SGP 73rd ANNUAL SYMPOSIUM / SOBLA ANNUAL MEETING

"Structural Basis of Electrical Signaling in the Nervous System and Heart"

September 4 to 7, 2019
Valparaíso, Chile

CENTRO INTERDISCIPLINARIO DE Neurociencia de Valparaíso

sobla2019.com

Society of General Physiologists (SGP) 73rd Annual Symposium Sociedad de Biofísicos Latinoamericanos (SOBLA) Annual Meeting

*Why a joint meeting between **SGP** and **SOBLA** in Valparaíso, Chile?*

The idea of a joint meeting emerges from the historical and long-standing relationship between many SGP and SOBLA members. It is well known that groundbreaking work in the field of membrane excitability was achieved in the 1950's using the squid giant axon preparation. This preparation was critical for identifying the voltage-dependent conductances that give rise to nerve impulses, notably discovered by Noble Prize recipients Alan Hodgkin and Andrew Huxley. In the 1960's, scientists working with the squid axon were concentrated in three international research stations, known as the "squid triangle" (Plymouth, UK; Woods Hole, USA; and Montemar, Chile). In Chile, the Humboldt squid can reach almost 2 m in length and have very large axons, typically reaching 1 mm in diameter and 20 cm in length. Not surprisingly, electrophysiologists at the time flocked to Montemar during the Chilean summer months, including notable scientists such as Robert Taylor and Clay Armstrong. Major discoveries regarding ion channel gating and permeation, and more recently Na+/K+ transport, have been achieved in the Montemar Laboratory (2 miles away from Valparaíso). Not less important, these international scientific interactions opened the doors for many Latin American scientists to successfully continue their careers (and lives) in the USA. These scientists paved the way for future generations of Latino biophysicists, who continue to train in laboratories across the USA and Latin America.

For the first time in more than 70 years, the SGP has agreed to host its annual meeting outside The Marine Biological laboratory in Woods Hole, MA, in recognition of the long-standing relationship with Hispanic biophysical community represented by SOBLA. This joint meeting is an ideal environment to present the recent and exciting discoveries in membrane excitability from members of both societies. It is a unique opportunity for scientists and trainees from both US and Latin America to exchange professional and cultural experiences to create a better and trusted global scientific community.

Organizing Committee

Dr. Ramon Latorre
Centro Interdisciplinario de Neurociencia de Valparaiso, Universidad de Valparaiso, Chile

Dr. Brad Rothberg
Lewis Katz School of Medicine, Temple University, USA

Dr. Jorge Contreras
New Jersey Medical School, Rutgers University, USA

Dr. Miguel Holmgren
National Institute of Neurological Disorders and Stroke, NIH, USA

Program

WEDNESDAY 4

14:00 – 17:00 Registration

17:00 – 17:30 Welcome
by **Ramon Latorre** (CINV Director), **Merritt Maduke** (SGP President) **and Jorge Contreras** (SOBLA President).

17:30 – 18:30 Friends of Physiology Keynote
Lecture Francisco Bezanilla (Univ. Chicago. USA)

19:00 – 21:00 Reception and Cocktail at Parque Cultural de Valparaíso

THURSDAY 5

9:00 – 10:15 Session I
"Structural perspectives on permeation and selectivity"
Chair: **Crina Nimigean,** (Weill Cornell Medicine. USA)

9:00 AM Speaker 1
Luis Cuello (Texas Tech Univ. USA)
"Dissecting the ion bound configurations within the selectivity filter of a potassium channel"

9:30 Speaker 2
Benoit Roux (Univ. Chicago. USA)
"Unraveling the mechanism of C-type inactivation in potassium channels"

10:00 Short talk 1
Maria Falzone (Weill Cornell Medicine. USA)
"Structural basis of lipid and ion transport by TMEM16 scramblases"

10:15 – 10:45
COFFEE BREAK

10:45 – 12:30 Session II
"Structural and functional approaches to gating"
Chair: **Alessio Accardi,**(Weill Cornell Medicine. USA)

10:45 Speaker 3
Carlos Gonzalez (CINV - Univ. de Valparaíso. Chile**)**
"Molecular gating mechanism of activation in (Hv1) Proton Channels"

11:15 Speaker 4
Baron Chanda (Univ. Wisconsin. USA)
"Hidden in plain sight: Temperature-dependent gating in model systems"

11:45 Speaker 5
Cecilia Bouzat (CONICET. Argentina)
"Activation and multilevel modulation of alpha7 nicotinic receptors"

12:15 Short Talk 2
Jerome LaCroix (Western Univ. Health Sci. USA) **"Shedding Light on Piezo1 Activation"**

12:30 – 14:00
LUNCH AT PARQUE CULTURAL DE VALPARAISO

14:00 – 16:00 Session III
"Calcium and electrical excitability"
Chair: **Alan Neely** (CINV – Univ. de Valparaíso. Chile)

14:00 Speaker 6
Claudia Moreno (Univ. Washington. USA)
"Coupling of L-Type Calcium Channels to facilitate calcium entry"

14:15 Speaker 7
Cathy Proenza (Univ. Colorado. USA)
"Allosteric regulation of HCN channels"

14:30 Speaker 8
Robert Dirksen (Univ. Rochester. USA)
"Exercise-dependent Activation of Store-operated Ca2+ Channels in Skeletal Muscle: Why do Mice Run Better with SOCCs?"

15:00 Speaker 9
Ana Gomez (INSERM-Univ Paris Sud. France)
"Ryanodine receptor role in heart rhythm and arrhythmias"

15:30 Speaker 10
Ariel Escobar (UC Merced. USA)
"Relationship between excitability and contractility: when Ca2+ influx happens"

16:00 – 18:00 Poster presentations
Beer and wine will be served at the poster session.

18:00 – 19:15 ROUND TABLE I
Establishing a diverse population of scientists in the study of membrane excitability
Panel:**Theanne Griffith** (Columbia Univ. USA); **Walter Duran** (Rutgers-NJMS. USA); **Julio Cordero-Morales** (Univ. Tennessee. USA); **Delany Torres Salazar** (NINDS. USA); **Susan Amara,** (NIMH. USA).

19:35
Please find your preferred dinner at Valparaiso´s restaurants. Information about restaurants will be available at the meeting

FRIDAY 6

9:00 – 10:15 Session IV
"Control of electrical excitability in neurons"
Chair: **Andres Chavez** (CINV – Univ. de Valparaíso. Chile)

9:00 Speaker 11
Chris Lingle (Washington Univ.-St. Louis. USA)
"Long-term inactivation of Nav currents in rodent chromaffin cells and its molecular underpinnings"

9:30 Speaker 12
Frank Bosmans (Ghent Univ. Belgium)
"The expanding role of Nav1.9 in sensory perception"

10:00 Short talk 3
Theanne Griffith (Columbia Univ. USA)
"Tetrodotoxin-sensitive sodium channels mediate action potential firing and excitability in menthol-sensitive Vglut3-lineage sensory neurons"

10:15 – 10:45
COFFEE BREAK

10:45 – 12:30 Session V
"TRP channels: from molecular mechanisms to physiology"
Chair: **Leon Islas** (UNAM. Mexico)

10:45 Speaker 13
Tamara Rosenbaum (UNAM. Mexico)
"The modes of action of an endogenous TRPV1 activator"

11:15 Speaker 14
Tibor Rohacs (Rutgers Univ. USA)
"Lipid regulation of Transient Receptor Potential Channels"

11:45 Speaker 15
Andres Jara-Oseguera (NINDS. USA)
"The role of the selectivity filter in gating of the TRPV1 channel"

12:15 Short talk 4
Lisandra Flores (Univ. Austral. Chile)
"Evolutionary analysis unveils a sequence motif associated with fast inactivation in TRPV5 and TRPV6 channels"

12:30 – 14:00
LUNCH AT PARQUE CULTURAL DE VALPARAISO

14:00 – 15:30 Session VI
"Expanding the ion channel toolkit"
Chair: **Matthew Trudeau** (U. Maryland. USA)

14:00 Speaker 16
Jon Sack (UC Davis. USA)
"Visualizing endogenous ion channel activation"

14:30 Speaker 17
Bill Kobertz (Univ. Mass. USA)
"Painting Cells with Fluorescent Ion Sensors: Spatiotemporal Visualization of Membrane Transporter and K+ Channel Activity"

15:00 Speaker 18
Teresa Giraldez (Univ. La Laguna. Spain)
"Bringing light to BK channel calcium sensing"

15:30 Short talk 5
Michael Rockman (Temple Univ. USA)
"Mechanism of NS11021 Activation of BK Channels"

15:45 – 17:45 Poster presentations
Beer and wine will be served at the poster session.

17:45
Please find your preferred dinner at Valparaiso´s restaurants. Information about restaurants will be available at the meeting.

SATURDAY 7

9:00 – 10:00 Session VII
"Junctional channels and cellular communication: P2X and connexin channels" Chairs: **Agustin Martinez/ Juan Carlos Saez** (CINV – Univ. de Valparaíso. Chile)

9:00 Speaker 19
Mufeng Li (NINDS. USA)
"Tuning P2X receptor channel function with divalent cations"

9:30 Short talk 6
Isaac Garcia (CINV - Univ. de Valparaíso. Chile) **"Human connexin mutations reveal a key role for the N-terminus in the gating mechanism of connexin hemichannels"**

9:45 Short talk 7
Mauricio Lillo (Rutgers-NJMS. USA)
"Opening of remodeled Connexin43 hemichannels promote arrhythmias upon cardiac stress"

10:00 – 10:30
COFFEE BREAK

10:30 – 12:30 Session VIII
"Channels under the influence"
Chair: **Valeria Vasquez** (Univ. Tennessee. USA)

10:30 Speaker 21
Andrea Brueggemann (Nanion. Germany)
"Opening up New Opportunities by Combining High Throughput Electrophysiology with Optical Tools"

11:00 Speaker 22
Susy C. Kohout (Montana State Univ.USA)
"Connecting electrical and chemical signaling through VSP"

11:30 Speaker 23
Gail A. Robertson (Univ. Wisconsin-Madison. USA)
"Cotranslational association of mRNAs encoding components of the action potential"

12:00 Speaker 24
Sudha Chakrapani (Case Western. USA)
"Gating and Drug Modulation in 5-HT3A Receptors"

12:30 – 14:00
LUNCH AT PARQUE CULTURAL DE VALPARAISO

14:00 – 15:30 Session IX
"Primary and Secondary Active Transporters"
Chair: Pablo Artigas (Texas Tech Univ. USA)

14:00 Speaker 25
Kazuhiro Abe (Nagoya Univ. Japan)
"Structural basis for the H+-extrusion and the K+-occlusion of the gastric proton pump"

14:30 Speaker 26
Nancy Carrasco (Vanderbilt Univ. USA)
"The Na+/I- symporter (NIS): an unending source of surprises"

15:00 Speaker 27
Joseph Mindell (NINDS. USA)
"Protons to patients:evaluating the role of the chloride transporter CLC-7 in lysosomal acidification"

15:30 – 16:00
COFFEE BREAK

16:00 – 17:00 ROUND TABLE II
Establishing rigorous benchmarks and reproducibility in science.
Chair: **Shai Silberberg** (NINDS)

17:00 – 18:00 SOBLA Lecture
Dr. Mario Amzel (Johns Hopkins School of Medicine. USA)**.**
Title: **"Regulation of voltage-gated sodium channels by calcium and calmodulin"**

19:30
Final Banquet at "Yacht Club de Chile".

About Valparaíso

The Society of General Physiologists (SGP) 73rd Annual Symposium / Sociedad de Biofísicos Latinoamericanos / (SOBLA) Annual Meeting will be held September 4th – 7th at the South American Port of Valparaíso, Chile. Valparaíso and its neighboring cities are home to about one million people. However, Valparaíso itself is a small city of about 300,000 inhabitants, making it easy for people to walk and use public transportation. The World Heritage List by UNESCO in 2004 included the historic quarter of Valparaíso. The hills, Cerro Alegre and Cerro Concepción, are at the heart of its historic quarter and are an important tourist attraction due to its many bars, pubs, restaurants, and hotels. The fifteen urban elevators (funiculares; a heritage from the industrial revolution) and the trolleybuses (the oldest in the world still in operation) are two unique characteristics of the city.

Valparaíso is just one and half hours from Santiago's International Airport and the city of Santiago, making national and international travel easy. From this airport, connections are possible to the major tourist attractions in the country, including Patagonia (Torres del Paine), the Atacama Desert (San Pedro de Atacama), and Rapa Nui (Easter Island).

The Symposium will take place at the Valparaíso Cultural Park (PCdV). The PCdV is a cultural center and public space of 1.5 ha (3.7 ac), which was recently created on the site of Valparaiso's former jail. It includes an auditorium with a capacity of around 300 seats, where all talks will be held. Posters will be displayed throughout the entire duration of the symposium in a designated location. The PCdV is a ten-minute bus ride and a 10-30 minute walk from most Valparaiso hotels.

Parque Cultural de Valparaíso (PCDV)

The Symposium will take place at the Parque Cultural de Valparaíso (PCdV). It is located in Cerro Cárcel, which was the prison of Valparaíso until 1994. Today it serves the community as a Cultural Park.

Address: Calle Cárcel 471, Cerro Cárcel, Valparaíso. www.parquecultural.cl

Yacht Club de Chile

The Yacht Club of Chile was founded in 1941, and became the first Institution of these characteristics in the Country. It is a club for sailing and sailing lovers. It focuses on the practice, promotion and promotion of water sports. It has hosted numerous local and international races and championships.

Address: Av Escuadra libertadora 1800, Viña del Mar, Chile http://www.yachtclubdechile.cl/

Useful Information for your visit to Valparaíso

- Please exchange money in official currency exchange offices (e.g., in Prat Street, one block from Plaza Sotomayor, across from Banco de Chile).
- Carry only small bills in your pockets.
- Businesses usually do not accept large bills.
- The voltage in Chilean outlets is 220V/50Hz. Electric Plugs/Outlets are type C or L.
- For your convenience, use comfortable shoes and clothes, since the majority of attractions are located on hills.
- The mean temperature in Valparaiso is mild at 14.2°C (57.56°F). in September the temperature ranges between 17° (63 °F) and 9° (48 °F), windy with occasional rain.
- Please watch your belongings (cell phone, cameras, wallet) while you are walking around Valparaíso.
- Keep your documents (IDs) such as passport, airfare, etc., in a safe place; do not carry them around with you.
- Public transportation is paid with cash (only Chilean pesos). However in Santiago the public transportation is paid with a card that you can buy at Metro Stations.
- ATM's with “Redbanc” label accept foreign credit cards.

Society of General Physiologists Council Members

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Sociedad de Biofísicos Latino Americanos

The Sobla, “Society of Latin American Biophysicists”, was founded in the mid-eighties by a group of colleagues who sought to open a forum to discuss and promote biophysics in Latin America. The Sobla is a society of individuals that for reasons of origin, network, and/or affections have decided to help strengthen biophysics in Latin America.

Sobla's mission is to catalyze interactions among Latin American Biophysicists worldwide, providing young scientists with the opportunity to reach biophysicists in Latin America and abroad to establish collaborations and/or internships, in order to promote the development of Biophysics.

President
- Jorge E. Contreras

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- Miguel Holmgren
- Valeria Vásquez

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- Mario Amzel

CENTRO INTERDISCIPLINARIO DE
Neurociencia de
Valparaíso

Centro Interdisciplinario de Neurociencia de Valparaíso

The CINV (www.cinv.cl) is a research institute, which is part of Universidad de Valparaiso in Chile (www.uv.cl). Its Director is Dr. Ramón Latorre, a biophysicist and a recipient of the Chilean National Science Award ("Premio Nacional de Ciencias"). The CINV specializes in research on the nervous system, and includes biophysicists, physiologists, neurobiologist and experts in bioinformatics and molecular modeling. Its scope of study spans from the inner workings of proteins transducing signals from the outside world to animal behaviors represented in neural networks.

The CINV includes core faculty members from graduate programs in neuroscience and biophysics of "Universidad de Valparaíso". In 2011 the CINV became the first research center within a public university in Chile to become a Millennium Institute, and from the field of science, it seeks to become an active contributor to reactivating the city of Valparaíso in terms of research and development.

CINV researchers:

Ramón Latorre (Director)
(Biophysics of Ion Channels)

Juan Carlos Sáez (Deputy Director)
(Connexins and Pannexins)

Ana María Cárdenas
(Vesicle Trafficking and Exocytosis)

Adrián Palacios
(Sensory and Neurodegenerative Process)

Agustín Martínez
(Connexins and Pannexins)

Alan Neely
(Biophysics of Ion Channels)

Andrea Calixto
(C. elegans Neurodevelopment)

Andrés Chávez
(Synaptic Transmission and Plasticity)

Carlos González
(Biophysics of Ion Channels)

Chiayu Chiu (CINV-Max Planck Research Leader)
(Synapses, Neurons and Circuits)

Fernando D. González-Nilo
(Molecular Modeling)

Francisco Bezanilla
(Biophysics of Ion Channels)

John Ewer
(*Drosophila* Behavior and Development)

Kathleen Whitlock
(Zebrafish Genetics and Development)

Oliver Schmachtenberg
(Sensory Physiology)

Pablo Moya
(Neuropsychiatric Diseases)

Patricio Orio
(Mathematical Modeling of Neuron Behavior)

Tomás Pérez-Acle
(Molecular Modeling)

Young Researchers

Alvaro Ardiles
(Synaptic Function and Structure)

Arlek González
(Cytoskeleton and Membrane Trafficking)

Helmuth Sánchez
(Connexins and Deafness Models)

Isaac García
(Molecular Physiology and Biophysics)

José Antonio Gárate
(Molecular Modeling)

Karen Castillo
(Biophysics and Physiology of Ion Channels)

Abstracts

1. Crystal structures of the gastric proton pump—structural basis for proton extrusion and the transport stoichiometry **KAZUHIRO ABE,[1,2]** *[1]Cellular and Structural Physiology Institute, Nagoya University, Nagoya, 464–8601, Japan; [2]Graduate School of Pharmaceutical Sciences, Nagoya University, Nagoya, 464–8601, Japan*

The gastric proton pump, H^+,K^+-ATPase is a P-type ATPase that is responsible for acidifying the gastric juice up to pH 1, and is thus an important drug target for treating gastric acid-related diseases. It mediates electro-neutral exchange of H^+ and K^+ coupled with ATP hydrolysis, but with an as yet undetermined transport stoichiometry. I will present the crystal structures of the H^+,K^+-ATPase in complex with two acid blockers, vonoprazan and SCH28080, in the luminal-open E2P state. These drugs have partially overlapped, but clearly distinguishable, binding modes, which are defined in the middle of a conduit running from the gastric lumen to the cation-binding site. The crystal structures also revealed a conserved lysine residue that points to the juxtaposed carboxyl residues in the cation-binding site. The unusual configuration of the cation-binding site enables the extrusion of a single proton even into the pH1 solution of the stomach.

We also show crystal structures of E2-P transition state, in which the counter-transporting K^+ is occluded. We found a single K^+ bound to the cation-binding site of H^+,K^+-ATPase, indicating an exchange of $1H^+/1K^+$ per hydrolysis of one ATP molecule. This fulfils the energy requirement for the generation of a six pH unit gradient across the membrane. The structural basis of K^+ recognition is resolved, supported by molecular dynamics simulations, and this establishes how the gastric pump overcomes the energetic challenge to generate an H^+ gradient of more than a million-fold - the highest cation gradient known in any mammalian tissue–across the membrane.

