral analysis), diameter and flow velocities of portal vein (PV), superior mesenteric artery (SMA), and hepatic artery (HA) by using USD. Second day study, hemodynamics of systemic and mesenteric blood circulation were assessed at baseline and during incremental doses of intravenous infusion of phenylephrine (dose-response fashion) during supine rest. Both studies were repeated after a standard 850 Kcal-meal.

**Results:** After meal rest supine cardiac sympathetic tone (LFrr/HFrr ratio) increased from  $0.86 \pm 0.1$  to  $1.44 \pm 0.2$  ( $p=0.001$ ) but sympathetic vascular tone (LF<sub>SBP</sub>) remained unchanged, and baroreflex sensitivity, decreased from  $31 \pm 5.9$  to  $22.7 \pm 3.2$  ms/mmHg,  $p=0.02$ . Diameter of PV, SMA and HA at baseline was  $11 \pm 0.2$ ,  $6.6 \pm 0.2$  and  $2.5 \pm 0.1$  mm, respectively, and after meal remained unchanged. Flow velocity of PV, SMA and HA at baseline was  $26 \pm 1.2$ ,  $78 \pm 4$ ,  $44 \pm 2$  cm/sec and after meal increased to  $37 \pm 2$  ( $p=0.001$ ),  $109 \pm 4$  ( $p=0.001$ ) and  $57 \pm 4$  ( $p=0.054$ ), respectively. During HUT after meal, cardiac sympathetic tone remained unchanged, while sympathetic vascular tone increased from  $5.7 \pm 1.2$  to  $8.4 \pm 1.3$  mmHg<sup>2</sup> ( $p=0.02$ ), and baroreflex sensitivity decreased from  $15.3 \pm 2.3$  to  $10.8 \pm 1.8$  ms/mmHg,  $p=0.005$ . During HUT, PV blood flow decreased from  $880 \pm 50$  to  $730 \pm 50$ ,  $p=0.07$ , and from  $1390 \pm 120$  to  $990 \pm 115$ ,  $p=0.03$ , during fasting and after meal, respectively. Plasma glucose, insulin, renin, and aldosterone increased significantly after meal, at rest supine.

During fasting, mean BP, systemic vascular resistance, SMA resistance and PV resistivity increased in a dose response fashion to phenylephrine ( $p > 0.01$ , all). Comparable dose-response curves effects were observed after meal for systemic vascular and SMA resistance. PV resistance did not show significant dose-response effect to phenylephrine after meal ( $p=0.01$ ). Phenylephrine did not affect the tested vessels' diameters during fasting and after meal.

**Conclusions:** Postprandial hyperemia, at rest, was only associated with increased sympathetic cardiac tone; however, during orthostatic stress the significant blood flow shift from the mesenteric circulation requires huge increase in sympathetic vascular tone. This was possible, because of the meal-induced baroreflex reset. Alpha1-adrenoceptor stimulation causes an increase in vascular resistance in SMA and PV territories, without changing their diameters. Meal causes a significant decrease in alpha1-adrenoreceptor sensitivity, only in the PV territory.

**Perspectives:** This is the first study, in healthy human, to explore in an integrated fashion the regulatory mechanisms involved in the control of mesenteric blood flow, during orthostatic stress and after meal. Because of the importance of the mesenteric circulation, we must perform similar investigation in patients with congestive heart failure and thereafter in those with liver cirrhosis. Undoubtedly, the results of the proposed studies will allow us to develop new treatment strategies in these common diseases.

## **MITOCHONDRIAL VDAC1-BASED PEPTIDE AS A NEW STRATEGY FOR FIGHTING STEATOSIS AND NASH**

Srinivas Pittala, Yakov Krelin and Varda Shoshan-Barmatz

*Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel*

[pittala@post.bgu.ac.il](mailto:pittala@post.bgu.ac.il)