2. Clathrin lattices are a dynamic signaling platform for the EGF receptor in human cells **MARCO A. ALFONZO-MENDEZ, KEM A. SOCHACKI, JUSTIN W. TARASKA** *National Heart, Lung, and Blood Institute. Bethesda, Maryland 20892, United States of America.*

Clathrin-mediated endocytosis (CME) is key to internalize solutes, lipids, and integral proteins from the plasma membrane of eukaryotic cells. It is well known that classical CME occurs through the assembly of clathrin-coated pits which invaginate to form clathrin-coated vesicles. Additionally, clathrin can assemble as flat honey-comb like structures called flat clathrin lattices (FCLs) or plaques. FCL biogenesis and its potential roles in cell signaling, however, remains elusive. Here we used quantitative fluorescence and electron microscopic imaging to show that clathrin remodels in response to external signals such as the Epidermal Growth Factor (EGF). Specifically, nanoscale analysis of the plasma membrane show a dramatic increase in plaque-covered areas and plaque size with EGF stimulation. These effects required Epidermal Growth Factor Receptor (EGFR) interactions with EGF and Src kinase and b5-integrin activity. Remarkably, agonist stimulation leads to persistent recruitment of EGFR and b5-integrin into clathrin structures and a corresponding loss of Src, Ras, and Akt. Our results suggest that clathrin plaques act as stable platforms capable of clustering and organizing specific signaling complexes at the plasma membrane.

3. Functional Coupling between NOX2 and Hv1 channels in myeloid-derived suppressor cells (MDSC) **JUAN JOSÉ ALVEAR,[1,3] ANTONIO PEÑA,[1,3] CHRISTIAN CARRILLO,[1,3] JAVIERA VILLAR,[1] RICHARD BETANCOURT,[1] EMERSON M. CARMONA,[1,3] OSVALDO ALVAREZ,[1,2] ALAN NEELY,[1] RAMON LATORRE,[1] CARLOS GONZALEZ[1]** *[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, Valparaíso, Chile; [2]Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile; [3]Doctorado en Ciencias mención Biofísica y Biología Computacional, Facultad de Ciencias, Universidad de Valparaíso, Chile.*

Myeloid-derived suppressor cells (MDSC) have an increased enzymatic activity of the NADPH oxidase protein complex (NOX2). In this work we show that functional expression of Hv1 channels are responsible of these findings. Primary culture of bone-marrow derived cells from C57BL/6 mice were induced to MDSC differentiation by culturing in the presence of GM-CSF for 4 d and then characterized by Gr-1 and CD-11b. We detected the presence of Hv1 in a population corresponding to 80–90% of MDSC by flow cytometry, immuno-fluorescence microscopy and by Western blot. We explore the presence of Hv1 at the transcriptional level by RT-PCR and found, alongside with the conventional transcript, two novel isoforms of the channel. To explore the functionality of Hv1 channel we performed electrophysiological experiments by whole-cell patch-clamp technique. Proton currents were elicited by voltage protocols and pH-gradients. It shows a slow kinetics activation and high proton selectivity according to a Hv1 channel activity. Additionally, MDSC proton currents were inhibited by 10 µM Zn+2 and 100 µM 5-Cl-2GBI respectively. The two found isoforms were studied by

inside-out macropatches in *Xenopus* oocytes heterologous system and their differences support the variability of native Hv1 wild-type. Finally, we showed by perforated patch clamp the activity of Hv1 by PMA and the modulation of the NOX2 activity alongside with the measurement of ROS in the presence of Hv1 inhibitors by flow cytometry using H2DCFDA. These results strongly suggest the functional expression of Hv1 channels are the main responsible of ROS production by a tight coupling with the NOX2 complex in MDSC. Supported by CONICYT-PFCHA/Doctorado Nacional/2017-21170395 to E.C., CONICYT-PFCHA/Doctorado Nacional/2019-21191239 to A.P., Fondecyt 1180464 to C.G. and 1150273 to R.L. The Centro Interdisciplinario de Neurociencia de Valparaiso is a Millennium Institute supported by the Millennium Scientific Initiative of the Chilean Ministry of Economy, Development, and Tourism (P029-022-F).

4. Hippocampal synaptic impairments in a mouse model of dynamin-2-linked Centronuclear Myopathy **J.ARRIAGADA,[1,4] L. PRADO,[1,4] I. GAJARDO,[2] M. BITOUN,[3] A. ARDILES,[1,2] A. GONZÁLEZ-JAMETT,[1]** *[1]Centro Interdisciplinario de Neurociencias de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile; [2]Escuela de Medicina, Facultad de Medicina, Universidad de Valparaíso, Valparaíso, Chile; [3]Research Center for Myology, Institute of Myology, Paris, France; [4]Magister en Ciencias Biológicas, mención Neurociencia, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile.*

Dynamins are large GTP-ases required for membrane remodeling in several cellular processes including endocytosis, exocytosis and vesicle trafficking. Three dynamin isoforms are expressed in the mammalian nervous system where they participate in synaptic vesicle recycling, neurite-morphogenesis, and signaling receptor turnover. Mutations in dynamin-2 cause centronuclear myopathy (CNM), a congenital neuromuscular disorder characterized by progressive weakness and atrophy of distal skeletal muscle. Although CNM-linked dynamin-2 mutations cause a muscle-specific disease, cognitive defects in CNM-patients have been reported, suggesting concomitant central nervous system involvement. To evaluate this possibility we used heterozygous knock-in (KI) mice harboring the R465W mutation in dynamin-2, a mammalian model of CNM. Spatial memory was assessed in KI and wild type (WT) adult mice (6 mo old) using a Barnes-maze test. Using visual cues mice were trained to find the entrance to a dark hidden escape chamber. Although both WT and KI mice learned the task, KI animals exhibited a significantly higher latency to enter to the escape chamber suggesting impairments in spatial memory flexibility. This effect was not due to defects in locomotor activity, as no differences between KI and WT mice were observed in an open-field test. Since memory formation and consolidation rely on the synaptic mechanisms that support plasticity of the synaptic strength, we also evaluated excitatory synaptic plasticity (SP) in KI and WT brain slices. Specifically we studied long-term-potentiation (LTP) and long-term-depression (LTD) at the Shaffer Collateral-to-CA1 hippocampal synapses, the most well-known forms of SP. We found that both, LTP and LTD are impaired in KI compared with WT hippocampal slices, suggesting that synaptic dysfunctions could underlie the pathological mechanisms of CNM.

This work has been supported by Fondecyt 3160311, Fondecyt 11180731 and ICM-MINECON P09-022-F.

5. Therapeutic Implications of Cav1.2 Mutations **MORADEKE BAMGBOYE, MARIA TRAFICANTE, JOSIAH OWOYEMI, IVY DICK** *University of Maryland, Baltimore, Maryland*

The Cav1.2 channel is responsible for the regulated entry of calcium into most excitable cells and is extensively expressed in many cell types, including cardiac and neuronal cells. An increasing number of mutations identified in this channel have been implicated in severe disruptions of electrical signaling in the heart and brain. Many of these mutations cluster near the S6 region of the channel, a locus widely recognized as important for channel activation. Thus, these mutations have often been shown not only to cause changes in channel activation, but to result in multifactorial changes in channel regulation, including calcium dependent (CDI) and voltage dependent inactivation (VDI); two important channel regulatory mechanisms.

Here we show that these S6 mutations are capable of distinctly disrupting either form of channel inactivation (CDI or VDI) without altering channel activation. This represents an important new insight into the complex and diverse roles of the S6 region in channel function. Furthermore, we show that such disruption of channel inactivation has important implications for patient treatment. Specifically, mutations in CaV1.2 which diminish channel inactivation reduce the state dependent block of the channel by clinically relevant calcium channel blockers (CCBs), such that the amount of channel block is strongly correlated to the fraction of channel inactivation. In fact, inactivation deficits alone are sufficient to attenuate the efficacy of CCBs on the channel, presenting a major impediment to the treatment of patients harboring these mutations. These results emphasize the importance of understanding how mutations disrupt normal S6 function, as well as the need to tailor patient therapies to the specific channel deficit produced by each mutation.

6. Selective blockade of connexin-based channels by 8-hydroxyquinoline via a kinase-dependent mechanism
IVÁN BARRÍA,[1] PALOMA A. HARCHA,[1] PABLO J. SÁEZ,[3] ANIBAL A. VARGAS,[4] PAOLA FERNÁNDEZ,[1] VIVIANA M. BERTHOUD,[5] JUAN C. SÁEZ[1,2] *[1]Instituto de Neurociencias, Centro Interdisciplinario de Neurociencias de Valparaíso, Universidad de Valparaíso, Chile; [2]Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile; [3]Institut Curie, PSL Research University, CNRS, UMR144, France; [4]Universidad de O'Higgins, Rancagua, Chile; [5]Department of Pediatrics, University of Chicago, Chicago, USA.*

Connexins (Cxs) are a family of membrane proteins that form gap junction channels (GJCs) and hemichannels (HCs). Functional GJCs participate in diverse physiological process. Whereas, low HC activity participates in physiological process, high HC activity is associated to pathological conditions. The identification of drugs with selective inhibitory effect on Cx-based channels could allow furthering our knowledge on functional role of these channels. It has been shown that quinine derivates selectively block different Cx GJC but their effect on HCs remains unknown. Here, we evaluated the effect of 8-hydroxyquinoline (HxQ) on HCs and GJCs formed by Cxs 26, 32, 37 or 43. Dye coupling (Lucifer yellow) and dye uptake (ethidium bromide) methods were used to evaluate the functional state of GJCs and HC, respectively, in Cx transfectant HeLa cells. The open probability of HCs was increase by Ca^{2+}/Mg^{2+}-free extracellular solution. Both GJCs and HCs were blocked by 100 µM HxQ in HeLa-Cx43 and -Cx37 cells, whereas no effect was found on Cx26 or –Cx32 channels. Because the main structural difference between these two Cx types is the length of their carboxyl-terminus, we tested the effect of HxQ on channels formed by Cx43 truncated in amino acid 251 (Cx43-251). In HeLa Cx43-251, both GJCs and HCs were not affected by 100 µM HxQ. Then, we evaluated whether the HxQ-induced inhibition of Cx43-based channels was mediated by protein kinases. To this end, we used inhibitors of ERK1/2 (U0126), Akt1/2(A6730), JNK (SP600125) or p38 (SB203580) kinase. In all cases, the effect of HxQ on Cx43 channels was prevented, suggesting that HxQ promotes indirect activation of intracellular signaling cascades that modulate Cx43 channel activity. Thus, the selectivity of HxQ dependent on amino acid residues located in the C-terminus of Cx43 and requires the activity of protein kinases.Acknowledgments: This work was funded by ICM-Economía P09-022-F (JCS).

7. Connexin and pannexin blockeage with carbenoxolone prevents the release of pathological extracelullar ATP (ATPe) levels in an asmathic mouse model
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Introduction: ATP is one of the chemical signals locally released by the airway epithelium associated with an autocrine/paracrine mechanism that controls basal ciliary beat frequency (CBF). A recent study has reported that ATPe has a dual effect on CBF, since ATP (µM) increases CBF, but high levels of ATP (mM) significantly decreases CBF in cultures of mouse tracheal epithelial cells. This nucleotide can be released through hemichannels of connexins (Cx43) and pannexins (Panx1), that could modify their opening state under pathological conditions. High levels of ATP have been measured in the bronchoalveolar fluid of patients with inflammatory diseases, such as asthma. One of the most widely used hemichannels blockers is carbenoxolone (CBX), known for its anti-inflammatory properties and currently used for the treatment of stomach ulcers in humans. With this background we proposed the following.

Objective: Determine the effect of pretreatment with CBX in an asthmatic mouse model on the pathological levels of ATPe in the trachea lavage.

Methods: We measured ATPe with a luminometric assay on asthmatic mice pretreated with Carbenoxolone (100 µM). The ethidium bromide (5 µM) capture for the trachea epithelium, were registered with a videomicroscopy system (Olympus). The protocols used in this study were approved by the ethics committee of the PUC.

Results: We established that high levels of ATPe present in asthmatic mice trachea lavage, were reduced to physiological levels after the pretreatment with CBX in vivo, with no significant difference with either the asthmatic control group with or without CBX treatment. This reduction in ATPe levels was probably associated to the blockage of connexin and pannexin hemichannels.

Conclusions: Pretreatment with an hemichannel blocker such as CBX could prevent the pathological release of ATPe in inflammatory diseases of the airways.

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8. Functional Local Crosstalk of SR-Calcium Release Events Caused by RyR2 and InsP3R2 Activity in Cardiomyocytes
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Augmented inositol 1,4,5-trisphosphate receptor (InsP3R2) expression and - function has been linked to a variety of cardiac pathologies including cardiac Ca^{2+}-dependent arrhythmogenicity. However, a detailed understanding of its potential role in myocyte excitation-contraction coupling under pathophysiological conditions remains elusive. This is especially true for functional local interaction between the two major Ca^{2+} release mechanisms: Ca^{2+}-release mediated by ryanodine receptors (RyR2s, Ca^{2+}-induced Ca^{2+}-release) and InsP3-induced SR-Ca^{2+} release (IP3ICR).

Here we test the hypothesis that at one hand the scenario might be plausible in which IP3ICR is part of an ECC protecting mechanism, resulting in a Ca^{2+}-dependent anti-arrhythmogenic response on the cellular scale in ventricular myocytes. On the other hand we confirmed that in atrial myocytes local IP3ICR events (Ca^{2+} puffs) in close proximity to RyR2s may directly activate RyRs and trigger/boost Ca^{2+}-induced Ca^{2+} release events (Ca^{2+} sparks) resulting in a proarrhythmogenic response

Our data reveals that stimulation of IP3ICR in ventricular myocytes isolated from a cardiac-specific InsP3R2 overexpressing mouse model leads to a decreased asynchronous SR-Ca^{2+}release, a decline in the SR-Ca^{2+} content and subsequent decrease in pro-arrhythmic Ca^{2+}-occurrence, suggesting a potential anti-arrhythmic role of IP3ICR.

Under cellular remodelling conditions the functional expression of InsP3R2 is up-regulated.

Although, no additional InsP3R2 mediated local SR-Ca^{2+} events (e.g., puffs) were identified, the SR-Ca^{2+} content appears to be under active control of IP3ICR. Interestingly, study of the SR-Ca^{2+} leak revealed a more prominent InsP3R2-dependent component that controls the SR-Ca^{2+} content.

These results support the view that IP3ICR contributes to the total SR-Ca^{2+} leak via "eventless" SR-Ca^{2+} release. The underlying mechanism actively stabilizes the SR-Ca^{2+} content and limits luminal RyR2s sensitization below the critical threshold for spontaneous SR-Ca^{2+} release thereby offering a protective mechanism against arrhythmias in ventricular myocytes.

9. Modulation of native and recombinant GIRK1,2 channels by analgesic α-conotoxins **ANUJA R. BONY, JEFFREY R. MCARTHUR, ROCIO K. FINOL-URDANETA, DAVID J. ADAMS.** ***Illawarra Health and Medical Research Institute (IHMRI), University of Wollongong, Wollongong, NSW 2522, Australia***

Activation of G protein-coupled inwardly-rectifying potassium (GIRK or Kir3) channels leads to membrane hyperpolarization conferring their critical role in inhibitory regulation of neuronal excitability. G protein-coupled receptors activate GIRK channels through the direct action of G protein βγ subunits (Dascal and Kahanovitch. 2015. Int Rev Neurobiol. 123:27-85). The analgesic α-conotoxins Vc1.1, RgIA and PeIA inhibit neuronal Cav2.2 and Cav2.3 channels via activation of G protein-coupled $GABA_B$ receptors ($GABA_BR$) providing a plausible mechanism for its analgesic actions (Sadeghi et al., 2017. Neuropharmacology 127:116-23). Neuronal GIRKs are predominantly hetero-tetramers of GIRK1 and GIRK2 subunits, consequently we investigated the properties of $GABA_BR$-active α-conotoxins on these channels comparing their actions to canonical $GABA_BR$ agonists, GABA and baclofen. In HEK293 cells cotransfected with human $GABA_BR$, GIRK1 and GIRK2 subunits, α-conotoxins Vc1.1, RgIA and PeIA potentiated GIRK1,2-mediated K^+ currents. K^+ current potentiation by Vc1.1 was reversible and concentration-dependent with a half-maximal effective concentration of ~70 nM. $GABA_BR$ dependent potentiation of GIRK1,2 channels by either baclofen or Vc1.1 was blocked by extracellular Ba^{2+} (1 mM), and prevented by incubation with *Pertussis* toxin (PTX) or the selective $GABA_BR$ antagonist CGP55845 (1 μM). The action of Vc1.1 was also investigated in dissociated dorsal root ganglion (DRG) neurons from adult mice. Under current clamp conditions, 1 μM Vc1.1 hyperpolarized the resting membrane potential by ≤10 mV in small DRG neurons (<30 μm) and increased their current threshold for action potential firing (rheobase). Similarly, baclofen (100 μM) reduced neuroexcitability in DRG neurons by increasing the rheobase. We surmise that potentiation of GIRK channels by activation of $GABA_BR$ causes cell hyperpolarization and concomitantly reduces excitability, consistent with Vc1.1 and baclofen analgesic effects in vivo (Klimis et al., 2012. Pain. 152(2):259-66). The analgesic α-conotoxins potentiate native and recombinant GIRK1,2 channels via $GABA_BR$ activation and is mediated by a PTX-sensitive G protein.

10. Chemical Activation and Lipid Modulation of Mechanosensitive Piezo1 Channel **WESLEY M. BOTELLO-SMITH,[1] WENJUAN JIANG,[1] HAN ZHANG,[1] JEROME J. LACROIX,[2] YUN LUO[1]** ***[1]College of Pharmacy, Western University of Health Sciences, Pomona CA 91766; [2]Graduate College of Biomedical Sciences, Western University of Health Sciences, Pomona, CA 91766***

Mechanosensitive Piezo1 and Piezo2 channels contribute to many important mechanotransduction processes such as osmotic homeostasis, epithelial growth and touch sensation. A small molecule Yoda1 activates Piezo1, but not Piezo2, by lowering its mechanical activation threshold. Using chimera engineering, we have recently identified a small protein region essential for Yoda1-mediated activation. However, the binding mechanism of Yoda1 remained elusive. Here, we used

a 8 µs all-atom molecular dynamic (MD) simulation of Piezo1 in conjunction with network analysis, principal component analysis, and free energy calculations, to investigate how Yoda1 interacts with and modulates Piezo1 in absence or presence of mechanical stimuli. Our work revealed the presence of an unanticipated Yoda1 binding site that was validated by experimental assays.

Piezo channels have a unique structure capable of locally bending the lipid bilayer and their activities are highly regulated by membrane tension and influenced by the physical properties of the surrounding lipid bilayer. We employed multiscale simulations to explore lipid-protein interactions within different symmetric and asymmetric lipid bilayers, including 1-palmitoyl-2-oleoy phosphatidylcholine (POPC), 1-palmitoyl-2-oleoyl phosphatidylserine (POPS), Phosphatidylinositol-(4,5)-bisphosphate (PIP2). Our study shows that most PIP2s in the lower leaflet are clustered around intracellular binding regions, while POPS lipids are evenly distributed throughout the lower leaflet. The difference between PIP2 and POPS binding can be attributed to the strong electrostatic interaction between the Piezo1 intracellular surface and the negatively charged PIP2. Lipid density maps in POPS bilayer membrane through 30 µs show several stable binding sites around Piezo 1, one of which located in the pore, which became vacant upon mechanical stimulation by membrane tension. Thus, our results suggest a polymodal lipid modulation of Piezo1, ranging from pore blocking, curvature modulation, to force-from-lipid enhancer

11. Elevated hemichannel activity in microglia and astrocytes in the serotonin transporter knockout mice **IVÁN D. BRAVO,[1] PABLO R. MOYA,[1,2] PAOLA FERNÁNDEZ,[1] JUAN C. SÁEZ[1]** *[1]Instituto de Neurociencias, Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, Valparaíso, Chile; [2]Instituto de Fisiología, Facultad de Ciencias, Universidad de Valparaíso, Chile*

Introduction: Depression is a disorder that affects millions of people worldwide and is responsible for substantial mortality, morbidity, and disability. Like other psychiatric diseases its etiology remains largely unexplained. Alterations in serotonergic transmission at cellular and molecular levels are associated with depressive disorder. Although the serotonin transporter (SERT) is a key target for anti-depressants its exact role in depression etiology remains unclear. A proposed explanation is that these changes may be associated with stress and neuroinflammation. Stress activates microglia, which release glutamate and/or ATP via hemichannels, whereas proinflammatory cytokines released by activated microglia enhance hemichannel activity of astrocytes. Furthermore, clinical and preclinical studies have implicated glial anomalies in major depression.