Non-alcoholic fatty liver disease (NAFLD) (steatosis) and steatohepatitis (NASH) are liver pathologies characterized by fat accumulation and severe metabolic alterations that lead to liver inflammation, parenchyma damage and fibrosis (cirrhosis). NASH is the most extreme form of non-alcoholic fatty liver disease (NAFLD), and is regarded as a major cause of cirrhosis. There are currently no specific therapies for NASH, therefore an unmet need for new modalities for NASH treatment. We present here, a novel strategy to suppress steatosis and NASH progression, based on the mitochondrial protein, voltage-dependent anion channel 1 (VDAC1). This protein is a dynamic regulator of global mitochondrial function and is positioned at the crossroads of metabolic and survival pathways. As a “proof of concept,” we selected the high-fat diet (HFD-32)-STAM mouse model. Using this model, we demonstrated that intravenous administration of a synthetic VDAC1-based peptide, Tf-D-LP4, at different time points during steatosis-NASH-HCC progression, eliminated steatosis, inflammation, ballooning degeneration, fibrosis, and development of HCC. Using metabolic cages, we demonstrated that peptide-treated mice have high energy expenditure. The results suggest that peptide-treated mice possess high fatty acid oxidation, energy expenditure. These results show that the VDAC1-derived peptide, R-Tf-D-LP4 represents a novel, and effective compound that can eliminate the pathogenesis of NAFLD, NASH, as liver cells degeneration, inflammation, fibrosis, and reduces blood glucose level

## **HEAT ACCLIMATION MEDIATED CROSS-TOLERANCE: HIF-1A CONTROLLED MITOCHONDRIAL PERFORMANCE IN CARDIO-PROTECTION.**

A. Mreisat<sup>1</sup>, A. Saada Reisch<sup>2</sup> M. Horowitz<sup>1</sup>

<sup>1</sup>Laboratory of Environmental Physiology, Faculty of Dentistry, The Hebrew University of Jerusalem and <sup>2</sup> School of Medicine- Dept of genetics and metabolic diseases Hadassah Medical Center Jerusalem, Israel.

*Heat acclimation* (AC) is a reversible “within lifetime” phenotypic adaptation to long-term elevations in environmental temperature. It evolves via a continuum of temporally varying cellular processes. Successful AC leads to enhanced thermal tolerance collectively delaying the onset of heat injuries. AC can reinforce or interfere with the ability to combat novel acute stressors. An essential component in AC is the hypoxia inducible factor HIF-1 $\alpha$  leading to a metabolic switch linked with changes in anaerobic vs aerobic metabolic pathways ratio. In this presentation we present two parameters signifying HIF-1 $\alpha$  impacts: (i) HIF-1 $\alpha$  mitochondrial targets expression modulation (ii) mitochondrial physiological performance. We hypothesize that AC-induced metabolic switch remodels mitochondrial function to maintain ATP production despite impairment in oxygen supplementation. Male *Rattus norvegicus* were subjected to heat acclimation (AC) without and with the presence of HIF-1 $\alpha$  dimerization blockade. Using cardiac ischemia (75%) as a testing tool, AC impacts on mitochondrial oxygen consumption and on HIF-1 $\alpha$  mitochondrial target transcript and protein levels in the left ventricle of the heart were studied. Here we confirm AC metabolic switch via the expressed PDK, and transcripts. Importantly, AC increased mitochondrial O<sub>2</sub> consumption and resulted in a switch of LDH-a to LDH-b predominance, leading to lactate-pyruvate shift. Others demonstrated in the brain that enhanced LDH-b is associated with improved hypoxic tolerance. Integrating the current observations with our previous NMR results demonstrating ATP preservation in AC hearts subjected to global ischemia and upregulation of COX 4.2 / COX 4.1 isoforms ratio (notably, COX isoform IV-2 suppresses the sensitivity of COX to its allosteric regulator ATP (Horvat, Beyer, Arnold, 2006), we can conclude that AC, via HIF-1 $\alpha$  target genes remodeling, leads to optimized mitochondrial performance during ischemia, and in turn, to cardio-protection. Acclimation with HIF-1 $\alpha$  dimerization blockade abolishes the heat acclimation mediated cross-tolerance thus confirming our hypothesis.