Aim: Here, we assessed the possible contribution of SERT and microglia and astrocytes cell activation in neuroinflammation associated with pathophysiology of neuropsychiatric disorders.

Methods: Adult C57BL/6 wild type (WT) and SERT-knockout (KO) mice were used and ethidium (Etd) bromide uptake experiments were performed to assess the glial hemichannel activity in hippocampal slices of mice. Images were taken with a confocal microscope and cells were identified in immunofluorescence assays by their Iba1 (microglia) and glial fibrillary acidic protein (GFAP, astrocytes). reactivity to specific antibodies.

Results: We found a significant induction of hemichannel activity in microglia and astrocytes of hippocampal slices from SERT-KO and heterozygous mice by Etd uptake as compared with WT mice. Moreover, they showed increased in hippocampal microglia and astrocytes immunoreactivity to Iba1 positive cells and GFAP positive cells, respectively.

Conclusions: These findings suggest that lack of SERT activity represents a potential condition that promotes neuroinflammation evident by activation of the microglia and astrocyte that might be relevant in neuropsychiatric disorders associated with stress and neuroinflammation.

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12. ΔpH modulates the voltage sensor domain in voltage-gated proton channel (Hv1) **EMERSON M. CARMONA,[1] MIGUEL FERNÁNDEZ,[1] JUAN JOSÉ ALVEAR,[1] OSVALDO ALVAREZ,[1,2] ALAN NEELY,[1] RAMON LATORRE,[1] CARLOS GONZALEZ[1]** *[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, Valparaíso, Chile; [2]Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.*

The voltage-gated proton channel (H_v1) is a protein responsible for maintaining the pH homeostasis in cells. This is accomplished by the increase of the channel opening probability by voltage and ΔpH across the membrane. However, the molecular mechanisms of the pH dependence and the modulation of currents kinetics are still poorly understood. H_v1 is a dimeric transmembrane protein of four transmembrane segments. When the N-and C-terminal domain are deleted a monomeric channel is produced, which maintains the biophysical properties of the dimer. In fact, the permeation pathway for protons, the voltage sensor, and the pH sensor are found in the same structural domain. We measured monomeric H_v1 currents to study the pH dependence in excised membrane patches of *Xenopus laevis* oocytes. As in the dimeric channel, the monomeric channel G-V curves were shifted according to the ΔpHestablished

across the membrane, but the kinetics of activation were affected by the absolute internal and external pH. To determine the mechanisms by means of which ΔpHchanges the H_v1 open probability and the kinetics, we measured the effect of pH in gating currents of the monomeric H_v1 using a nonconducting mutant channel. The Q-V curves and the kinetics of decay of the ON-gating currents were changed according to the ΔpHestablished across the membrane. We used different voltage protocols to study the kinetics of gating currents at different DpHs with the aim to determine the transitions in the channel activation pathway modulated by pH. Our results suggest that ΔpHmodulate the voltage sensor in H_v1 channel.

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13. Mechanisms involved in $K_v1.3$-induced proliferation: $K_v1.3$ channels as voltage-sensors **PILAR CIDAD, ESPERANZA ALONSO, MARÍA SIMARRO, MIGUEL ÁNGEL DE LA FUENTE, JOSÉ RAMÓN LÓPEZ-LÓPEZ, MARÍA TERESA PÉREZ-GARCÍA.** ***Departamentos de Bioquímica y Biología Molecular y Fisiología, Biología Celular y Enfermería, and Instituto de Biología y Genética Molecular, Universidad de Valladolid and CSIC, Valladolid, Spain***

The voltage-dependent potassium channel $K_v1.3$ has been involved in proliferation in many different systems. K^+ channels can influence cell cycle regulation by modulating membrane potential (EM), cell volume and/or Ca^{2+} influx. In addition, noncanonical functions of the channel could contribute to cell proliferation. In vascular smooth muscle cells (VSMCs) the specific requirement of $K_v1.3$ channels for proliferation suggests the involvement of molecule-specific interactions, but the underlying mechanisms are poorly identified. Using heterologous expression of $K_v1.3$ channels, we found an increase in cell proliferation that does not require K^+ fluxes but needs an intact voltage-sensing mechanism. The molecular determinants of $K_v1.3$-induced proliferation are located at the C-terminal domain, where two individual point mutations (Y447A and S459A) abolished $K_v1.3$-induced proliferation.

Here we explore $K_v1.3$-induced proliferation by analyzing: 1) the effect of EM changes on $K_v1.3$-induced proliferation in transfected HEK cells 2) the effect of $K_v1.3$ gating mutants on proliferation, 3) the interactions of $K_v1.3$ with proteins that activate signaling pathways and 4) the possible extrapolation of these mechanisms to native VSMCs. $K_v1.3$ or the poreless mutant $K_v1.3$-WF channels were cotransfected with WT or gain of function (GOF) KATP channels. Co-expression of GOF-KATP hyperpolarized resting EM and abolished $K_v1.3$-induced proliferation, which could be restored by increased $[K^+]e$. The effect on proliferation of $K_v1.3$ mutants with modified activation threshold confirmed the voltage-dependence of $K_v1.3$ induced proliferation. $K_v1.3$ interacts with IQGAP3, a scaffold protein involved in proliferation which facilitates MEK/ERK signaling. $K_v1.3$-induced proliferation and $K_v1.3$ phosphorylation were impaired by MEK/ERK inhibitors, and both $K_v1.3$ phosphorylation and IQGAP3 interaction were facilitated by depolarization. Moreover, IQGAP3 also contributes to VSMC proliferation. Altogether, these data indicate that voltage-dependent conformational changes of $K_v1.3$ are an essential element in $K_v1.3$-induced proliferation in HEK cells, contributing also to phenotypic modulation of VSMCs.

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14. Intrinsic Ligand Dynamics of hERG Potassium Channels **SARA J. CODDING AND MATTHEW C. TRUDEAU** ***Department of Physiology, Baltimore School of Medicine, University of Maryland, Baltimore, Maryland***

Human ether-á-go-go related gene (hERG) voltage-activated potassium channels are critical for cardiac excitability. Characteristic slow closing (deactivation) in hERG is regulated by a direct interaction between the N-terminal Per-Arnt-Sim (PAS) domain and the C-terminal cyclic nucleotide binding homology domain (CNBHD). An intrinsic ligand in hERG is located at the PAS-CNBHD interface, but its role in hERG gating is not well understood. Our recent work with coexpressed channel fragments in which the hERG PAS domain fused to CFP was expressed in trans with the 'core' of the hERG channel (i.e., a channel lacking the PAS domain that is fused to Citrine at the C-terminus, hERG DPAS-Citrine) showed that deactivation gating was disrupted (was made faster) by intrinsic ligand mutations and that the activation time course of the channel was perturbed (was made faster), indicating that the intrinsic ligand is necessary for the functional and structural interaction of the PAS domain and the CNBHD. To better understand the dynamics of the intrinsic ligand during hERG gating we used amber codon suppression technology and transition metal FRET to measure intramolecular movements at short range (10-20Å). We report that channels with an amber stop codon in the intrinsic ligand incorporate the fluorescent noncanonical amino acid L-ANAP shown by robust currents measured with two-electrode voltage-clamp. The addition of di-histidine mutations structurally adjacent to this L-ANAP are also well

tolerated. We performed PCF recordings of excised patches with L-ANAP dihistidine containing hERG channels that also additionally had a C-terminal Citrine fluorescent protein label for identification and found that when the internal face of excised patches were exposed to transition metals a voltage dependent change in L-ANAP fluorescence was observed. This indicates a voltage dependent role of the intrinsic ligand during deactivation in hERG.

15. Molecular determinants of voltage gating in CNBD family channels **JOHN COWGILL,[1] VADIM A. KLENCHIN,[1] CLAUDIA ALVAREZ-BARON,[1] DEBANJAN TEWARI,[1] ALEXANDER BLAIR,[1] BARON CHANDA[1,2]** *[1]Department of Neuroscience, University of Wisconsin, Madison, Wisconsin; [2]Department of Biomolecular Chemistry, University of Wisconsin, Madison, Wisconsin*

Hyperpolarization-activated channels, cyclic nucleotide-gated (HCN) channels show an inverted gating polarity compared with virtually all other voltage-gated ion channels (VGICs). The basic motions of voltage sensing and pore gating are thought to be conserved, implying that these domains are inversely coupled in HCN channels. Although the structure of the HCN1 channel was recently solved, the structural element(s) responsible for the inverted gating polarity of HCN are not known. We used a top-down, protein engineering approach to assemble an array of chimeras between HCN1 and the depolarization-activated EAG. These chimeras display the full complement of voltage-gating phenotypes observed in the VGIC superfamily and highlight the functional contribution of the various structural elements in HCN channels. Surprisingly, our chimeras reveal that the voltage-sensing domain of the HCN channel has an intrinsic ability to drive pore opening in either direction. Specific contacts at the voltage sensor-pore interface and unique interactions near the pore gate force the HCN channel into a hERG-like inactivated state, thereby obscuring their opening upon depolarization. Our findings reveal an unexpected common principle underpinning voltage gating in the CNBD family of ion channels and identify the essential determinants of gating polarity.

16. Regulation of Piezo2 Channel Currents by Gi-protein Coupled Receptors **JOHN DEL ROSARIO, TIBOR ROHACS** *Pharmacology, Physiology & Neuroscience, School of Graduate Studies, Rutgers New Jersey Medical School*

Mechanotransduction is a critical biological process for organisms to discriminate between environmental cues. However, little is known about the molecular and cellular components that contribute to its regulation. Piezo2 channels have been identified as key channels responsible for mechanosensation and mechanical pain. These channels are highly expressed in primary sensory neurons in vertebrates [Dorsal Root Ganglion (DRG) neurons] and genetic mutations in these channels have been shown to impair physiological processes such as light touch and proprioception in humans and mice. Reports have shown that Piezo2 currents are enhanced through the activation of the Gq-coupled bradykinin beta 2 receptor (BDKRB2) involving a PKC and cAMP- dependent mechanism. However, whether Gi-coupled receptors in DRG neurons play a role in the regulation of Piezo2 channels is still unexplored. Electrophysiogical experiments in our laboratory show that activation Gi-protein coupled receptors potentiate Piezo2 currents in DRG neurons and heterologous systems and inhibit Piezo1 currents in HEK293 cells, thus suggesting a general mechanism of action. The potentiation of Piezo2 currents by Gi- protein coupled receptors is also long-lasting, persisting after the removal of the agonist for several minutes. Interestingly, blocking G-protein βγ using the C-terminal domain of beta-adrenergic kinase (βARKct) inhibits the potentiation of Piezo2 currents. In addition, inhibition of G-protein βγ-downstream kinases such as mitogen-activated protein kinase (MAPK) or phosphoinositide 3-kinase (PI3K) also abolishes the potentiation of Piezo2 currents by Gi-protein coupled receptors. Hence, our studies illustrate a potential G-protein βγ-indirect mechanism of action to sensitize Piezo2 channel currents. We aim to investigate GPCR signaling in the regulation of mechanoreceptors and dissect specific molecules and proteins that can potentially serve as a basis for the development of new drug targets for the treatment of mechanical pain.

17. Tracking the allosteric pathways during the heat-driven TRPV1 channel gating **IGNACIO DIAZ-FRANULIC,[1,2] FELIPE GOMEZ,[2] FERNANDO GONZALEZ-NILO,[1,2] RAMON LATORRE[1]** *[1]Centro Interdisciplinario de Neurociencias de Valparaíso, Universidad de Valparaíso, Chile; [2]Centro de Bioinformática y Biología Integrativa, Universidad Andrés Bello, Chile.*

The TRPV1 channel is a polymodal receptor whose gating is under control of several stimuli, including capsaicin, peptides, voltage, protons, lipids and heat [1]. With the exception of heat, the underlying mechanism for these stimuli have been revealed by combining structural biology, mutagenesis and electrophysiology. Several channel regions have showed to be critical for temperature sensing, including the pore domain and the carboxy and amino terminus. However, the identity of the temperature-sensing domain remains unknown [2].

To identify the allosteric pathways followed by the thermal energy captured from the environment in the TRPV1 channel we used a computational method called anisotropic thermal

diffusion (ATD). The expectation is to reveal the identity of the temperature sensor by inverse tracking. The amino acids of the network identified using ATD were replaced by alanine, expressed channels in *Xenopus laevis* oocytes and the mutant channel response to heat determined by electrophysiological recordings.

Using ATD we identified a branched network connecting the different channel regions with the activation gate. Side chain removal at specific nodes *increases* the Q_{10} channel :3-4 times. We hypothesize that this increase in enthalpic change can occur when "unplugging" some regions of the channel that make a negative contribution to the enthalpy change, i.e., undergoing conformational changes that include the heat-induced formation of bonds.

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18. Structural basis of lipid and ion transport by TMEM16 scramblases **MARIA FALZONE,[1] GEORGE KHELASHVILI,[2] XIAOLU CHENG,[2] BYOUNG-CHEOL LEE,[3] JAN RHEINBERGER,[4] ASHLEIGH RACZKOWSKI,[5] EDWARD ENG,[5] CRINA NIMIGEAN,[2,4,6] HAREL WEINSTEIN,[2] ALESSIO ACCARDI,[2,4,6]** ***[1]Graduate School, [2]Physiology and Biophysics, Weill Cornell Medical College, New York, NY, USA; [3]Department of Structure and Function on Neural Network, KBRI, Deagu, Republic of Korea; [4]Anesthesiology, Weill Cornell Medical College, New York, NY, USA; [5]Simons Electron Microscopy Center, New York Structural Biology Center, New York, USA; [6]Biochemistry Weill Cornell Medical College, New York, NY, USA.***

The plasma membranes of eukaryotic cells are asymmetric, with polar and charged lipids sequestered to the inner leaflet when the cell is at rest. Activation of phospholipid scramblases causes the rapid collapse of this asymmetry and externalization of negatively charged phosphatidylserine molecules, activating extracellular signaling networks that control processes such as blood coagulation, membrane fusion and repair. The TMEM16 family is comprised of Ca^{2+}-dependent Cl^- channels and phospholipid scramblases which also have nonselective ion channel activity. Structural and functional analyses TMEM16 scramblases identified a membrane-exposed hydrophilic groove that serves as the lipid translocation pathway. The mechanisms underlying Ca^{2+}-dependent gating of TMEM16 scramblases/nonselective channels as well as the characteristics of the ion pore in these proteins remain poorly understood. Here we describe cryo-electron microscopy structures of a fungal scramblase/nonselective channel from *Aspergillus fumigatus*, afTMEM16, reconstituted in lipid nanodiscs in the presence and absence of Ca^{2+}. These structures reveal that Ca^{2+} binding induces a global rearrangement of the transmembrane and cytosolic regions, resulting in opening of the lipid permeation pathway by way of rearrangements of TM4 and TM6. Molecular dynamics simulations of another fungal scramblase, nhTMEM16, revealed that a hydrophobic lock between TM3 and TM4 is essential to maintain the open, lipid-conductive conformation of the scramblase. Disruption of this lock, via interactions with lipid tails or in silico mutagenesis, favors the positioning of TM4 giving rise to a Ca^{2+}-bound closed conformation. Mutations at the TM3/TM4 lock convert the dual activity scramblase/channel into a channel only protein and the cryoEM structure of a channel-only mutant reveal a continuous, protein delimited pore of sufficient size to allow ion permeation. Our results show that TM6 and TM4 are the gating elements of TMEM16 scramblases where TM6 movement is directly controlled by Ca^{2+}-binding and TM4 movements are controlled by a hydrophobic lock between TM3 and TM4.

19. The suppressive effects of amyloid beta peptides (1–42) and (25–35) on macroscopic and single-channel $K_V1.1$ currents: evidence for direct peptide-peptide interactions **JOSEPH FARLEY, KRISTI DEBOEUF, AND NICHOLAS THELEN.** ***Neuroscience, Indiana University, Bloomington, Indiana USA.***

The beta amyloid peptides (Aβs) have long been associated with Alzheimer's Disease (AD). Aβ accumulation has been linked to the disruptions of neuronal Ca^{2+} homeostasis, synaptic communication, long-term potentiation (LTP) and learning and memory. The mechanisms underlying these effects are still largely unclear. Because $K_V1.1$ and related channels are activated during an action potential, regulate depolarization-produced Ca^{2+} influx, and inhibition of K_V1 channels can be neurotoxic, K_V1 channels are intriguing Aβ target candidates in early AD pathogenesis. Our previous studies have shown that Aβ(1–42) suppressed macroscopic murine $K_V1.1$ currents in *Xenopus* oocytes by ~50% within 30 min. Suppression occurred in part through intracellular Ca^{2+}-dependent signaling pathways involving PP2B and PKC. Here, we sought to answer three questions. Using patch-clamp and artificial membrane techniques, we asked whether suppression of $K_V1.1$ by Aβ(1–42) also involved direct peptide-peptide interactions. Second, using capacitance measurements and Western blot analyses of plasma membrane proteins, we asked if suppression of $K_V1.1$ activity by Aβ(1–42) involved endocytosis. Third, we asked whether the core peptide, Aβ(25–35), was sufficient for $K_V1.1$ suppression. We found that application of Aβ(1–42) to the

intracellular face of K_V1.1 channels, in both ripped-off patch and tip dip experiments, produced dramatic reductions in p(open), with no observable current ~2 min post-addition. BLM experiments also showed clear reductions in *p*(open) in response to intra- and extra-cellular Aβ application but did not fully eliminate channel activity (~45% reduction). Suppression of K_V1.1 by Aβ(1–42) did not involve channel endocytosis. Finally, the core fragment, Aβ(25–35), was sufficient to produce K_V1.1 suppression, but less so than Aβ(1–42). Suppression of K_V1.1 and related K^+ channels presynaptically could lead to larger and longer action potentials, permitting greater influx of Ca^{2+} and subsequent increase in glutamate release. Postsynaptically, the increased glutamate release, through activation of AMPA and NMDA receptors, may contribute to excitotoxicity.

20. Conserved voltage-dependent gating elements between Shaker and hERG Kv channels **ANA I. FERNANDEZ-MARIÑO AND KENTON J. SWARTZ** ***Molecular Physiology and Biophysics Section, Porter Neuroscience Research Center, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.***

Voltage-gated potassium (Kv) channels are of crucial importance in neural excitability and cardiac function. The crystal structure of the K_V1.2/2.1 paddle chimera has provided a foundation for investigating the mechanisms of voltage-dependent gating and illustrates how these ion channels are tetramers with each subunit containing a peripheral S1-S4 voltage-sensing domain and contributing S5-S6 segments to a central pore domain. The domain-swapped architecture evident in these channels places the voltage-sensing domain of one subunit adjacent to the pore-forming helices of the adjacent subunit, a feature also seen in voltage-gated calcium and sodium channels. In such domain-swapped channels, the S4-S5 linker helix forms an intracellular cuff around the pore that physically couples movement of the voltage-sensing domains to opening of the pore. A noncanonical coupling mechanism was also recently proposed to involve interactions between the two domains within the membrane. Surprisingly, the recent cryo-EM structures of the "Ether a-go-go" EAG channel family (EAG, hERG and ELK; K_V10-12) reveal that these Kv channels adopt a nondomain-swapped architecture, and functional studies have shown that the integrity of the S4-S5 linker is not required for voltage-dependent gating. We set out to identify structural elements that are critical components of the gating machinery in the EAG family and to explore whether the noncanonical coupling mechanism is conserved. To address this question, we successfully engineered chimeras where we transferred the pore of the hERG Kv channel into the Shaker Kv channel. Several of these chimeras exhibit partial constitutive activity, while retaining voltage-dependent changes in macroscopic conductance, potassium ion selectivity and remarkably, the properties of C-type inactivation that are typical of hERG Kv channels. Our results demonstrate that pore domains can be transplanted between Kv channels with and without domain-swapped architecture, providing new approaches for localizing structural elements that are critical for the functional properties of Kv channels.

21. Exocytosis in immortalized myoblasts from patients harboring dysferlin mutations **C. FIGUEROA-CARES, H. ALMARZA-SALAZAR, M.J. GUERRA-FERNÁNDEZ, M.C. MALDIFASSI, A. GONZALEZ-JAMETT, A.M. CÁRDENAS** ***Centro Interdisciplinario de Neurociencias de Valparaíso (CINV), Facultad de Ciencias, Universidad de Valparaíso.***

Dysferlin is a skeletal muscle protein that plays an important role in sarcolemma repair (Escobar et al. 2016. Molecular Therapy-Nucleic Acids, 5, e277). Mutations in the gene that codes for dysferlin cause a group of muscular dystrophies known as dysferlinopathies, which are characterized by high serum levels of creatine kinase, weakness and muscle atrophy (Cárdenas et al. 2016. Experimental neurology, 283:246-254). Dysferlin has a transmembrane C-terminal domain and seven cytosolic C2 domains that bind Ca^{2+} and phospholipids (Abdullah et al. 2014. Biophys J 106:382-389). The expression of the transmembrane region together with the C2F and C2G domains recovers the membrane repair function in an animal model of the disease. However, the progressive degeneration remains unabated (Lostal et al. 2012. PLoS One 7:e38036), suggesting that other critical functions, which depend on additional dysferlin regions, are also disturbed in the disease. Based on evidences showing that dysferlin directly interacts with the SNARE proteins and promotes SNARE-dependent fusion of liposomes (Codding et al. 2016. J Biol Chem 291:14575-14584), we hypothesized that dysferlin also has a critical role in Ca^{2+}-induced exocytosis. Therefore, we studied exocytosis in immortalized myoblasts from unaffected individuals and from patients suffering from dysferlinopathy using total internal reflection fluorescence microscopy (TIRFM) and the pHluorin reporter fused to IRAP, an aminopeptidase that localizes in GLUT4-carrying vesicles. Exocytosis events were induced with the Ca^{2+} ionophore ionomycin. For each exocytotic event was analyzed the amount of events, and their duration, size, lag time and diffusion pattern. We have found that dysferlinopathy myoblasts display impaired ionomycin induced-exocytosis of IRAP-pHluorin with no changes in Ca^{2+} signals. Durations of the exocytotic events were also smaller in dysferlinopathy myoblasts, suggesting that dysferlin also influences exocytosis kinetics.

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22. Evolutionary analyses unveil a sequence motif associated with fast inactivation in TRPV5 and TRPV6 channels **LISANDRA FLORES-ALDAMA,[1] MICHAEL W. VANDEWEGE,[2] KATTINA ZAVALA,[3] CHARLOTTE K. COLENSO,[1] SEBASTIAN E. BRAUCHI,[1] JUAN C. OPAZO[3]** *[1]Instituto de Fisiología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile; [2]Department of Biology, Eastern New Mexico University, Portales, New Mexico, USA; [3]Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.*

Fundamental for calcium homeostasis in vertebrates, TRPV5 and TRPV6 are calcium-selective channels belonging to the Transient Receptor Potential (TRP) gene family. Detailed phylogenetic analyses unveil paralogs in mammals, sauropsids, amphibians and chondrichthyans, suggesting that TRPV5 and TRPV6 arose via independent gene duplication events rather than speciation. Coincident with the conquest of land and the physiological changes needed to maintain calcium homeostasis, our expression analyses suggest a change in their expression pattern. Within amniotes, we identified a traceable sequence signature of three amino acids located at the amino-terminal intracellular region that correlates well with both the duplication events and the phenotype of fast inactivation observed in mammalian TRPV6 channels. Electrophysiological recordings and mutagenesis suggest that calcium-induced fast inactivation represent an evolutionary innovation that emerged recursively on almost every vertebrate clade after each duplication event.

23. Pannexin1: a "brake" for actin remodeling and structural synaptic plasticity in hippocampal neurons **CAROLINA FLORES-MUÑOZ,[1,2,7] FRANCISCA GARCÍA-ROJAS,[3,7] MIGUEL A. PEREZ,[3] DANIELA LOPEZ-ESPÍNDOLA,[4,5] AGUSTÍN D. MARTÍNEZ,[2] ARLEK GONZALEZ-JAMETT,[2] ÁLVARO O. ARDILES[1,2,6]** *[1]Centro de Neurología Traslacional de Valparaíso, Facultad de Medicina, Universidad de Valparaíso, 2341386 Valparaíso, Chile; [2]Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, 2360102 Valparaíso, Chile; [3]Centro de Neurobiología y Plasticidad Cerebral, Instituto de Fisiología, Universidad de Valparaíso, 2360102 Valparaíso, Chile; [4]Escuela de Tecnología Médica, Facultad de Medicina, Universidad de Valparaíso, 2529002 Valparaíso, Chile; [5]Centro de Investigaciones Biomédicas, Escuela de Medicina, Universidad de Valparaíso, 2341386 Valparaíso, Chile; [6]Centro Interdisciplinario de Estudios en Salud, Facultad de Medicina, Universidad de Valparaíso, Viña del Mar, Chile; [7]Programa de Doctorado en Ciencias, mención Neurociencia, Universidad de Valparaíso, Valparaíso, Chile.*

Neurons are highly specialized cells whose polarized morphology allows them to process information within the brain. The highly branched and complex morphology of their dendritic tree is crucial to establish contacts and neural circuits. Rearrangements of the neuronal architecture accompany the modifications in the synaptic functionality that lead to synaptic plasticity (SP). This latter is manifested as long-lasting changes in the synaptic strength that is been widely pointed as the molecular basis of learning and memory. In fact, long-term-potentiation (LTP) and long-term depression (LTD) of the synaptic efficacy are the most prominent forms of SP and the mechanisms governing their induction have been proposed to be finely tuned during experience-induced neuronal activity, as well as during central nervous system development and upon neuropathological conditions. Previously we demonstrated that Pannexin 1 (Panx1), a nonselective membrane channel, modulates the induction of excitatory SP by preventing LTP and favoring LTD-mechanisms in hippocampal neurons. Here we show that the absence of Panx1 in knock-out mice (Panx1-KO) promotes the structural remodeling of neuronal architecture by favoring dendritic branching, spine maturation, spine-innervations and by increasing the size of the post-synaptic density (PSD) in hippocampal neurons. Consistently, modifications in the frequency of mEPSCs and in the number of functional synaptic contacts are also observed in Panx1-KO mice compared with wild-type littermates. These data strongly suggest a "stabilizing role of Panx1 in neuronal morphology and structural SP. Remarkably these modifications are associated with increased expression of actin-related proteins and enhanced F-actin content in hippocampal tissue of Panx1-KO mice, suggesting that the role of Panx1 in neuronal morphology and structural SP relies on actin organization and dynamics.

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24. Acute Pannexin 1 blockade mitigates synaptic plasticity defects in a mouse model of Alzheimer's disease **CAROLINA FLORES-MUÑOZ,[1,4] BÁRBARA GÓMEZ,[1,2] ELENA MERY,[1,2] IVANA GAJARDO,[1] PAULA MUJICA,[1,4] PABLO MUÑOZ,[1,3] DANIELA LOPEZ-ESPÍNDOLA,[2,3] ALAN NEELY,[4] ARLEK GONZALEZ-JAMETT,[4,#] ÁLVARO O. ARDILES,[1,4,5,#]** *[1]Centro de Neurología Traslacional, Facultad de Medicina, Universidad de Valparaíso, 2341386 Valparaíso, Chile; [2]Escuela de Tecnología Médica, Facultad de Medicina, Universidad de Valparaíso, 2529002 Valparaíso, Chile; [3]Centro de Investigaciones Biomédicas, Escuela de Medicina, Universidad de Valparaíso,*

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Synaptic loss induced by soluble oligomeric forms of the amyloid β peptide (sAβos) is one of the earliest events in Alzheimer's disease (AD) that is thought to be the major cause of the cognitive deficits. These abnormalities rely on defects in synaptic plasticity, a series of events manifested as activity-dependent modifications in synaptic structure and function. It has been reported that Panenxin1 channels (Panx1), a membrane protein implicated in cell communication and intracellular signaling, modulate the induction of excitatory synaptic plasticity under physiological contexts and contribute to neuronal death under inflammatory conditions. Here, we decided to study the involvement of Panx1 channels in the sAβos-mediated defects observed in excitatory synapses of the APP/PS1 transgenic (Tg) mice, an animal model of AD. We found an enhanced expression of Panx1 channels in hippocampal slices from Tg mice and an exacerbated Panx1 activity in response to glutamate receptors activation. Importantly, the acute inhibition of Panx1 activity with the drug probenecid (PBN) attenuates excitatory synaptic defects in the AD model. Specifically, PBN normalized long term potentiation and depression, and rescue the limited dendritic arborization and spine density observed in hipocampal neurons of the Tg mice to levels comparable to those observed in wild type (Wt) animals. These findings correlates with a decreased levels of activated p38MAPK, as part of a possible mechanisms underlying sAβos-induced synaptotoxicity.

Our data support Panx1 as a potential AD therapeutic target and suggest PBN as a promising treatment to ameliorate synaptic defects of the disease.

25. A novel two-electrode voltage clamp/dye uptake assay enables quantitative exploration of molecular permeation in wide-pore channels **P.S. GAETE, M.A. LILLO, W. LOPEZ, Y. LIU, A.L. HARRIS, J.F. CONTRERAS** *Department of Pharmacology, Physiology and Neuroscience. New Jersey Medical School, Rutgers, The State University of New Jersey, Newark, NJ, USA*

Channels that are permeable to small molecules such as ATP, in addition to atomic ions, are emerging as important regulators in health and disease. Nonetheless, mechanisms of molecular permeation and selectivity of these channels remain largely unexplored due to lack of quantitative methodologies. To address this need, we developed a novel two-electrode voltage clamp (TEVC)/dye uptake assay to examine the kinetics of molecular permeation of channels formed by human connexins (Cx), pannexins (Panx) and the calcium homeostasis modulator (CALHM1). hCx26, hCx30, hPanx1 and hCALHM1 were individually expressed in *Xenopus laevis* oocytes. To quantify the uptake of small molecular dyes through these channels, we developed a protocol that renders oocytes translucent—thereby amenable to optical detection techniques—without affecting functional properties of the expressed channels. To control membrane potential and to determine channel expression accurately, dye uptake was evaluated in parallel with TEVC. Using this methodology, we found that: (1) All of these channels display saturable transport of molecules that could be described by Michaelis-Menten kinetics, with apparent K_M and V_{max}; (2) Kinetic parameters for molecular transport through CALHM1 are sensitive to extracellular calcium; (3) Permselectivity differs significantly among closely-related Cx channel isoforms and, in the case of Panx1, may depend on membrane potential; (4) Significant transport of molecules occurs through CALHM1 when there are little or no ionic currents through the channels; (5) Cx mutations in the N-terminal region significantly affect kinetics of transport and permselectivity. Our results reveal that molecular permeability of these channels empirically displays enzyme-like properties of transport and that the kinetic parameters of molecular transport and permselectivity are sensitive to modulators of channel gating. Our methodology allows analysis of how mutations associated with human diseases or specific conditions affect kinetic properties and permselectivity of molecular signaling, and enables the study of molecular mechanisms, including selectivity and saturability, associated with molecular transport in wide-pore channels.

26. A Na,K-ATPase with Reduced Stoichiometry is Vital for Brine Shrimp Adaptation to High Salinity **CRAIG GATTO,[1] DYLAN J. MEYER,[1,2] VICTORIA C. YOUNG,[1] JESSICA EASTMAN,[1] JASMINE HATCHER-MOORMAN,[2] MATTHEW A. BIRK,[3] JOSH J. ROSENTHAL,[3] PABLO ARTIGAS 1*** *[1]School of Biological Sciences, Illinois State University, Normal, IL; [2]Department of Cell Physiology and Molecular Biophysics, Center for Membrane Protein Research, Texas Tech University Health Sciences Center, Lubbock, TX; [3]The Marine Biological Laboratory Woods Hole, MA, USA*

Brine shrimp (*Artemia salina*) are osmoregulators that survive in ~4.5 M salt. They express two Na/K pump isozymes formed by different α-submits associating with a common β-subunit; a "normal" α-subunit ($\alpha1_{NN}$) or a special α-subunit ($\alpha2_{KK}$) containing two asparagine-to-lysine substitutions in the ion-binding region. We introduced the equivalent substitutions (N333K and N785K) in the *Xenopus* α1-subunit and functionally evaluated the mutants with electrophysiology

in *Xenopus* oocytes. N785K reduced apparent affinity for K^+_o. Both individual mutants reduced Na^+_i apparent affinity. The ion affinity change observed in N333K/N785K was not the sum of individual mutants' effects, indicating these residues are coupled. Simultaneous determination of $^{86}Rb^+$ (a K^+ congener) uptake and charge extruded under two-electrode voltage clamp yielded ratios of Rb^+ uptake/charge extruded of 2.11 ± 0.07 (n = 40) for WT and 1.07 ± 0.04 (n = 21) for N333K/N785K, indicating stoichiometries of $3Na^+$:$2K^+$ and $2Na^+$:$1K^+$, respectively. Inhibition of $\alpha1_{NN}$ by ouabain (IC_{50}~100 µM) reveals a ~ten-fold lower affinity than $\alpha2_{KK}$ inhibition (IC_{50}≤10 µM) (Cortas et al. J. Memb. Biol. 108:187-195, 1989). The LD_{50} for larvae (nauplii) incubated for 24-h in ouabain was LD_{50}~300 µM at 0.12 M and LD50~30 µM at 2 M salt, indicating that $\alpha2_{KK}$ Na/K pumps are required for high-salinity adaptation. We reared *Artemia* at 0.25 M, 2M and 4 M salt to quantify the changes in mRNA expression at different salinities using transcriptome analysis and qPCR. Compared with animals in 0.25 M, *Artemia* at 4 M salt increased expression of $\alpha2_{KK}$ (≥10-fold) and β (~2-fold) while $\alpha1_{NN}$ expression remained unaltered. Immunostaining of salt-extrusion organs and guts of adult *Artemia* show exclusive basolateral Na/K pump localization. Therefore, the need for a reduced stoichiometry must come from an extreme basolateral membrane voltage, probably required to extrude Cl^- passively by mechanisms under investigation. (NIH/GM061583 & NSF/MCB-1515434.)

27. A "receptophore model" for local anesthetics binding site in cardiac ion channels **WENDY GONZÁLEZ,[1] GABRIEL NÚÑEZ-VIVANCO,[1] DAVID RAMÍREZ,[2] AYTUG K. KIPER,[3] SUSANNE RINNÉ,[3] MAURICIO BEDOYA,[1] LAURA SÁNCHEZ,[1] JOSÉ C.E. MÁRQUEZ-MONTESINOS,[1] MIGUEL REYES-PARADA,[4] VLADIMIR YAROV-YAROVOY,[5] NIELS DECHER[3]** ***[1]Center for Bioinformatics, Simulations and Modeling (CBSM), Universidad de Talca, Talca, Chile; [2]Department of Chemistry and Biochemistry, Worcester Polytechnic Institute, Worcester, MA, USA; [3]Institut für Physiologie und Pathophysiologie, Philipps-Universität Marburg, Germany; [4]Facultad de Ciencias Médicas, University of Santiago, Chile; [5]Department of Physiology and Membrane Biology, School of Medicine, UC Davis Health, Davis, CA, USA***

Intoxication with local anesthetics may provoke arrhythmias by interaction with cardiac ion channels such as $Na_V1.5$, $K_V1.5$ and TASK-1. While $Na_V1.5$, $K_V1.5$ and TASK-1 channels have different sequences and structures, they share local similarities in the architecture of the binding site for local anesthetics. Using computational polypharmacology and multi-target/structure-based methods such as Geomfinder (https://jcheminf.biomedcentral.com/articles/10.1186/s13321-016-0131-9), PocketMatch (http://proline.physics.iisc.ernet.in/pocketmatch/), and MultiBind (http://bioinfo3d.cs.tau.ac.il/MultiBind/) we searched for local anesthetics Binding Site Similarities (BSS) between $Na_V1.5$, $K_V1.5$ and TASK-1 channels, converting 3D coordinates of proteins into easy patterns to compare. We report a "receptophore model" revealing features of the cardiac ion channels that are essential to ensure optimal interactions with the local anesthetics. This knowledge of promiscuous drug action will help in the multi-target drug design of novel cardiac ion channels modulators that may simultaneously interact with $Na_V1.5$, $K_V1.5$ and TASK-1. This approach might yield innovative anti-arrhythmic compounds having a more promising risk–benefit ratio than currently available drugs in clinic.

28. Theoretical and experimental study of the thermodynamics parameters that govern the activation process of TRPV1 **FELIPE A. GÓMEZ-ALVEAR,[1,2] IGNACIO DIAZ-FRANULIC,[2] DANIEL R. AGUAYO-VILLEGAS,[1] RAMÓN LATORRE,[2] FERNANDO D. GONZÁLEZ-NILO[1,2]** ***[1]Center for Bioinformatics and Integrative Biology, Facultad de Ciencias de la Vida, Universidad Andrés, Bello, Chile; [2]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile.***

The TRPV1 channel is a polymodal receptor whose activation is controlled by vanilloid ligands, heat, voltage, lipids, protons and peptides from venomous animals. The knowledge of its structure in different conditions of activation (open and closed) provides a framework to identify the thermodynamic parameters that drive the channel activation . . Here we describe the difference of free energy profile between the open and closed state of TRPV1, both already characterized by CryoEM. To describe the process of activation we used the Adaptive Biasing Force (ABF) method to determine the reaction coordinate with the RMSD function as a collective variable. . The results of this study are consistent with our experimental measurements for the wild type and mutations designed to disturb the heat sensitivity. Thus, our theoretical and experimental results contribute to the better understanding of the thermodynamics parameters that govern the activation process of TRPV1 at the molecular level.

29. Deficient activity of LRRC26-associated BK CHANNELS EXPRESSED IN COLONIC Goblet cells DRAMATICALLY enhances susceptibility to colitis in mice **VIVIAN GONZALEZ-PEREZ,[1] PEDRO L. MARTINEZ-ESPINOSA,[1] MONICA SALA-RABANAL,[1] NIKHIL BHARADWAJ,[1] XIAO-MING XIA,[1] DAVID ALVARADO,[2] MATTHEW A CIORBA2 AND CHRISTOPHER J LINGLE[1]** ***[1]Department of Anesthesiology, Washington University School of Medicine, St Louis, MO,63110; [2]Department of Internal Medicine, Division of Gastroenterology, Inflammatory Bowel Disease Program, St Louis, Missouri.***

BK channels are membrane complexes enabling K^+-efflux from cells in response to an increase in intracellular calcium concentration and/or membrane depolarization. BK channels can contain regulatory subunits which critically define the functional properties of the channel. One of those regulatory proteins is LRRC26 (also known as BK-γ1 subunit) and its presence produces a shift of the activation range so dramatic that LRRC26-associated BK channels can contribute to K^+ efflux near normal physiological resting conditions. However, the physiological implications of this unique type of BK channel in native cells are unknown. Using a LRRC26 KO mouse model generated in our laboratory, we have recently identified that LRRC26 expression is specific to secretory epithelial cells and, within the gut, specifically localizes to Goblet cells (GCs). Intestinal GCs are epithelial cells contributing critically to intestinal barrier function and mucosal immune homeostasis. GC dysfunction is linked to human inflammatory bowel diseases (IBD) such as Ulcerative colitis and Crohn's Disease. However, the cellular mechanisms that regulate GC function are incompletely understood and the physiological impact of BK channel activity in those cells is totally unknown. In this study, by using a fluorescence-tagged GC mouse, we have confirmed that murine colonic GCs have functional LRRC26-BK channels which become inoperant in the absence of LRRC26. Surprisingly, the nonfluorescent colonocytes appear to have no BK channels at all. Furthermore, we have found that the genetic ablation of either LRRC26 or the BK-pore forming subunit in mice results in a dramatically enhanced susceptibility to colitis induced by dextran sulfate sodium. The fact that BK channels seem to be found only in GCs among all colonic epithelial cells contrasts with the general view regarding BK channel expression in enterocytes. Our results also suggest that normal potassium flux though LRRC26-associated BK channels in GCs has protective effects against colitis.