## **STRUCTURE-DYNAMIC COUPLING OF REGULATORY DOMAINS IN TISSUE-SPECIFIC VARIANTS OF NCX**

Moshe Giladi and Daniel Khananshvili

*Department of Physiology and Pharmacology, Sackler School of Medicine,  
Tel-Aviv University, Tel-Aviv, 69978, Israel*

*E-mail: dhanan@post.tau.ac.il*

**Purpose** - Mammalian Na<sup>+</sup>/Ca<sup>2+</sup> exchangers (NCX1, NCX2, and NCX3) and their splice variants are expressed in a tissue-specific manner and are regulated by Ca<sup>2+</sup> binding CBD1 and CBD2 domains. NCX2 does not undergo splicing, whereas in NCX1 and NCX3, the splicing segment (with mutually exclusive and cassette exons) is located in CBD2. The purpose of the present work was to highlight the general and special mechanisms underlying the Ca<sup>2+</sup>-dependent allosteric regulation in tissue-specific isoform/splice variants of mammalian NCXs.

**Results** - Ca<sup>2+</sup> binding to CBD1 results in Ca<sup>2+</sup>-dependent tethering of CBDs through the network of interdomain salt-bridges, which is associated with NCX activation, whereas a slow dissociation of "occluded" Ca<sup>2+</sup> inactivates NCX. Although NCX variants share a common structural basis for Ca<sup>2+</sup>-dependent tethering of CBDs, the Ca<sup>2+</sup> off-rates of occluded Ca<sup>2+</sup> vary up to 50-fold, depending on the exons assembly. The Ca<sup>2+</sup>-dependent tethering of CBDs rigidifies the interdomain movements of CBDs without any significant changes in the CBDs' alignment; consequently, more constraining conformational states become more populated in the absence of global conformational changes. Although the Ca<sup>2+</sup>-dependent "population shift" is a common mechanism among NCX variants, the strength and span of backbone rigidification from the C-terminal of CBD1 to the C-terminal of CBD2 is exon dependent. The mutually exclusive exons differentially stabilize/destabilize the backbone dynamics of Ca<sup>2+</sup>-bound CBDs in NCX1 and NCX3 variants, whereas the cassette exons control the stability of the interdomain linker.

**Conclusions** – The combined effects of mutually exclusive and cassette exons permit a fine adjustment of two different regulatory pathways involving the Ca<sup>2+</sup>-dependent activation (controlled by CBD1) and the Ca<sup>2+</sup>-dependent alleviation of Na<sup>+</sup>-induced inactivation (controlled by CBD2). Exon-controlled dynamic features match with cell-specific regulatory requirements in a given variant. The present information can be used for structure-based design of potential drug candidates selectively affecting the tissue-expressed NCX isoform/splice variants.

## **A VOLTAGE SENSOR MODE OF REGULATION OF THE MITOCHONDRIAL $\text{Na}^+/\text{Ca}^{2+}$ EXCHANGER, NCLX**

Marko Kostic\*, Tomer Katoshevski\* and Israel Sekler

*Dept. of Physiology and Cell Biology, Ben Gurion University, Beersheva, Israel*

\*Both authors contributed equally to this work

Mitochondrial  $\text{Ca}^{2+}$  signaling plays several key roles in the cell, for example in regulating ATP production, controlling free  $\text{Ca}^{2+}$  content in different cell compartments, as well as in cell apoptosis. Overload of mitochondrial  $\text{Ca}^{2+}$  can cause mitochondrial permeability pore (MPP) to open which is a sign of cell death. The  $\text{Ca}^{2+}$  flows into the mitochondria through the MCU, thanks to the mitochondrial membrane potential, and out through the much slower (100fold) NCLX a  $\text{Na}^+/\text{Ca}^{2+}/\text{Li}^+$  exchanger, that mediates  $\text{mCa}^{2+}$  removal. Because NCLX is much slower than MCU it constitutes the rate limiting step in  $\text{mCa}^{2+}$  transport and is therefore an essential transporter. Previous studies show that NCLX is regulated by phosphorylation on the Serin 258 residue (NCLX s258). But, how phosphorylation regulates NCLX activity is unknown. We hypothesized that NCLX activity is regulated by the mitochondrial membrane potential. A mild depolarization was induced by mitochondrial uncouplers, such as BAM15 and UCP1 (a physiological brown fat uncoupler) and  $\text{mCa}^{2+}$  transport was determined by *Cepia*. Both uncouplers caused partial mitochondrial depolarization and an inhibition of  $\text{mCa}^{2+}$  efflux; in contrast,  $\text{Ca}^{2+}$  influx was not affected, suggesting that the MCU is not affected by changes in mitochondrial membrane potential. Rescue of  $\text{mCa}^{2+}$  efflux in partial mitochondrial depolarized cells was achieved by expressing mutant NCLX S258D; mimicking a constitutively phosphorylated state of NCLX. The physiological implications of this regulation were tested in the  $\beta$  Cells line (INS1). Low glucose that induces mitochondria depolarization, failed to accelerate  $\text{mCa}^{2+}$  efflux. In contrast, application of high glucose that caused hyperpolarization in  $\beta$  Cells led to acceleration of  $\text{mCa}^{2+}$  efflux, which was suppressed by co-treating the cells with BAM15. The results of this study identify a new voltage sensor mode of regulation of the mitochondrial  $\text{Na}^+/\text{Ca}^{2+}$  exchanger NCLX, and show that it is controlled by mitochondrial membrane potential, and modulated by NCLX phosphorylation.