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30. Tetrodotoxin-sensitive sodium channels mediate action potential firing and excitability in menthol-sensitive Vglut3-lineage sensory neurons **THEANNE N. GRIFFITH,[1,2] TREVOR A. DOCTER,[3] ELLEN A. LUMPKIN[1,2,4]** ***[1]Columbia University, Dept. of Physiology & Cellular Biophysics, New York, NY; [2]Neurobiology Course, Marine Biological Laboratory, Woods Hole, MA; [3]Columbia University, Undergraduate Program in Neuroscience & Behavior, New York NY; [4]Columbia University, Dept. of Dermatology, New York, NY.***

Small-diameter vesicular glutamate transporter 3-lineage (Vglut3^{lineage}) dorsal root ganglion neurons exemplify the functional heterogeneity typical of sensory neurons. They are mechanosensitive and also contribute to thermal hypersensitivity in pathological states. Nonetheless, the physiological basis of this diversity is poorly understood. Thus, we set out to identify mechanisms of excitability within this subpopulation. Using calcium microfluorimetry in adult DRG neurons (< 24 h in culture) harvested from male and female *Slc17a8i^{Cre};Rosa26^{Ai14}* mice, we show the cooling agent, menthol, selectively activates a subset of Vglut3lineage neurons. Targeted whole-cell electrophysiological recordings from small-diameter Vglut3^{lineage} neurons (≤ 25 pF) found that 50% of these cells fired menthol-evoked action potentials. Surprisingly, the vast majority of these neurons fired robust action potential discharges at room temperature, which were dependent upon transient potential receptor melastatin 8 activation. To interrogate the mechanisms mediating this unique excitability profile, we performed current-clamp recordings and action potential phase plot analysis. Menthol-sensitive Vglut3^{lineage} neurons had membrane potentials that were more depolarized and firing thresholds that were more hyperpolarized, compared with menthol-insensitive Vglut3^{lineage} neurons. Additionally, they produced more action potentials in response to evoked current. Voltage-clamp experiments revealed that sodium channel (NaV) currents in menthol-sensitive neurons we overwhelmingly resistant to entry into slow inactivation (t = 1485 ms, n = 6) compared with menthol-insensitive neurons (t = 376.5 ms, n = 5). Using a pharmacological approach, we determined that menthol-sensitive Vglut3^{lineage} neurons engage tetrodotoxin (TTX)-sensitive NaVs during action potential firing, with a notable role for $Na_V1.1$ channels. Conversely, menthol-insensitive neurons largely fired TTX-resistant action potentials. Interestingly, blocking $Na_V1.1$ channels completely reversed Na_V slow inactivation kinetics in menthol-sensitive Vglut3^{lineage} neurons, with the residual Na_V current readily sequestered into the slow inactivated state. Collectively, these data demonstrate a previously unknown requirement for tetrodotoxin-sensitive NaVs, in particular $Na_V1.1$, channels in mediating excitability of small-diameter somatosensory neurons.

31. A new member of the gap junction family that be able to form functional channels in the protozoa Trypanosoma cruzi **JUAN GÜIZA,[1] FRANCISCO SOLÍS,[2] VALESKA MOLINA,[3] PEDRO ZAMORANO,[2,3] JONATHAN CANAN,[4] ROMINA SEPÚLVEDA,[4] NIEVES NAVARRO,[5] JONATHAN SAAVEDRA,[5] GUIDO MELLADO,[5] ALAN NEELY,[5] FERNANDO GONZÁLEZ-NILO4,5, JUAN C.SÁEZ,[5] JOSÉ L. VEGA[1]** ***[1]Laboratory of Gap Junction & Parasitic Diseases (GaPaL), Instituto Antofagasta, Universidad de Antofagasta; [2]Laboratorio de Microorganismos Extremófilos, Instituto Antofagasta, Universidad de Antofagasta; [3]Laboratorio Neurobiología, Facultad de ciencias de la salud, Universidad de Antofagasta; [4]Center for Bioinformatics and Integrative Biology, Universidad Andrés Bello; [5]Instituto de Neurociencias, Centro Interdisciplinario de***

Neurociencias de Valparaíso, Universidad de Valparaíso.

Introduction: The gap junction proteins of vertebrates (connexins) and invertebrates (innexins) are well described. However, it remains unknown whether unicellular organisms express functional equivalent proteins. The objective of this work was to identify and characterize a member of gap junction protein family in the protozoa *Trypanosoma cruzi* (*T. cruzi*).

Methodology: The search of genomic sequences were performed using TritrypBD genomic database. The protein topology was analyzed with PROTTER software. The channel structural stability was performed in Modeller 9.10 and dynamic simulation performed in Amber16 software. The function of channel was evaluated by dye uptake assay in HeLa cells transfected with *T. cruzi* genes (pCDNA3.1+Trynx-C-eGFP vector). Membrane current was studied in *Xenopus* oocytes injected with 30 ng cRNA and recorded under cut open voltage clamp.

Results: We identified a protein sequence with a length of 257 amino acids whose membrane topology has 4 transmembrane domains, cytoplasmic N- and C-termini and a highly conserved innexin motif "YYQWV". Three-dimensional modeling (10 ns) revealed a stable channel structure with 8 subunits with pore diameter of 10-12Å. Whit a gradient of electrostatic potential, being electropositive in extracellular regions and electronegative in intracellular regions. HeLa cells transfected with *T. cruzi* sequence showed permeability to DAPI when exposed to extracellular Ca^{2+}/Mg^{2+} free solution, which was blocked by La^{3+}. Currents recorded in cRNA injected oocytes in Ca^{2+} free solution displayed little voltage-dependence and were fully blocked by La^{3+}.

Conclusion: These results suggest the *T. cruzi* present a channel that could be a new member of the gap junction family. This channel can provide a target for future studies on the parasite biology that can help in the search for new therapeutic targets.

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32. Comparative Description of the Expression Profile of ATPase Isoforms in Adult Mouse Nervous System **SONG JIAO,[1] KORY JOHNSON,[2] CRISTINA MORENO,[1] MIGUEL HOLMGREN[1]** *[1]Molecular Neurophysiology Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland; [2]Bioinformatics Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland*

Mutations in the Na^+/K^+-ATPase α2 and α3 subunits have recently been identified as the genetic cause of three neurological disorders: familial hemiplegic migraine, rapid-onset dystonia parkinsonism and alternating hemiplegia of childhood. To fully understand the pathogenesis of these diseases, it is important to know the expression patterns of these different ATPase subunits within the brain regions and the types of brain cells of each region. To address this issue, we investigated the mRNA expression patterns at the single cell level using the newly available scRNA-Seq databases of adult mouse nervous system from Dr. McCarroll's laboratory (Saunders et al., 2018) and Dr. Linnarsson's laboratory (Zeisel et al., 2018). We examined the cellular heterogeneity in the expression of the different ATPase a subunits among brain regions and various cell populations. We further identified the cell types which expressed the highest level of α3 and the genes that correlated with α3 expression. This information will allow us to classify and predict relevant cell populations which are potentially important in the pathogenesis of these diseases.

33. Dynamics of the PAS domain and cyclic nucleotide-binding homology domain interaction probed with a fluorescent noncanonical amino acid (L-ANAP) in hERG potassium channels **ASHLEY A. JOHNSON AND MATTHEW C. TRUDEAU** *Department of Physiology, School of Medicine, University of Maryland, Baltimore*

The voltage-gated potassium channel hERG plays a critical role in cardiac repolarization and is characterized by unusually slow deactivation kinetics. Slow deactivation in hERG channels is regulated by a direct interaction between the N-terminal PAS domain and the C-terminal CNBHD. The PAS-CNBHD interaction is sensitive to point mutations at the domain interface in hERG but less is known about potential dynamic rearrangements of the PAS domain relative to the CNBHD and the rest of the channel. Here, we report that hERG channels formed from PAS-CFP domains and hERGΔPAS–Citrine channels showed FRET that is sensitive to voltage and potassium-induced cell depolarization, consistent with a PAS-CNBHD rearrangement. To examine this in more detail, we took advantage of a fluorescent noncanonical amino acid (L-ANAP) and a metal ion bound to a dihistidine motif that can be used to monitor small structural rearrangements within ion channels using patch-clamp fluorometry (PCF) and transition metal FRET (tmFRET). Using amber stop codon suppression technology, we show that amber stop codon-containing mutants in the PAS domain incorporate L-ANAPs as shown by robust currents measured with two-electrode voltage-clamp. Likewise, the addition of dihistidine mutations in the CNBHD also result in functional channels. We performed

PCF recordings of excised patches containing L-ANAP-incorporated channels labeled with a C-terminal Citrine fluorescent protein for identification. We show that hERG channels are stable in excised patches with robust L-ANAP and Citrine fluorescence that is linearly correlated, suggesting that L-ANAP has been site-specifically incorporated into the channel. We then exposed the internal face of the excised patch to transition metal ions, which bind to the dihistidine motif to quench nearby L-ANAP in a distance dependent matter. We report a voltage-dependent change in L-ANAP fluorescence in the presence of metal ions, demonstrating a rearrangement of the PAS and CNBHD during slow deactivation of the channel.

34. Opening of remodeled Cx43 hemichannels promote arrhythmias upon cardiac stress **MAURICIO A. LILLO,[1] ANDREW LEE,[1] ERIC HIMELMAN,[2] LAI-HUA XIE,[2] DIEGO FRAIDENRAICH,[2] JORGE E. CONTRERAS[1]** *[1]Department of Pharmacology, Physiology, and Neuroscience; [2]Department of Cell Biology and Molecular Medicine. Rutgers University, New Jersey Medical School.*

Connexin 43 (Cx43) is most abundant connexin that forms gap junction channels in the heart. Cellular biogenesis of Cx43 gap junctions is affected in several cardiac pathologies. Unhealthy cardiomyocytes display abnormal plasma membrane Cx43 distribution patterns, with significant levels at lateralized regions, a phenomenon known as cardiac remodeling. Previously, we found that remodeled Cx43 protein functions as nonjunctional channels (hemichannels) in a Duchene muscular dystrophy (DMD) mouse model (D*md*^{mdx}). These mice are susceptible to arrhythmias upon β-adrenergic stress; however, arrhythmias were prevented using Cx43 hemichannel blockers and by genetically reducing Cx43 levels. Nonetheless, the mechanisms of isoproterenol-induced arrhythmias in dystrophic mice remain elusive. Using isolated cardiomyocytes from WT and D*md*^{mdx} mice, we studied the role of Cx43 hemichannels in cardiac action potentials. D*md*^{mdx} cardiac cells showed an increase in Cx43 hemichannel activity, which resulted in membrane potential oscillations following the upstroke of consecutive action potentials, a process called triggered activity. Blockade of Cx43 hemichannels and nitric oxide production restored isoproterenol-induced resting membrane potential depolarization, triggered activity and rescued arrhythmic phenotypes observed in D*md*^{mdx} mice. We then tested whether opening of lateralized Cx43 hemichannels is general mechanism to alter cardiomyocyte excitability or specific to cellular dysfunction associated with the DMD pathology. We examined knockin mice with mutations in three serine residues (S325/S328/S330) by nonphosphorylatable alanines (S3A), which leads to reduced gap junction formation and increased remodeling of Cx43. In S3A isolated cardiomyocytes, b-adrenergic stimulation promoted prolongation of cardiac action potential, membrane plasma depolarization, and subsequently triggered activity. Opening of Cx43 hemichannels is mediated by direct S-nitrosylation as previously observed in dystrophic mice. Thus, we propose that opening of remodeled Cx43 hemichannels is sufficient to promote arrhythmias upon cardiac stress stimulation.

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35. STRUCTURAL RESPONSE OF THE PIEZO CHANNEL UPON APPLICATION OF FORCE **YI-CHIH LIN1,# YUSONG R GUO2,# ATSUSHI MIYAGI,[1] JESPER LEVRING,[2] RODERICK MACKINNON,[2]* SIMON SCHEURING[1]*** *[1]Department of Anesthesiology, Department of Physiology and Biophysics, Weill Cornell Medicine, New York, United States; [2]Laboratory of Molecular Neurobiology and Biophysics, Howard Hughes Medical Institute, The Rockefeller University, New York, United States*

Piezo proteins are mechanosensitive, nonselective cation channels that mediate force-detection in eukaryotic cells through translating a mechanical stimulus into an electrical signal. Recent cryo-EM studies have revealed the structure of most parts of the channel, and gating mechanisms have been suggested. However, it is intrinsically difficult to acquire a structural view of the channel exposed to force. High-speed atomic force microscopy (HS-AFM) is a powerful technique that provides dynamic movies of biomolecules and simultaneously permits varying the applied force during imaging; thus representing an excellent tool for the characterization of potential mechano-induced conformational changes in Piezo1. Here, we show that the Piezo1 channel undergoes significant reversible conformational changes under force: the channel reversibly flattens into the membrane plane during a designed force-sweep imaging cycle.

36. Lysophosphatidic acid is an endogenous agonist of the TRPV4 ion channel **ANA ELENA LÓPEZ ROMERO*, LEÓN DAVID ISLAS SUÁREZ+, TAMARA LUTI ROSENBAUM EMIR*** ***Circuito Exterior s/n Ciudad Universitaria, Departamento de Neurociencia Cognitiva, División Neurociencias, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Coyoacán, 04510, Mexico City, Mexico. +Circuito Interior Ciudad Universitaria, Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, Mexico***

The TRPV4 ion channel responds to stimuli such as hypoosmotic conditions (Strotmann et al., 2000. Nat. Cell. Biol. 2:695-702); Liedtke et al., 2000. Cell. 103: 525–535), moderate temperatures (Güler et al., 2002. J. Neurosci. 22: 6408–6414) and phorbol derivates from plants (Watanabe et al., 2002. J. Biol. Chem. 277:47044-47051).

The only endogenous agonist for TRPV4 for which a binding site has been described is epoxyeicosatrienoic acid (Berna-Erro et al., 2017. Sci. Rep. 7: 10522). The present study shows that lysophosphatidic acid 18:1 (LPA) can directly activate TRPV4.

We studied TRPV4's activity using the inside-out configuration of the patch-clamp technique in HEK293 cells expressing human wild-type (WT) or mutant TRPV4 channels. We recorded currents activated by 1 mM GSK1016790A (GSK101), a synthetic agonist of TRPV4. Moreover, we observed that LPA activates TRPV4 in a dose-dependent fashion (Kd = 4.05 ± 0.1 µM and n_H = 3.6) and that activation is most probably independent of LPA's G protein-coupled receptors.

Furthermore, we show that LPA can activate TRPV4 through an interaction with an arginine at position 746 in the C-terminus of the channel. When this residue is substituted by a negative charge (R746D), the channels remain responsive to GSK101, but unable to respond to LPA. Conversely, the TRPV4-R746K mutation maintains its activation by LPA, similar to what is observed in the WT channels.

The single-channel current remained unchanged in the presence of 5 µM LPA (6.47 ± 0.37 pA), as compared with 100 nM GSK101 (6.45 ± 0.55 pA). However, the open probability in the presence of LPA, was significantly lower when compared with that obtained with GSK101.

In conclusion, we have described a novel endogenous agonist of the TRPV4 ion channel that interacts with the C-terminus region of the channel.

37. Electrophysiological Characterization of Sea Urchin Sperm Ionic Currents **VERÓNICA LOYO CELIS, GERARDO ORTA, CARMEN BELTRÁN, ALBERTO DARSZON** ***Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Morelos 62250, México***

Sea urchins are widely used in studies of developmental biology since they are external fertilizers and possess a large number of gametes. Their sperm have a 2–4 µm diameter head and the flagella is 50 µm long. Speract, a decapeptide released from *L. pictus* and *S. purpuratus* egg jelly, induces chemotaxis in sea urchin sperm and triggers intertwined biochemical and electrophysiological processes that result in intracellular increases in cyclic nucleotides (cGMP, cAMP), calcium concentration ($[Ca^{2+}]_i$) and pH (pH_i), as well as membrane potential changes caused by ionic fluxes of Cl^-, K^+, Na^+ and Ca^{2+}. These events lead to $[Ca^{2+}]_i$ fluctuations that control flagellar beating. The identity of the ionic transporters associated with the $[Ca^{2+}]_i$ changes required for chemotaxis is still not fully known. CatSper, a sperm exclusive Ca^{2+} channel expressed in the flagellum has been detected by proteomic and immunocytochemical analysis in sea urchin sperm and there is some evidence for its involvement in chemotaxis (Seifert et al. 2015. EMBO J. 34(3):379-92, Espinal-Enríquez et al. 2017. Sci Rep. 7(1):4236). In mammals, this channel is mildly voltage dependent and activated with alkaline pHi. This work presents initial findings in an endeavor to electrophysiologically characterize CatSper in sea urchin sperm using the patch-clamp technique. Due to the morphology and size of the sea urchin sperm, we have resorted the technique previously described (Sánchez et al. 2001.FEBS Lett. 503(1):111-5), that swells sperm diluting artificial sea water (ASW) 10 fold, increasing the probability of obtaining high resistance seals to record ion currents. Applying this strategy, we are now obtaining patch-clamp recordings in the cell-attached configuration and detecting a voltage-dependent ion channel that permeates K^+ and Na^+ in the absence of divalent cations, is sensitive to pH_i alkalinization and is blocked with NNC55-0396 and Mibefradil. All these findings are consistent with the CatSper channel properties.

38. Pannexin-1 Channels are functionally coupled to the α7 Nicotinic Acetylcholine Receptor **M. CONSTANZA MALDIFASSI, M. JOSÉ GUERRA, DANIELA PONCE, XIMENA BÁEZ-MATUS, JAIME MARIPILLÁN, AGUSTÍN D. MARTÍNEZ, ANA MARÍA CÁRDENAS** ***Centro Interdisciplinario de Neurociencias de Valparaíso (CINV), Instituto de Neurociencias Facultad de Ciencias, Universidad de Valparaíso.***

Introduction: Pannexin-1 (Panx1), the most widely expressed pannexin family member, is a plasma membrane glycoprotein that forms channels permeable to signaling molecules such as ATP. A preliminary study by our group suggests a functional coupling between Panx1channels and nicotinic acetylcholine receptors (nAChR), however the involvement of a specific nAChR such as the α7 nAChR has not been studied. Furthermore, the mechanism that governs the cross-talk between these two proteins remains unknown.

Materials and methods: A stably transfected SH-SY5Y-α7 nAChR cell line was donated by Dr. Feuerbach (Novarits). Non-transfected SH-SY5Y cells were used as a control. Opening of Panx1 channels was determined by DAPI uptake and ATP release assays. Ca^{2+} signals were monitored in Fluo-4

loaded cells. The physiological relevance of this functional coupling was evaluated in bovine chromaffin cells through DAPI uptake, Ca+2 signals and amperometry to study exocytosis. Specific agonists, antagonists, inhibitors and Ca^{2+} chelating agents were used to study the involved mechanism.

Results: Activation of α7 nAChR increases DAPI uptake through Panx1 in a Ca^{2+}-dependent way. In turn, Panx1 channels amplify the Ca^{2+} signals induced by α7 nAChR activation. In bovine chromaffin cells, activation of α7 nAChR induced DAPI uptake through Panx1. In turn Panx1 regulated the number of exocytotic events modulated by the α7 nAChR through the control of Ca^{+2} signals.

Discussion: Our data show that α7 nAChR is able to open Panx1 in a Ca^{2+}-dependent manner. Panx1 amplifies Ca^{+2} transients, and as such controls the number of secretory events and the amount of catecholamine released by the activation of the α7 nAChR.