## **STRUCTURAL DYNAMICS OF ENGINEERED Na<sup>+</sup>/Ca<sup>2+</sup> EXCHANGER CAPABLE OF Li<sup>+</sup>-TRANSPORTING (NCLX\_Mj)**

Bosmat Refaeli, Reuben Hiller, Moshe Giladi and Daniel Khananshvili

*Department of Physiology and Pharmacology, Sackler School of Medicine,  
Tel-Aviv University, Tel-Aviv, 69978, Israel*

*E-mail: dhanan@post.tau.ac.il*

**Purpose** - Na<sup>+</sup>/Ca<sup>2+</sup> exchangers (NCX) extrude cytosolic Ca<sup>2+</sup> to control Ca<sup>2+</sup> homeostasis. The Sext, and Sint sites of the archaeal NCX (NCX\_Mj) exhibit high selectivity for Na<sup>+</sup> binding, whereas the SCa site can alternatively bind either Na<sup>+</sup> or Ca<sup>2+</sup>. Although this site-specific ion-selectivity could be related to ion-induced alternating access, the underlying mechanisms remain unclear. Here, NCLX\_Mj chimera (derived from NCX\_Mj) is analyzed due to its capacity to transport either Na<sup>+</sup> or Li<sup>+</sup> in exchange with Ca<sup>2+</sup>, although the ion-exchange rates are comparable in two proteins. Notably, the Km values for Na<sup>+</sup> transport are 10-fold higher in NCX\_Mj as compared with Na<sup>+</sup> (or Li<sup>+</sup>) transport in NCLX\_Mj, while exhibiting akin Km for Ca<sup>2+</sup>.

**Results** - Fluorescent labeling of apo-NCLX\_Mj reveals a comparable accessibility of extracellular and cytosolic vestibules to bulk phase, whereas apo-NCX\_Mj preferentially adopts extracellular orientation. HDX-MS (hydrogen-deuterium exchange mass-spectrometry) identifies a rigid core of TM2B and TM7B segments, which predefines “symmetric” preorganization of ion-transport passageway in apo-NCLX\_Mj, but not in NCX\_Mj. Characteristic patterns of HDX in NCLX\_Mj reveal specific changes in ion selectivity at distinct sites, where either Na<sup>+</sup>, Li<sup>+</sup> or Ca<sup>2+</sup> bind to SCa and Sext lacks the Na<sup>+</sup> binding capacity. The initial rates of Na<sup>+</sup>/Ca<sup>2+</sup> or Li<sup>+</sup>/Ca<sup>2+</sup> exchange are insensitive to membrane potential clamping in NCLX\_Mj reconstituted liposomes, which is incompatible with electrogenic stoichiometry of 3Na<sup>+</sup>:1Ca<sup>2+</sup>.

**Conclusions** - Compiling data reveal that local conformational changes at “nonselective” SCa predefine ion-induced alternating access in NCX\_Mj and NCLX\_Mj. Present findings have valued implications for understanding the underlying mechanisms of ion selectivity and transport in NCX and similar proteins.