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39. Modulation Of Glycine Receptors By β Auxiliary Subunit: A Critical Target For Central Pain Sensitization

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Impairment in Glycine mediated inhibitory neurotransmission is thought to play a critical role in the disinhibition that accounts for the development of central pain hypersensitivity. Glycine receptors (GlyR) are heteropentamers chloride channels, formed by α, and β subunits, and are mainly expressed in the spinal cord and brainstem. Our results show that the expression levels of auxiliary GlyR β subunit (β GlyR) increase in tissue derived from animals subjected to neuropathic and inflammatory pain conditions. The expression of β GlyR subunit in neuropathic (Chronic Constriction Injury, CCI) and inflammatory (Zymosan injected) rat pain models, were evaluated by RT-qPCR and Western blot analysis and reveal increased expression of the gene encoding b subunit and the respective protein 3 d after CCI surgery. Similarly, augmented expression levels of β GlyR subunit were observed after 4 h of zymosan A injection. Thus, a relevant role of β GlyR levels regulation may operate as a common target in both pain models. These results correlate with increased expression of interleukin 1β (IL 1β), which is reported to reduce glycine-induced currents, thus promoting spinal neural hyper-excitability, a signature of chronic pain status. We are performing electrophysiological and bioinformatics experiments to unravel the molecular elements responsible for this modulation. Our results suggest that sites of interaction of IL-1β localize exclusively in the auxiliary b subunit. The residues involved in this interaction, are able to form hydrogen bonds between Lys93 (IL 1β) with Tyr218 - Glu217 (β-subunit) and Arg4 (IL 1β) with Asp197-Glu214 (β-subunit). The interaction is associated with residues belonging to both regions, described that interact with the cytokine receptor corresponding to residues of the back of the loop C of GlyR subunit. Together, this evidence suggests that auxiliary β GlyR subunit may play a substantial role in establishing GlyR-mediated pain sensitization during neuropathic and inflammatory injury.

40. Arachidonic acid modulation of BK (Slo1) channels: Role of the β1 accessory subunit

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Arachidonic acid (AA) is a fatty acid involved in modulation of several ion channels. Previously, we reported that AA activates the high conductance Ca^{2+}- and voltage-dependent K^+ channel (BK) in vascular smooth muscle cells where the channel is expressed with the accessory β1-subunit (β1) [Martín et al., 2014. Pflugers Arch. 466(9):1779-92]. Here, we studied in depth the action mechanism of AA using the patch-clamp technique on BK channel heterologously expressed with β1. 10 μM AA activated the BK channel by a left shift on G-V curve ($\Delta V_{1/2}$ = -55.2 mV ± 4,4; n = 3; P < 0,05). We also demonstrated that the modulation of the channel by AA is direct, since activation persisted in the presence of AA metabolic enzymes blockers (Indomethacin, CDC and 17-ODYA to block the COX, LOX and CYP450, respectively). Considering that activation by AA requires the presence of β1, which modulates the apparent Ca^{2+} sensitivity, stabilizes the voltage sensor domain in its active configuration, and the intrinsic opening of the channel, we analyzed whether AA acts in these processes. By measuring the gating currents, we evaluated if the voltage sensor domain is affected by AA, observing that it produces a significant left shift in the Q-V curve ($\Delta V_{1/2}$= -17.2 ±8.1 mV, n = 5, P < 0.05). We also studied the effect of AA on the intrinsic channel opening probability (NPoi). The results showed that AA increases NPoi in all tested cells (control: NPoi= 0.0013 ±0.0008; AA: NPoi= 0.0245 ±0.0051; n = 4; P < 0.05). Finally, the AA-induced BK channel activation was independent of the intracellular Ca^{2+} concentration ($\Delta V_{1/2}$= -59.8 mV ± 4.8 and -67.5 mV ± 8.8 at 3 nM and 1 μM Ca^{2+},

respectively, n = 5–6, P > 0.05). These results indicated that BK activation by AA depends on the presence of β1-subunit, affecting the voltage sensor domain and increasing the intrinsic opening of the channel.

41. LRRC52 regulates BK channel function and localization in mouse cochlear inner hair cells **PEDRO L MARTINEZ-ESPINOSA,[1] CHRISTOPHER J. LINGLE,[1] AIZHEN YANG-HOOD,[2] LUIS BOERO,[2,4] SHELBY PAYNE,[2] DORA PERSIC,[3] BABAK V-GHAFFARI,[2] MAOLEI XIAO,[2] YU ZHOU,[1] XIAOMING XIA,[1] SONJA J. PYOTT,[3] MARK A. RUTHERFORD[2]** *[1]Department of Anesthesiology, Washington University School of Medicine, St. Louis MO, USA; [2] Department of Otolaryngology, Washington University School of Medicine, St. Louis MO, USA; [3] Department of Otorhinolaryngology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands; [4] Instituto de Investigaciones en Ingeniería Genética y Biología Molecular "Dr. Héctor N. Torres", Consejo Nacional de Investigaciones Científicas y Técnicas, 1428 Buenos Aires, Argentina.*

The perception of sound relies on sensory hair cells in the cochlea that convert the mechanical energy of sound into release of glutamate onto postsynaptic auditory nerve fibers. The hair cell receptor potential regulates the strength of synaptic transmission and is shaped by a variety of voltage-dependent conductances. Among these conductances, the Ca^{2+}- and voltage-activated BK current is prominent and in mammalian inner hair cells (IHCs) displays unusual properties. First, BK currents activate at unprecedentedly negative membrane potentials (-60 mV) even in the absence of intracellular Ca^{2+} elevations. Second, BK channels are positioned in clusters away from the voltage-dependent Ca^{2+} channels that mediate glutamate release from IHCs. Here, we test the contributions of two recently identified leucine-rich-repeat-containing (LRRC) regulatory subunits, LRRC26 and LRRC52, to BK channel function and localization in mouse IHCs. Whereas BK currents and channel localization were unaltered in IHCs from Lrrc26 KO mice, BK current activation was shifted more than +200 mV in IHCs from Lrrc52 KO mice. Furthermore, the absence of LRRC52 disrupted BK channel localization in the IHCs. Given that heterologous coexpression of LRRC52 with BK subunits shifts BK current gating about -90 mV, to account for the profound change in BK activation range caused by removal of LRRC52, we suggest that additional factors may help define the IHC BK gating range. LRRC52, through stabilization of a macromolecular complex, may help retain some other components essential both for activation of BK currents at negative membrane potentials and also appropriate BK channel positioning.

42. Negatively Charged Gating Modifying Toxin Snx-482 insert into lipids membranes through an hydrophobic patch and polar residues **GUIDO MELLADO,[1] ALAN NEELY,[2] JOSE ANTONIO GÁRATE[2]** *[1]Doctorado en Biofisica y Biologia Computacional, Universidad de Valparaiso; [2]Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaiso*

Spider gating modifier toxins (GMTs) are a class of venom peptides that modify the kinetics and voltage dependence of voltage-gated ion channels (Swartz KJ. 2007. Toxicon 49:213–230). These are members of the inhibitory cystein knot (ICK) family of peptides for the presence of a conserved disulfide bridges connecting cystein in a conserved pattern: Cys I–Cys IV, Cys II–Cys V and Cys III–Cys VI (Agwa et al., 2017. Toxins 9:248). Several studies suggest that GMTs, voltage sensor domains and the plasma membrane form a tripartite complex (Agwa et al., 2017. Neuropharmacology 127:32). The emerging view from studies of positively charged GMTs (>+2) is that a hydrophobic patch surrounded by positively charged residues stabilize the membrane-GMTs complex. SNX-482, isolated from the African Tarantula Hysterorates gigas, that inhibit CaV2.3 channels carry a net charge of -2 instead (Newcomb et al., 2000. CNS Drug Reviews. 6:153-173). To investigate SNX-482-membrane interactions we carried 0.5 ms full-atom Molecular Dynamic simulations of a comparative model of the toxin with a partially charged bilayer membrane consisting of a mixture of 2-oleoyl-1-pamlitoyl-sn-glyecro-3-phosphocholine (POPC) and 2-oleoyl-1-pamlitoyl-sn-glyecro-3-glycerol (POPG). These simulations revealed that SNX-482 bind to the membrane within 100 ns simulation by insertion of one or two aromatic N-temini residues followed by embending a large patch of hydrophobic or polar residues. All residues involved in this interaction form a contigous surface that is not surrounded by charged residues. However, there are two arginines on the oposite side of the hydrophobic patch that may also contribute to stabilize membrane binding.

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43. Modulation of Na/K pump function by FXYD2, FXYD4, FXYD6, & FXYD7 **DYLAN J. MEYER,[1] SHARAN BIJLANI,[1] CRAIG GATTO,[2] AND PABLO ARTIGAS[1]** *[1]Department of Cell Physiology and Molecular Biophysics, Center for Membrane Protein Research, Texas Tech University Health Sciences Center, Lubbock TX; [2]School of Biological Sciences, Illinois State University.*

Normal, IL.The Na^+ and K^+ gradients required for excitability of cell membranes are built by the heterodimeric (αβ) Na/K pump. Frequently, a heterotrimer is formed when FXYD

subunits associate to the αβ complex, regulating it. Modulation of human a1β1-pump function by FXYD2, FXYD4, FXYD6, and FXYD7 was studied following expression in *Xenopus* oocytes. Two-electrode voltage clamp of Na^+-loaded oocytes was used to evaluate interaction with external ions (K^+_o and Na^+_o). The K0.5,K^+ from the K^+_o-dependent activation of pump current in the presence of Na^+_o ($[Na^+_o]+[K^+_o]=150$ mM) was increased by FXYD4 and FXYD7 (~2-fold between -100 and -40 mV, ~1.5-fold at voltages above -20 mV). FXYD2 and FXYD6 produced milder effects. Square-voltage pulses with Na^+_o without K^+_o induce transient currents due to voltage-dependent transition between outward-open (Na^+-free) and Na^+-occluded states. Transient-current decay at positive voltage (Na^+-deocclusion) was accelerated by FXYD6 and to a lesser extent by FXYD2. In contrast, FXYD4 and FXYD7 increased current-decay rate at negative voltages (Na^+-occlusion). The center ($V_{1/2}$) of the Boltzmann describing the charge-voltage curve obtained from transient-current integration was, in mV, -36 ±2, -61 ±3, -4 ±3, -54 ±2, and -13 ±1 (n = 7–22), for α1β1, α1β1FXYD2, α1β1FXYD4, α1β1FXYD6, and α1β1FXYD7, respectively. A left-shifted $V_{1/2}$ (compared with α1β1) indicates reduced Na^+_o affinity while a right-shifted one indicates increased Na^+_o affinity. FXYD6 increased the turnover rate (pump current in K^+_o/total charge moved without K^+_o) by ~1.5-fold. Inside-out patches with 5 mM K^+_o and 140 mM NMG^+o in the pipette were used to study the Na^+_i-dependence of ATP-activated pump currents at 0 mV ($[Na^+_i]+[K^+_i]=140$ mM), which gave K0.5,Na^+ (in mM) of 13.8 ±0.5, 21.5 ±3.0, 10.1 ±0.5, 19.5 ±1.1, and 9.7 ±0.8 (n = 7–18), for α1β1, α1β1FXYD2, α1β1FXYD4, α1β1FXYD6, and α1β1FXYD7, respectively. FXYD-mediated changes in Na^+_i apparent affinity and FXYD6-induced increase in turnover must be physiologically relevant. NSF-MCB-1515434.

44. Local synthesis of membrane proteins in axons **PABLO MIRANDA,[1] CHHAVI MATHUR,[1] SUPRAJA CHITTARI,[1] DEEPA SRIKUMAR,[1] FRANCISCO BEZANILLA,[2] MIGUEL HOLMGREN[1]**
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Local membrane protein synthesis has been described in neuron subcellular domains like soma, dendrites, axon termini and more recently in isolated axons in the presence of Schwann cells. The synthesis mechanism, canonical or not, is still unclear, especially in the case of axon local synthesis. Here we show the local synthesis of heterologously expressed tagged membrane proteins in isolated axons and in extruded axoplasm obtained from giant axons of the squid *Loligo pealeii*. Using the giant axon, we have been able to express fluorescently labeled membrane proteins like Shaker Kv channel after incubation of the mRNA in the isolated axoplasm. According to a canonical mechanism of synthesis, mRNA translation to a polypeptide is mediated by ribosomes. We have identified the presence of functional ribosomes in isolated axons and extruded axoplasm. Using taggable puromycin we have labeled and detected newly synthesized proteins and the signal of these proteins colocalize with ribosomal proteins detected using antibodies. Even though these ribosomes do not seem to be associated in polyribosomes, the results suggest that the local synthesis of membrane proteins could be done by a relatively small number of ribosomes locally present in the axon. These results obtained in extruded axoplasm indicated that the axoplasm contains the elements required for membrane protein synthesis without the contribution of the Schwann cells that surrounds the axon membrane and only requires a supply of mRNA to produce membrane proteins.

45. Characterization of the Epilepsy- and Dyskinesia-Linked KCNMA1-N999S Mutation **HANS J. MOLDENHAUER, COLE S. BAILEY, AND ANDREA L. MEREDITH** ***Dept. of Physiology, University of Maryland School of Medicine, Baltimore, MD, 21201***

Mutations in the *KCNMA1* gene are linked to severe neurological disorder. To date, 7 unrelated patients have been identified with the de novo gain-of-function (GOF) mutation N999S, with one patient harboring an additional mutation, R1128S. All these patients share: developmental delay, epilepsy and/or paroxysmal nonkinesigenic dyskinesia (PNKD). Because it is not yet known how the N999S and N999S/R1128W mutations manifest in the brain, in this study we recorded BK currents from WT and mutant channels, in the context of a brain-expressed splice variant (BK_{ZERO}), with physiological solutions: 140/6 mM K^+, with 1 and 10 μM Ca^{2+}. Our results show that the N999S voltage of half maximal activation ($V_{1/2}$) was shifted toward negative potentials by ~50 mV at 1 and 10 μM [Ca^{2+}] compared with WT. At +40mV, near the peak voltage of an action potential (AP), N999S activation was 13 times faster, while at -60mV, near to the neuronal resting potential, deactivation was two-times slower than WT. These biophysical changes resulted in massively larger AP-evoked N999S current compared with WT. The double N999S/R1128W mutation produced no significant differences in BK current properties compared with N999S alone. Finally, we compared N999S with previously characterized GOF mutation, D434G, associated with epilepsy and/or PNKD. The N999S $V_{1/2}$ was shifted by 16 mV toward more hyperpolarized potentials. N999S activation was three-times faster, while deactivation was twice as slow, and the AP-evoked current was two-times larger than D434G. In summary, we demonstrate N999S is a GOF mutation with

respect to BK current properties under physiological conditions, dominant over R1128W. Interestingly, development delay was reported only in N999S patients, raising the possibility that this symptom could be the result of the stronger GOF effects of N999S compared with D434G.

46. Two heads are better than one: Cooperative gating of clustered CaV1.3 channels in neurons **CLAUDIA M MORENO** ***University of Washington, Department of Physiology and Biophysics School of Medicine***

CaV1.3 channels regulate excitability in many neurons. As is the case for all voltage- gated channels, it is widely assumed that individual CaV1.3 channels behave independently with respect to voltage-activation, open probability, and facilitation. Here, we report the results of super-resolution imaging, optogenetic, and electrophysiological measurements that refute this long-held view. We found that the short channel isoform (CaV1.3S), but not the long (CaV1.3L), associates in functional clusters of two or more channels that open cooperatively, facilitating Ca^{2+}influx. CaV1.3S channels are coupled via a C-terminus-to-C-terminus interaction that requires binding of the incoming Ca^{2+} to calmodulin (CaM) and subsequent binding of CaM to the preIQ domain of the channels. Physically-coupled channels facilitate Ca^{2+} currents as a consequence of their higher open probabilities, leading to increased firing rates in rat hippocampal neurons. We propose that cooperative gating of CaV1.3S channels represents a mechanism for the regulation of Ca^{2+} signaling and electrical activity in excitable cells.

47. The role of IL-1β on Atrial Fibrillation Physiopathology **OSCAR MORENO-LOAIZA,[1] AINHOA RODRIGUEZ DE YURRE GUIRAO,[1] NARENDRA VERA NÚÑEZ,[1] ARIEL ESCOBAR,[2] EMILIANO MEDEI[1]** ***[1]Laboratory of Cardio-immunology. Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [2]University of California, Merced, USA.***

Atrial fibrillation (AF) is the most frequent sustained cardiac arrhythmia. Around 33 million people suffer from this arrhythmia and the physiopathology of this complex disease is not fully understood. There is evidence about the involvement of the innate immune response in AF. Our group has previously demonstrated the crucial role of IL-1β in different models of cardiac ventricular arrhythmias. Thus, in this work, we aim to test the hypothesis that IL-1β is involved in the physiopathology of AF. We compared Adult C57BL/6 mice subcutaneously daily-treated with IL-1β or with vehicle (Control) for 15 d. The EKG showed that IL-1β was able to reduce the P wave duration (Control = 10.9 ± 0.8 ms vs. IL-1β = 9.3 ± 1.2 ms). We studied atrial action potential in an intact whole heart, using a modified Langendorff system for mouse heart, with constant perfusion of an oxygenated Tyrode solution at 36–37°C. IL-1β treated mice showed shorter atrial action potential duration (APD) when compared with control hearts at 30, 50 and 70% of repolarization. Pacing at 10Hz, Control vs IL-1β APD_{30}: 11.2 ± 2.5 vs 8.6 ± 2.7, APD_{50}: 18.1 ± 3.7 vs 13.1 ± 2.9, and APD_{70}: 31.4 ± 7.2 vs 22.3 ± 5.6. There were no differences in depolarization rates (dV/dt). Additionally, the IL-1β treated group presented a higher number of spontaneous triggered events (Control = 1/8 vs. IL-1β = 7/13 mice) after a specific S1/S2 simulation protocol. In conclusion, the results presented here strongly suggest that IL-1β induce electrical remodeling that could be involved on the physiopathology of atrial fibrillation.

48. GHSR activation by ghrelin selectively inhibits $Ca_V3.3$ subtype of low-voltage-gated calcium channels **MUSTAFÁ, EMILIO ROMÁN, CORDISCO GONZALEZ, SANTIAGO, RAINGO JESICA.** ***Electrophysiology Laboratory of the Multidisciplinary Institute of Cell Biology [Argentine Research Council (CONICET), Scientific Research Commission of the Province of Buenos Aires (CIC-PBA) and National University of La Plata (UNLP)], Calle 526 S/N entre 10 y 11, 1900 La Plata, Buenos Aires, Argentina.***

The mechanisms by which ghrelin controls electrical activity in the hypothalamus are not fully understood. One unexplored target of ghrelin is Ca_V3, responsible for transient calcium currents (T-currents) that control neuronal firing. We investigated the effect of ghrelin on Ca_V3 subtypes and how this modulation impacts on neuronal activity. We performed whole-cell patch-clamp recordings in primary mouse hypothalamic cultures to explore the effect of ghrelin on T-currents. We also recorded calcium currents from transiently-transfected tsA201 cells to study the sensitivity of each Ca_V3 subtype to GHSR (growth hormone secretagogue receptor) activation. Finally, we ran a computational model combining the well-known reduction of potassium current by ghrelin with the Ca_V3 biophysical parameter modifications induced by ghrelin to predict the impact on neuronal electrical behavior. We found that ghrelin inhibits native T-currents in hypothalamic neurons. We determined that $Ca_V3.3$ is the only Ca_V3 subtype sensitive to ghrelin. The modulation of $Ca_V3.3$ by ghrelin comprises a reduction in maximum conductance, a shift to hyperpolarized voltages of the current-voltage (I-V) and steady-state inactivation curves, and an acceleration of activation and inactivation kinetics. Our model-based prediction indicates that the inhibition of $Ca_V3.3$ would attenuate the stimulation of firing originating from the inhibition of potassium currents by ghrelin. In summary, we discovered a new target of ghrelin in neurons: the $Ca_V3.3$. This mechanism would imply a negative feed-forward regulation of the neuronal activation exerted by ghrelin. Our work expands the knowledge of the wide

range of actions of GHSR, a receptor potentially targeted by therapeutics for several diseases.