## **IONIC Na<sup>+</sup> AND Ca<sup>2+</sup> DEPENDENT COMMUNICATION OF ASIC1a WITH MITOCHONDRIA CONTROLS NEURONAL METABOLIC ACTIVITY**

Ivana Savic<sup>1</sup>, Liu Fan<sup>2</sup>, Israel Sekler<sup>1</sup>

*1 - Department of Physiology and cell biology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.*

*2 - Discipline of Neuroscience and Department of Anatomy, Histology and Embryology, Institute of Medical Sciences, Shanghai Jiao Tong University School of Medicine, 280 South Chongqing Road, Shanghai 200025, China*

E-mail: savic@post.bgu.ac.il

A major hallmark of neuronal metabolic activity is the buildup of lactic acid resulting in mild drop in pH. The decrease in extracellular pH can be sensed by acid-sensing ion channels (ASIC). ASIC1a, which is main ASIC subunit in neurons, is predominantly Na<sup>+</sup> conducting channel and is expressed on both plasma membrane and inner mitochondrial membrane. Previous studies linked ASIC1a to pathophysiological insults in particular brain ischemia, which is followed by a very large pH drop. Here we ask how physiological pH decrease can affect mitochondrial Na<sup>+</sup> and Ca<sup>2+</sup> signals as well as mitochondrial metabolic activity and if ASIC1a is related to these processes. We hypothesize that ASIC1a is a metabolic sensor that by communicating with the mitochondria can control metabolic activity of neurons. We show that lowering of extracellular pH to 7.0 (physiological range) triggers increase in both cytosolic and mitochondrial Na<sup>+</sup> influx in WT cortical neurons. We further demonstrate that the mitochondrial Na<sup>+</sup> accumulation is accompanied by mitochondrial depolarization and cytosolic and mitochondrial Ca<sup>2+</sup> influx. Psalmotoxin 1 (PcTx 1), a selective inhibitor of ASIC1a, attenuates mitochondrial membrane potential as well as both cytosolic and mitochondrial Na<sup>+</sup> and Ca<sup>2+</sup> accumulation in mitochondria. Using same paradigms for WT vs ASIC1a KO cortical neurons, we confirm the role of ASIC1a in this scenario. More importantly, ASIC1a dysfunction strongly reduces basal respiration, maximal respiration and ATP production both in cortical neurons treated with PcTx1 and ASIC1 KO at ~ pH 7.0, suggesting that ASIC1a channel via propagation of Na<sup>+</sup> and Ca<sup>2+</sup> signals, plays significant role in the modulation of mitochondrial metabolic activity.

## **POSTTRANSLATIONAL MODIFICATION OF NCLX VIA SUMOylation**

Kuntal Dey, Israel Sekler

*Department of physiology and cell biology, Faculty of health sciences*

*Ben-Gurion University of the Negev, Beer Sheba, Israel*

*kd.bcku@gmail.com*

Mitochondrial Na<sup>+</sup>/Ca<sup>2+</sup> exchanger (NCLX), an inner membrane protein that by extruding Ca<sup>2+</sup> from the mitochondria in Li<sup>2+</sup> and Na<sup>+</sup> dependent manner controls mitochondrial as well as cytosolic Ca<sup>2+</sup> signals. Impaired NCLX activity leads to mitochondrial Ca<sup>2+</sup> overload and that is linked with multiple disorders. However, mechanisms controlling mitochondrial expression as well as NCLX activity are still elusive.

SUMOylation is a known posttranslational modification for the conjugation of the Small Ubiquitin-like MODifier (SUMO) at the lysine residues of the target protein.

The present study was undertaken to investigate (i) whether NCLX is a target for SUMOylation; (ii) if so, considering the known role of SUMOylation on protein stability and/alteration in protein function, we want to gain a better understanding how SUMOylation regulates NCLX activity and/stability.

"SUMOplot" analysis of human NCLX showed several potential SUMOylation sites (ΨKXE, Ψ is any hydrophobic amino acid). In western blot as well as in co-immunoprecipitation study, we detected the association of SUMO-1 with NCLX from the cells either treated with N-ethylmaleimide (NEM, 25mM) or transiently transfected with SUMO1. Among the three potential SUMOylation sites in NCLX (Lys 519, 331 and 328), only mutation at Lys519 effectively prevented NCLX SUMOylation. Importantly, decimating this SUMOylation site reduced the expression of NCLX in mitochondria and decreased mitochondrial Ca<sup>2+</sup> efflux. Moreover, we found that SUMOylation protects NCLX from ubiquitin mediated degradation. Interestingly, in the beta-cells, under glucolipotoxic condition, we found a diminished SUMOylation of NCLX along with the lowered protein expression into the mitochondria. Additionally, we found that impaired localization of NCLX leads to mitochondrial damage as evidenced by an increase in cytochrome C release. Corroborating with this data, we observed that NCLX downregulation enhances cell death through apoptosis.

In summary, our data suggest that NCLX is SUMOylated through the action of SUMO1 and reveal the existence of a crosstalk between SUMOylation and ubiquitination of NCLX. We are also able to show that impairment of SUMOylation downregulates the expression of NCLX into mitochondria.

## **PHARMA WEBSITES AND "PROFESSIONALS-ONLY" INFORMATION: THE IMPLICATIONS FOR PATIENT TRUST AND AUTONOMY.**

Hershkop Eliyakim<sup>2</sup>; Graber MA<sup>1</sup>; Graber RI<sup>3</sup>

*1Departments of Emergency and Family Medicine, University of Iowa Carver College of Medicine, Iowa City, IA, United States*

*2Medical School, Technion Israel Institute of Technology, Haifa, Israel*

*3Unaffiliated, Washington DC, DC, United States*

eliyakimhershkop@gmail.com

**BACKGROUND:** Access to information is critical to a patient's valid exercise of autonomy. One increasingly important source of medical information is the Internet. Individuals often turn to drug company ("pharma") websites to look for drug information.

**OBJECTIVE:** The objective of this study was to determine whether there is information on pharma websites that is embargoed: Is there information that is hidden from the patient unless she attests to being a health care provider? We discuss the implications of our findings for health care ethics.

**METHODS:** We reviewed a convenience sample of 40 pharma websites for "professionals-only" areas and determined whether access to those areas was restricted, requiring attestation that the user is a health care professional in the United States.

**RESULTS:** Of the 40 websites reviewed, 38 had information that was labeled for health care professionals-only. Of these, 24 required the user to certify their status as a health care provider before they were able to access this "hidden" information.

**CONCLUSIONS:** Many pharma websites include information in a "professionals-only" section. Of these, the majority require attestation that the user is a health care professional before they can access the information. This leaves patients with two bad choices: (1) not accessing the information or (2) lying about being a health care professional. Both of these outcomes are unacceptable. In the first instance, the patient's access to information is limited, potentially impairing their health and their ability to make reasonable and well-informed decisions. In the second instance, they may be induced to lie in a medical setting. "Teaching" patients to lie may have adverse consequences for the provider-patient relationship.

## Index of Posters

Anbtawi.....	11	Kotler .....	24
Arazi.....	9	Levi.....	19
Attali .....	15	Lezmy.....	23
Barkai.....	27	Marom David .....	1
Ben.....	25	Melamed.....	30
Ben Abu .....	29	Meshulam .....	37
Brandwine.....	7	Mreisat.....	39
Buzaglo .....	34	Olszakier .....	14
Chakraborty .....	31	Peretz.....	16
Cohen.....	32	Pittala.....	38
Danino.....	6	Rachminov .....	22
Dey.....	44	Refaeli .....	42
Eisen.....	35	Reis.....	5
Ferofontov .....	2	Rhodes-Mordov .....	18
Giladi.....	40	Savic.....	43
Goldstein.....	28	Shalom .....	21
Gutorov.....	8	Shvartsman .....	12
Handklo.....	36	Stoler.....	20
Hershkop.....	45	Tabak.....	3
Hodes.....	33	Tsemakhovich .....	17
Kamaysi.....	10	Uzan-Gueta .....	26
Katoshevski .....	41	Wessal.....	13
Kostic .....	41	Zaguri .....	4