49. Evidences of Slo1 K^+ channel participate in the capacitation-associated hyperpolarization in mouse sperm **G. ORTA,[1] J.L. DE LA VEGA-BELTRÁN,[1] I. MENDOZA-LUJAMBIO,[2] C. SANTI,[3] AND A. DARSZON[1]** *[1]Instituto de Biotecnología, Universidad Nacional Autónoma de México. Cuernavaca, Mor., México; [2]CINVESTAV, Instituto Politécnico Nacional, Ciudad de México; [3]Department of Obstetrics and Gynecology Department of Neurosciences, Washington University School of Medicine, St. Louis, Missouri 63110, USA,*

Mammalian sperm must undergo a series of biochemical transformations in the female reproductive tract through a process known as capacitation. This process comprises functional modifications rendering sperm competent to fertilize. Sperm capacitation in several mammals involves plasma membrane reorganization, appearance of hyperactive motility, increases in intracellular pH and Ca^{2+} and membrane potential hyperpolarization. There is evidence suggesting that in mouse, sperm membrane hyperpolarization during capacitation is regulated by a cAMP/protein kinase A dependent pathway involving activation of K^+ channels. Here we present RT-PCR, immunocytochemistry and Western Blot experiments that indicate the presence of Slo1 K^+ channels in mouse sperm. In addition, whole-cell patch clamp recordings in cauda epididymal mouse sperm revealed a K^+ current that is activated by intracellular Ca^2 and is sensitive to Slo1 antagonists, namely slotoxin, charybdotoxin and iberiotoxin. Importantly, these 3 toxins partially inhibited the capacitation-induced hyperpolarization but did not affect the acrosome reaction (AR). Furthermore, this Ca^{2+} activated K^+ current and an ionomycin-Ca^{2+} induced hyperpolarization were recorded in sperm from Slo3 null mice. Potassium currents obtained in sperm from Slo3 null mice show clear single channel activity (G = 252 ±5 pS) in positive voltage potentials and these currents was inhibited by low concentration of TEA. All our results suggest that Slo1 K^+ channels are present in mouse sperm and participate in capacitation but not in the AR

50. Shedding Light on Piezo1 Activation **ALPER D. OZKAN AND JEROME J. LACROIX** *Western University of Health Sciences, College of Integrative Biomedical Sciences, Pomona,CA, 91709 USA*

Mechanosensitive Piezo channels open upon a variety of physiological processes including fluid shear stress, osmotic swelling, adhesion forces and hydrostatic pressure. It is currently unclear whether Piezo proteins sense distinct or common cues produced by such a variety of mechanical stimuli. Here, to address this question, we used a cyclic permuted green fluorescent protein (cpGFP) as a conformational probe to track local conformational changes in response to different mechanical modalities. To this aim, cpGFP probes were individually inserted at different intracellular and extracellular loops into the putative mechanosensory domain of Piezo1. Mammalian cells expressing Piezo1-cpGFP constructs were exposed to incrementally increased levels of fluid shear stress, osmotic swelling and mechanical indentation. The insertion of cpGFP in two regions, one intracellular and one extracellular and separated by more than 1500 residues in the primary amino acid sequence, produces large fluorescence signals in response to low-intensity fluid shear stress but little to no signal in response to other mechanical modalities. These signals depend on the integrity of the actin cytoskeleton and temporally and spatially correlate with Piezo1-mediated intracellular Ca^{2+} entry. In addition, no fluorescence signal was observed when cpGFP was directly bound to the membrane using a genetic fusion with a lipid-modified protein tag. These results show the observed fluorescence signals are Piezo1-dependent and do not come from direct modulation of cpGFP during mechanical stimulation. These work identifies a long-distance shear stress specific conformational pathway in the Piezo1 protein and suggests Piezo channels use distinct gating mechanisms to sense specific mechanical stimuli.

51. Electrical field modulates the water flow through aquaporin channels **MARCELO OZU,[1,2] MIGUEL FERNANDEZ,[3] EMERSON CARMONA,[3] JOSÉ A. GARATE,[3] RAMÓN LATORRE,[3] GABRIELA AMODEO,[1,2] CARLOS GONZALEZ[3]** *[1]Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina; [2]Instituto de Biodiversidad y Biología Experimental y Aplicada (IBBEA), Universidad de Buenos Aires y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) Buenos Aires, Argentina; [3]Centro Interdisciplinario de Neurociencias de Valparaíso and Universidad de Valparaíso, Valparaíso, Chile*

Aquaporins (AQPs) are transmembrane tetrameric proteins that transport water and solutes. Each subunit has its own permeable pathway. Molecular dynamic (MD) simulations predict how the water molecule move through the permeable pathway. However, experimental records are lacking and the effects of the transmembrane electric field are still unknown. Using the heterologous *Xenopus* oocytes system we studied the mechanosensitivity of the plant FaPIP2;1 and the animal AQP4 channels. Functional parameters were obtained from the kinetics of cell volume changes with different osmotic gradients. To test the combined effects of both electric fields and membrane tension on the water transport rate we performed molecular dynamic simulations on homotetramers

of FaPIP2;1 and AQP4. Molecular dynamic simulations were performed with NAMD v.2.7 and the CHARMM27 force field, using an homology model of FaPIP2;1 developed with the crystal of SoPIP2;1 (PDB 2B5F) and the structural data of human AQP4 (PDB 3GD8).

Our experimental results show that FaPIP2;1 behaves as a mechanosensitive aquaporin. In analogy with the study of ion channels, the transport capacity of AQPs can be evidenced in a plot of water flux versus osmotic gradient (Jw-Dosm). For mechanosensitive AQPs the Jw-Dosm plots show deviations from linearity with high gradients. AQP4 is also mechanosensitive and molecular dynamic simulations predict that the water transport rate can change with the applied electric field. Similar results of MD were obtained with FaPIP2;1. Our results suggest that changes in the electrical field perturbates the movement of water flow through aquaporin channels.

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52. Isoform-specific regulation of HCN4 channels by a family of novel interacting proteins **COLIN H. PETERS, JOHN R. BANKSTON, CATHY PROENZA** ***Department of Physiology and Biophysics, University of Colorado, Anschutz Medical Campus***

The hyperpolarization-activated cyclic-nucleotide sensitive channel, HCN4, is a key contributor to the pacemaking ability of the cardiac sinoatrial node (SAN). The "funny current" (I_f) through HCN4 is active during the diastolic depolarization phase of the sinoatrial action potential. In part, I_f determines the rate of spontaneous action potential generation in the node. An important physiological regulator of HCN4 function is cAMP, which is increased by b-adrenergic stimulation of the SAN. cAMP binding increases the open probability of HCN4 ultimately causing an increase in heart rate. In contrast, one of the known accessory proteins of HCN channels, TRIP8b, decreases cAMP sensitivity by direct binding to the channel. While TRIP8b can bind HCN4 in vitro, in humans TRIP8b is expressed exclusively in the brain and therefore cannot be a major player in heart rate regulation. Using mass spectroscopy of HCN4 coimmunoprecipitates, we have identified two novel, isoform-specific modulators of HCN4 function, referred to here as hINT1 and hINT2. Similar to TRIP8b, hINT1 decreased the cAMP sensitivity of HCN4 channel activation. Conversely, hINT2 increased HCN4 channel open probability in the absence of cAMP. Compared with TRIP8b, which can functionally regulate HCN1, HCN2, and HCN4, neither hINT1 nor hINT2 altered the function of HCN2, suggesting distinct mechanisms of action. qPCR experiments found both hINT1 and hINT2 transcript in mouse SAN tissue and we were able to confirm protein expression of hINT2 using Western blot analysis. Unfortunately, there is currently no high quality hINT1 antibody available. In addition, immunofluorescence indicated that hINT2 was present in isolated HCN4 expressing SAN myocytes. Overall, these results suggest that both hINT1 and hINT2 are novel, isoform-specific regulators of HCN4 channel function and, based on its expression in the SAN, hINT2 likely plays a physiologically relevant role in defining pacemaker function.

53. A study of the effect of myoplasmic BAPTA on Ca^{2+} release in frog skeletal muscle based on the measurement of $[Ca^{2+}]$ transients inside the sarcoplasmic reticulum **GONZALO PIZARRO AND J. FERNANDO OLIVERA.** ***Departamento de Biofísica, Facultad. de Medicina, Universidad de la República, Gral. Flores 2125, 1800, Montevideo, Uruguay.***

$[Ca^{2+}]$ transients inside the sarcoplasmic reticulum (SR) of frog skeletal muscle were recorded under voltage clamp with Mag Fluo 4 a.m. to study the effect on Ca^{2+} release of BAPTA added at high concentration to the myoplasm. BAPTA added to the myoplasm binds part of the released Ca^{2+}, reducing the Ca^{2+} signal reported by a myoplasmic indicator and hindering the quantification of the amount of Ca^{2+} released. Monitoring release by measuring $[Ca^{2+}]$ inside the SR ($[Ca^{2+}]_{SR}$) avoids this problem. The application of extrinsic buffers reduced the resting $[Ca^{2+}]_{SR}$ continuously, starting close to 400 µM and reaching the range of 100 µM in about half an hour. The effect of reducing resting $[Ca^{2+}]_{SR}$ on the Ca^{2+} permeability of the SR activated by a voltage clamp pulse to 0 mV was studied, simultaneously recording the myoplasmic $[Ca^{2+}]$ ($[Ca^{2+}]_{myo}$) with Rhod2. The Ca^{2+} release flux was calculated from $[Ca^{2+}]_{myo}$ and divided by $[Ca^{2+}]_{SR}$ to obtain the permeability. Peak permeability was significantly reduced, from 0.026 ± 0.005 ms-1 at resting $[Ca^{2+}]_{SR}$ = 372 ± 5 µM to 0.021 ± 0.004 ms-1 at resting $[Ca^{2+}]_{SR}$ = 120 ± 16 µM (n = 4, P = 0.03). The time averaged permeability was not significantly changed (0.009 ± 0.003 and0.010 ± 0.003 ms-1, at the higher and lower $[Ca^{2+}]_{SR}$ respectively). After equilibrating with the high buffer intracellular solution, the change in $[Ca^{2+}]_{SR}$ ($\Delta[Ca^{2+}]_{SR}$) elicited by voltage clamp depolarization (0 mV, 200 ms) in 20 mM BAPTA was significantly lower ($\Delta[Ca^{2+}]_{SR}$ = 30.2 ± 3.5 µM from resting $[Ca^{2+}]_{SR}$ = 88.8 ± 13.6 µM, n = 5) than in 40 mM EGTA ($\Delta[Ca^{2+}]_{SR}$ = 72.2 ± 10.4 µM from resting $[Ca^{2+}]_{SR}$ = 98.2 ± 15.6 µM, n = 4),

supporting that a Ca^{2+} activated component of release was suppressed by BAPTA.

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54. The Friend Zone: Functional coupling of L-type Ca^{2+} channels and BK K^+ channels during the action potential **AMBER E. PLANTE, JOSHUA P. WHITT, ANDREA L. MEREDITH** ***Department of Physiology, University of Maryland School of Medicine, Baltimore, MD, USA***

BK channels are activated by both membrane depolarization and intracellular Ca^{2+}. However, little is known about how these two mechanisms activate BK currents during a physiological stimulus, such as an action potential. To be activated at physiological voltages in neurons, BK channels require high concentrations of Ca^{2+} (>10 μM), indicating BK channels must be located close to Ca^{2+} channels. Physical interactions between BK and several voltage-gated Ca^{2+} channels have been observed in neurons and heterologous cells. In neurons of the hypothalamus, L-type Ca^{2+} channels (LTCCs) are the predominant Ca^{2+} source for BK channel activation (Whitt et al., 2018). In these neurons, inhibiting LTCC currents with 10 μM Nimodipine reduces the magnitude of peak steady-state BK currents by 77% and peak action potential-evoked BK currents by 74%. LTCC inhibition also produces changes in the spontaneous action potential waveform that are consistent with BK current inhibition, including depolarization of the baseline potential (Control, -55 ± 2 mV; Nimodipine, -48 ± 1 mV, P = 0.007), and prolonged half width (Control, 6 ± 2 ms; Nimodipine, 11 ± 3 ms, P = 0.004). Two LTCC isoforms are expressed in these hypothalamic neurons, Cav1.2 and Cav1.3. Cav1.3 currents are activated at more hyperpolarized potentials compared with Cav1.2 channels, with peak Ca^{2+} influx at -10 mV and +10 mV respectively. To characterize the functional coupling between BK and each LTCC isoform, we coexpressed the BK channel α subunit with Cav1.2 or Cav1.3 channels in heterologous cells. Co-expression of Cav1.3 and BK channels activated BK currents during the interspike interval before threshold (-41 mV) and at the action potential peak (+8 mV). These data suggest that Ca^{2+} influx through Cav1.3 could couple to the activation of BK currents during an action potential. Further studies will test whether BK currents activated by Cav1.2 differ from BK currents activated by Cav1.3 during an action potential.

55. An alternate substrate-binding conformation in the glutamate transporter homologue GltPh **KRISHNA REDDY, YUN HUANG, AMANDA SCOPELLITI, HATICE DIDAR CIFTCI, OLGA BOUDKER** ***Department of Physiology and Biophysics, Weill Cornell Medical College, New York, New York***

A family of secondary active glutamate transporters imports glutamate into the cell through coupling to electrochemical gradients. In mammals, these proteins clear the synaptic cleft of excess neurotransmitter, while in prokaryotes they serve in nutrient uptake. Simple kinetic models of transport have been developed in the well-characterized archaeal aspartate transporter homologue (Glt_{Ph}), which couples aspartate transport to three sodium ions, and is also accompanied by an uncoupled chloride conductance. In the conformation where the binding sites face the extracellular side (outward-facing state), binding of two sodium ions induces formation of the aspartate site and a third sodium site, and subsequent binding of these solutes induces HP2 closure and translocation to the inward-facing state. However, an increasing body of evidence suggests that there are multiple outward-facing states which may translocate to inward-facing states at different rates. Therefore, simple kinetic models may not be sufficient to explain the overall transport cycle of Glt_{Ph}. Using isothermal titration calorimetry, a double mutant of Glt_{Ph} has revealed a second aspartate-binding conformation in the presence of increased salt concentrations. ^{19}F-NMR and single-molecule dynamics attribute these two aspartate-binding conformations to different outward-facing states. The distribution of these two conformations appears to be cation-independent and anion-dependent, suggesting that the second outward-facing state may be related to chloride conductance. Further functional and structural studies of this mutant may reveal the nature of multiple outward-facing states in glutamate transporters.

56. Inducible heteromerization in a stable hERG1a/1b cell line **ERICK B. RÍOS-PÉREZ, FANG LIU, CATHERINE A. EICHEL, WHITNEY A. STEVENS-SOSTRE, JONATHAN SILIGNAVONG, GAIL A. ROBERTSON** ***Department of Neuroscience, Wisconsin Institutes of Medical Research, University of Wisconsin School of Medicine and Public Health, Madison, WI***

Heterologously expressed hERG channels represent the mainstay of drug safety screens even as more channel elements are added to elevate specificity required to both mitigate the risk of sudden cardiac death and enhance throughput of promising lead drugs. Previous efforts to create cell lines stably expressing hERG1a and hERG1b, the components of the native cardiac IKr compromised in acquired long QT syndrome, were met with unpredictable silencing of the hERG1b component despite its stable integration into the HEK293 cell genome. Here we report a new cell line stably expressing hERG1a with hERG1b controlled by an inducible promoter sensitive to doxycycline. The expression of both subunits is quantitative, producing heteromeric channels with electrophysiological and

pharmacological properties consistent with native IKr. Interestingly, coexpression enhanced the number of channels at the surface membrane as revealed by an increase in hERG tail current. Because IC_{50} values can differ between homomeric and heteromeric channels, the cell line should be advantageous in drug safety screening, which in the future will likely use these values to estimate risk in computational models of ventricular cardiomyocytes.

57. Mechanism of NS11021 Activation of BK Channels MICHAEL E. ROCKMAN, ALEXANDRE G. VOUGA, BRAD S. ROTHBERG *Department of Medical Genetics and Molecular Biochemistry. Temple University Lewis Katz School of Medicine*

BK channels (Slo1, $K_{Ca}1.1$) are activated by a combination of intracellular calcium and a depolarized membrane potential under physiological conditions, and thus control neuronal excitability and smooth muscle contractility by hyperpolarizing the membrane in response to Ca^{2+} signals. The small molecule NS11021 is a relatively selective activator of BK channels, although its specific molecular mechanism of action remains unclear. In this study, we use patch-clamp electrophysiology to quantify effects of NS11021 on BK channel gating kinetics, to gain insight toward mechanism. We observe that NS11021 shifts the activation $V_{1/2}$ for BK channels toward more hyperpolarized voltages, in both in the presence and nominal absence of Ca^{2+}, suggesting that NS11021 facilitates BK channel activation by a mechanism that is distinct from Ca^{2+} activation. Based on analysis of channel activation and deactivation kinetics, NS11021 (30 µM) slows the time course of BK channel closing by 10.5-fold compared with control, while having no substantial effect on the time course of activation. This action persists at very negative voltages, at which the BK channel voltage sensor is in the resting conformation. Analysis of single-channel openings and closings further shows that 30 µM NS11021 increases the mean open time from 0.13 ms to 0.42 ms in nominally 0 Ca^{2+} at voltages <-70 mV, where the voltage sensors are at rest. Together these results are consistent with NS11021 binding to and stabilizing the open conformation of the channel, independent of the Ca^{2+}- or voltage-activation mechanisms.

58. Isoflurane anesthesia rapidly blocks calcium activity in mouse brain vascular endothelial cells in vivo ADRIAN RODRIGUEZ-CONTRERAS[1] AND LINGYAN SHI[1,2] *[1]The City University of New York, City College Biology Department and Center for Discovery and Innovation, New York, NY, USA; [2]University of California San Diego, Bioengineering Department. San Diego, CA, USA.*

Isoflurane is a fast acting volatile anesthetic widely used in animals and in humans undergoing surgery. Isoflurane exposure is linked to headaches, hypertension, cardiac, respiratory, cognitive and reproductive issues, which suggests that isoflurane targets multiple cell types. In this study we hypothesized that isoflurane acts directly on brain vascular endothelial cells directly (VECs). To test this idea we performed VEC Ca^{2+} imaging experiments in vivo. We used thinned-skull transcranial two-photon imaging in 3–8 wk old double transgenic mice expressing Cre recombinase and the genetically encoded calcium indicator GCaMP6 under the control of the Tie2 promoter, and in mice that have stable expression of GCaMP8 driven by the Cdh5 promoter. We report that independent of the mouse strain used, brain VECs in awake mice exhibit Ca^{2+} transients at frequencies that range from 1 mHz to 10 mHz and show localized and wave-like spatial patterns. Upon anesthesia induction (5% isoflurane), Ca^{2+} transients in brain VECs were partially or entirely blocked, failing to return to preexposure levels 10 min after isoflurane washout (n = 10 mice). Systematic monitoring of Ca^{2+} transients in awake mice during preexposure normoxia (air), vehicle (O_2 only), isoflurane (O_2 + 5% isoflurane), and washout normoxia (air) showed two types of inhibitory effects: a very rapid inhibition of Ca^{2+} transients within a few seconds of exposure to anesthetic, followed by a slower decrease in fluorescence baseline with an exponential time course of tens of seconds (n = 6 mice). To the best of our knowledge, this study provides the first measurements of Ca^{2+} transients in brain VECs in vivo, demonstrates a direct inhibitory effect of isoflurane on VEC Ca^{2+} activity and homeostasis, and motivates future experiments to analyze the mechanisms by which isoflurane alters neurovascular physiology under minimally invasive conditions.

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59. Screening the blocking capacity of novel 4-aminopyridine analogues toward Shaker potassium channel MARINA S. RODRÍGUEZ-RANGEL,[1] ALYSSA D. BRAVIN,[2] KARLA RAMOS-TORRES,[2] PEDRO BRUGAROLAS,[2] JORGE E. SANCHEZ-RODRIGUEZ[1] *[1]Departamento de Física, Universidad de Guadalajara, Guadalajara, Jalisco 44430, Mexico; [2]Gordon Center for Medical Imaging, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.*

4-Aminopyridine (4AP) is a specific blocker of voltage-gated potassium channels (K_v family) that is clinically approved for the symptomatic treatment in patients with multiple sclerosis (MS). Recently, it has been shown that [^{18}F]3F4AP, a radiofluorinated form of 4AP, can be used as a PET tracer to detect demyelinated lesions in rodent models of MS (P. Brugarolas, et. al., 2018, Sci. Rep., 8:607). Here, we investigated the structure-activity relationships of novel 4AP derivatives containing methyl (-CH_3), methoxy (-OCH_3)

and trifluoromethyl (-CF_3) groups and their potential as candidates for PET imaging. We characterized the physicochemical properties of these compounds (pK_a and logD) and analyzed their ability to block KV channels in terms of the half-maximal inhibitory concentration (IC_{50}). To this end, Shaker ion channel, with inactivation removed, was expressed in *Xenopus* oocytes and voltage-clamped using cut-open voltage-clamp (COVC) methodology. Our findings show that 3-methyl-4-aminopyridine (3Me4AP), a methylated analogue of 4AP, is approximately sevenfold more potent than 4AP, whereas the methoxy and trifluoromethyl containing compounds are 3- and 4- fold less potent, respectively. The blockage of Shaker by these compounds was voltage (V) and pH dependent. IC_{50}(V) curves were analyzed with a two-step model of inhibition (A. Woodhull, 1973, JGP, 61(6):687-708; A. Hermann & A. L. F. Gorman, 1981, JGP, 78:63-86) which allowed to determine the electric distance (δ) that each 4AP analogue has to cross through the electric field generated by the channel to bind to its site. Woodhull fits produced a δ of 0.4–0.5, indicating that these analogues bind to the channel in the same location as 4AP. We conclude that 3Me4AP shows good potential for PET radiotracer development. Supported by PROSNI-UdeG 2017–18 and PRODEP-SEP-2018 Mexico to JESR, MSRR hold Fellowships from CONACyT, Mexico (886951) and NIH grant R00EB020075 to PB.

60. TRPM3 contribution to agonist-induced changes in renal blood flow **JORGE ROJO-MENCÍA,[1] LUCÍA ALONSO-CARBAJO,[1,2] PILAR CIDAD,[1] KAREL TALAVERA,[2] M. TERESA PÉREZ GARCÍA,[1] JOSÉ R. LÓPEZ-LÓPEZ[1]** *[1]Departamento de Bioquímica y Biología Molecular y Fisiología and Instituto de Biología y Genética Molecular, Universidad de Valladolid y CSIC, Valladolid, Spain; [2]Department of Cellular and Molecular Medicine, Laboratory of Ion Channel Research, KU Leuven, Leuven, Belgium*

TRPM3 is a Ca^{2+}-permeable nonselective cation channel activated by the neurosteroid pregnenolone sulfate (PS). PS contracts mouse aorta by activating TRPM3 in vascular smooth muscle cells (VSMCs). However, TRPM3 channels are absent in VSMCs from mesenteric arteries, where PS induced vasodilation by activating TRPM3 channels in sensory nerve endings. Due to these opposing effects, the physiological role of TRPM3 channels in blood pressure control remain unknown.

Using a *Trpm3* KO mouse strain and their WT controls we studied the role of TRPM3 channels activation in integrated cardiovascular responses. *Trpm3* KO mice displayed lower systemic blood pressure (BP) than WT animals, indicating that TRPM3 contributes to mean BP regulation. *Trpm3* KO mice were resistant to Angiotensin II (AngII)-induced hypertension, while in WT mice AngII-induced hypertension was accompanied by an increased in PS-induced vasodilation, suggesting that the main contribution of TRPM3 to the hypertensive phenotype may be dependent on their function in other nonvascular tissues. As TRPM3 expression is high in the kidneys, which regulate many of the systemic hypertensive effects of AngII, we explored the role of TRPM3 channels in renal blood flow and BP control. We found that *Trpm3* mRNA expression was elevated in kidney cortex and was higher in BPH kidneys. We measured renal flow in isolated, pressurized, whole kidneys obtained from normotensive (BPN) and hypertensive (BPH) mice in organ bath. We study the dose–response effect on renal blood flow to Phenylephrine (Phe) and AngII application, and the effect of PS on agonists responses. 10 uM PS attenuates Phe responses both in BPN and BPH kidneys. However, it potentiates AngII response in BPN and attenuated it in BPH kidneys. Altogether, these data suggest the involvement of renal TRPM3 channels in AngII responses, and point to differences in their functional contribution associated with the hypertensive phenotype.

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61. Determining the mechanism of mechanosensitive ion channel regulation by fatty acids **LUIS O. ROMERO, JULIO CORDERO-MORALES, VALERIA VÁSQUEZ.** ***The University of Tennessee Health Science Center. Translational Science Research Building (TSRB).***

Mechanosensitive ion channels respond to changes in bilayer deformation. These channels play a crucial role in the ability of living organisms to respond to mechanical stimuli. They are involved in physiological processes, such as: osmoregulation and cell differentiation. Fatty acids are among the membrane lipid components that dynamically regulate membrane mechanics and ion channel function. Enriching the plasma membrane with saturated fatty acid or polyunsaturated fatty acids (PUFAs) differentially modified Piezo1 channel response to mechanical stimulation (Romero et al., 2019. Nat. Commun. 10:1200). Particularly, we found that saturated fatty acid margaric acid (MA, C17:0) inhibits Piezo1 activation, C20 PUFAs enhance inactivation, and C22 PUFAs decrease inactivation. Atomic force microscopy experiments show that membranes enriched in MA are more rigid than control membranes (» 78 vs. 62 pN); whereas C20 and C22 PUFAs-enriched membranes are less rigid (» 45 pN). The discrepancy in Piezo1 inactivation observed between C20 and C22 PUFAs remains unknown. Hence, we seek to determine whether this modulation is due to protein-fatty acids specific interactions. Here, we use fatty acid supplementation and electrophysiology to study the mechanism by which the mechanical properties of the membrane modulate the

function of bona fide mechanosensitive ion channels. We are characterizing the effect of MA and C20 and C22 PUFAs in plant (Osca1.2) and prokaryotic (MscL and MscS) mechanosensitive ion channels expressed in a Piezo1-KO cell line. Thus far, we found that MA inhibits Osca 1.2, MscL, and MscS by increasing their mechanical threshold for gating. Future experiments are aimed to determine the effect of C20 and C22 PUFAs in these channels. Our current findings demonstrate that saturated and polyunsaturated fatty acids contained in the plasma membrane modulate the cell's response to mechanical cues and that this might represent a common principle for mechanosensitive ion channels of different kingdoms.

62. Experimental Evidence for an Allosteric Gating Mechanism Governing Human Calcium Channel (CaV1.2) Voltage-dependent Activation and its Modulation by a2d Subunit **JONATHAN SAAVEDRA,[1] GUIDO MELLADO,[1] GUSTAVO CONTRERAS,[1] NICOLETTA SAVALLI,[2] CARLOS GONZALEZ,[1] RICCARDO OLCESE,[2,3] ALAN NEELY1,[2]** *[1]Centro Interdisciplinario de Neurociencias de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile; [2]Division of Molecular Medicine, Dept. of Anesthesiology, [3]Dept. of Physiology, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA, USA.*

In voltage-gated Ca+2 channels the gap along the voltage axis separating charge movement from channel opening varies over a wide range according to the subunit composition as if the work of voltage sensing domains (VSDs) movement transferred to the pore gate is variable. Incorporating voltage-clamp fluorometry and a global fit strategy we developed an allosteric model to estimates the energies coupling the different VSDs to the pore in CaV1.2 (Pantazis et al., 2014, PNAS,111:18381) with and without α2δ (Savalli et al., 2016, J. Gen. Physiol. 148:147) and showed that VSDs contribute little to channel opening without α2δ and that when present, VSD II and III contributed the most. Strictly coupled models predict that the slope of log(Po) vs voltage increases asymptotically and is limited by the total number of charges contributing to channel opening. In contrast, if coupling is allosteric, at very negative voltages the slope of log(Po) will decrease to the intrinsic voltage-dependence of the pore. By increasing signal to noise ratio by 10 folds through a masking technique that remove noise in the frequency domain, we extended the conductance measurement with the cut-open-open voltage-clamp technique in below -60 mV in oocytes expressing CaV1.2 in the presence and absence of α2δ, in 20 mM Ba^{2+}.Around this voltage there is a clear reduction in the slope of log(Po) with both subunit combination with the maximum slope being larger when coexpressing α2δ. Combining tail currents measurement and ramps to cover a wide range of voltages we generated Hill plots to obtain a direct measure of the total energies coupling VSDs work to the pore and report that together VSDs contribute :200 meV to the opening CaV1.2 channel when α2δ is present. In its absence this contribution drops to less than 60 meV.

ICM-Economía P09-022-F and FONDECYT 1161672 (AN), NIH R01GM110276 (RO).

63. Membranes Matter: Predicting Drug Cytotoxicity **R. LEA SANFORD,[1] JEANNE CHIARAVALLI-GIGANTI,[2] WESLEY CHAO,[1] ANTONIO LUZ,[2] J. FRASER GLICKMAN,[2] OLAF S. ANDERSEN[1]** *[1]Department of Physiology and Biophysics, Weill Cornell Medical College, New York, NY; [2]The Rockefeller University, New York, NY*

It remains a challenge to predict whether a new drug candidate will have undesirable side effects. Here we explore a general mechanism that may cause side effects, namely that many biologically active molecules, including drugs and drug-leads, are amphiphiles that partition into lipid bilayers. This may alter bilayer physical properties, thereby causing indiscriminate changes in membrane protein function, which may lead to undesirable changes in cell function that, if large enough, may cause cytotoxicity. Thus, it may be possible to predict whether a compound will have important off-target effects based on quantitative studies of the compound's bilayer-modifying potential. Using a gramicidin-based fluorescence assay (GFA), which reports the extent of membrane perturbation caused by a compound, we have shown that many drugs and drug-leads alter lipid bilayer properties at the concentrations where these compounds become indiscriminate modifiers of membrane protein function. Such indiscriminate modifiers of membrane protein function are likely to have off target effects; we pursued this question in a blinded study on a library of 488 compounds (289 nontoxic, 199 toxic) that had been tested for cytotoxicity in "high-content" screening assays. We found that the GFA can be used to predict cellular toxicity. We also explore a computational approach to gain insight into which physicochemical parameters drive a compound's bilayer perturbing propensity. We can predict which compounds will produce bilayer-modifying effects at either extreme (i.e., little membrane effect or significant membrane perturbation). Our results support a mechanism by which amphiphiles exert their toxicity, namely by altering lipid bilayer physical properties and that in vitro measurement could be used as a warning sign for off-target biological effects in drug discovery efforts. Furthermore, this approach demonstrates a physical mechanism for a small molecule's cytotoxicity; one mediated by the lipid bilayer and can in turn be used to gain insight into the bilayer's potential role in the mechanism of action of numerous small molecules and their interactions with ion channels from multiple families.

64. Zoledronic Acid Modulation of TRPV1 Channel Currents in Differentiated Neuronal SH-SY5Y Cells: A New Possible Target for Pain-relieving Effect of Bisphosphonates SCALA ROSA,[1] MAQOUD FATIMA,[1] SCILIMATI ANTONIO,[2] TRICARICO DOMENICO [1] *[1]Section of Pharmacology, Department of Pharmacy- Pharmaceutical Sciences, University of Bari (Italy); [2]Section of Medicinal Chemistry, Department of Pharmacy-Pharmaceutical Sciences, University of Bari (Italy); emails: rosa.scala@uniba.it; fatima.maqoud@uniba.it; antonio.scilimati@uniba.it; domenico.tricarico@uniba.it phone contact 00390805442795*

Bisphosphonates (BPs) are the most used bone specific anti-resorptive agents. BPs exert antinociceptive, anti-hyperalgesic and anti-allodynic properties against noxious stimuli. Nevertheless, targets involved in these analgesic effects remain largely unknown. Conversely, an FDA alert reported an increased risk of musculoskeletal pain in patients taking BPs; mechanisms underlying this pain sensation are mostly elusive.

TRPV1 channel is actually recognized to play a key role in bone formation process; as well, it appeared to be involved in bone pain sensation. Capsaicin, a selective agonist of TRPV1 channel, is used in fibromyalgia and osteo-arthritis. In addition, new blockers of TRPV1 might reduce the pain associated with inflammatory arthritis and bone metastasis. We recently demonstrated that Zoledronic acid (ZOL) (10–100 μM) elicited stronger outward currents on preosteoblast like cells MC3T3-E1 cells and bone marrow cells, abolished by the TRP antagonist ruthenium red (10 μM) and capsazepine (Capsz) (1 μM). In excised inside-out macropatches in *Xenopus* oocytes, ZOL (50 nM) enhanced TRPV1 currents of +51.25% at +30 mV, +21.58% at +60 mV, and +32.06% at +100 mV (Vm) with respect to the current activated by capsaicin (1 μM) at the same voltages (Scala et al., Cancer 2019).

We examined the ZOL effects (100–500 μM) on TRPV1 ionic channels by patch-clamp experiments in neuronal SH-SY5Y cell lines and in TRPV1-transfected HEK293 cells. In whole-cell patch clamp experiments on neuronal SH-SY5Y cells, ZOL (100 μM) enhanced ion channel currents both at negative and positive voltages; in particular, ZOL enhanced the currents by +351.9% at +100 mV (Vm) in respect to the maximal current measured at +120 mV (Vm) in the controls. At positive membrane voltages ZOL (100 μM)- evoked currents were abolished by the application of Capsz. ZOL also activates recombinant TRPV1 channel expressed in HEK293 cells. This interaction may contribute to the well-known effects of ZOL on pain.

65. Native-state prolyl isomerization is involved in the activation of a CNG channel PHILIPP A.M. SCHMIDPETER AND CRINA M. NIMIGEAN *Weill Cornell Medicine, Department of Anesthesiology*

The cyclic nucleotide-gated channel SthK activates biphasically with cAMP application, with the slow phase reminiscent of the cAMP-induced activation of eukaryotic HCN channels. The mechanistic underpinning for this effect is elusive. Here we show that SthK employs regulatory prolyl cis/trans isomerization in the cyclic-nucleotide binding domain to slow down cAMP-induced activation kinetics and fine-tune activity. Substitution of a single Pro in SthK by Ala abolishes the slow activation phase and increases the apparent affinity of SthK for cAMP fourfold, as measured in stopped-flow assays. The same effects are observed for WT SthK in the presence of prolyl isomerases (PPIases), in a PPIase concentration-dependent way. Neither the P-A mutation nor application of PPIases affect the steady-state single-channel characteristics in planar lipid-bilayer recordings. This suggests a mechanism where two channel conformations differentiated by a Pro in cis or trans configuration exist in equilibrium: while cis Pro is favored in the apo-state, addition of cAMP shifts the equilibrium toward trans Pro in the open state. Activation of these two SthK conformations with different rates can explain the biphasic activation kinetics. Removal of the cis species in P-A SthK or addition of PPIases that help to rapidly shift the equilibrium toward trans Pro in WT, will both lead to the disappearance of the slow phase. The cryoEM structure of P-A SthK revealed subtle differences from the WT structure, suggesting that the mutant indeed adopts a preactive conformation. We propose that prolyl isomerization functions as molecular pacemaker for SthK that can be modulated by PPIases.

66. Voltage-Sensing Residues in the Voltaje Sensor of the BK Channel IGNACIO SEGURA*,[1] WILLY CARRASQUEL-URSULAEZ*,[3] KAREN CASTILLO,[1,2] YENISLEIDY LORENZO-CEBALLOS,[4] DARÍO BASAEZ,[1,2] RAMÓN LATORRE,[1,2] *[1]Centro Interdisciplinario de Neurociencias de Valparaíso, Valparaíso, Chile; [2]Universidad de Valparaíso, Facultad de Ciencias, Valparaíso, Chile; [3]University of Wisconsin-Madison, Department of Neuroscience, Wisconsin, USA; [4]Washington University School of Medicine, Department of Anesthesiology, USA. *These authors contributed equally to the work.*

Voltage- and Ca^{2+} -activated K^+ channels are modular proteins with allosteric gating. BK channels damp excitatory stimuli mediated by voltage-dependent Ca^{2+} channels, and consequently they are directly implicated in the modulation of physiological processes such as muscle contraction and neuronal excitability. To identify the voltage-sensing residues that contribute to the gating charge movement, it is necessary

to establish the molecular mechanism of the BK channel voltage-dependent activation. In this work, to determine which charged aminoacids in the transmembrane segments S1-S4 contribute to the voltaje sensitivity of the BK channel, we measured the gating currents in BK channels containing neutralization mutations on most charged residues present in the voltage sensor domain (VSD). Exclusively the neutralization of basic residues R210 and R213, present in the transmembrane S4, can decrease the voltage dependence of the BK channel voltage sensor. Furthermore, the neutralization of other charged residues in the VSD shifted the equilibrium of the active-resting states, revealing a modulating role in the free energy necessary to activate the voltage sensor. These results reveals that in BK channels the S4 segment is solely responsible for the gating charge movement.

67. Labeling and purification of BK channel for single molecule experiments **SHUBHRA SRIVASTAVA,[1] PABLO MIRANDA,[1] TERESA GIRALDEZ,[2] MIGUEL HOLMGREN[1]** *[1]National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, United States; [2]Instituto de Tecnologias Biomedicas, Universidad de La Laguna, San Cristóbal de La Laguna, Tenerife, Spain.*

BK channels are potassium channels essential for the regulation of several key physiological processes such as neurosecretion, neuronal firing and smooth muscle tone. In humans, defects in BK channels can cause hypertension, cancer and epilepsy.

BK channels are characterized by both their large single-channel conductance and their synergistic activation by Ca^{2+} and membrane depolarization. The pore-forming α subunit consist of seven membrane-spanning regions (S0–S6) and a large intracellular C-terminal domain. The voltage sensor resides within the membrane, whereas Ca^{2+} binds to sites located within the large C-terminal intracellular region where eight regulator of conductance for K^+ (RCK) domains form the "gating ring".

Using fluorescently labeled BK channels, we have shown that there are remarkably large rearrangements of the gating ring upon Ca^{2+} binding by using FRET. However, little is known about the dynamics at the single molecule level. We have begun this approach by substituting the fluorescence label CFP and YFP used in our FRET studies with self-labeling tags (SNAP and CLIP) optimized for labeling with fluorophores suitable for single molecule recordings. We have expressed these constructs in mammalian cells and labeled them with the fluorophores. Then we unroof the cells using probe sonicator to image the plasma membrane isolated from organelles and cytosolic components and look for the localization of the expressed channel by using TIRF microscopy. In parallel, we are establishing the optimal conditions for purification of the tagged BK channel protein expressed in insect cells and preparing nanodisc for stabilization of purified membrane protein. These studies set the background for future single molecule FRET experiments.

68. Antibody epitope mutations disrupt hERG deactivation and define PAS-channel interaction sites **WHITNEY A. STEVENS-SOSTRE,[1] CAROL A. HARLEY,[2] GANEKO BERNARDO-SEISDEDOS,[3] OSCAR MILLET,[3] JOÃO H. MORAIS-CABRAL,[2] GAIL A. ROBERTSON[1]** *[1]Department of Neuroscience, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA; [2]I3S-Instituto de Investigação e Inovação em Saúde and IBMC, Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; [3]Protein Stability and Inherited Disease Laboratory, CIC bioGUNE, Bizkaia Technology Park, Building 800, 48170, Derio, Spain*

The human ether-à-go-go-related gene (hERG) potassium channel conduct IKr currents, which are essential for the repolarization of the cardiac action potential. The interaction between the N-terminal Per-Arnt-Sim (PAS) domain and the C-terminal cyclic nucleotide binding homology domain (CNBhD) is an important regulator of IKr currents. Previous studies suggest that the PAS interaction is transient, as shown by biochemical and functional analyses with a single-chain variable fragment (scFv) that binds to the PAS domain. In this study, we have mapped the epitope for scFv 2.12, which binds to the globular PAS domain, using NMR and ELISA assays. Functional analyses of hERG channel mutants targeting scFv 2.12 epitope residues using two-electrode voltage clamp in *Xenopus* oocytes revealed that single-residue mutations accelerate deactivation. Thus, the scFv 2.12 antibody and its epitope may be useful to probe for conformational changes of the PAS domain and the functional effects of the PAS-CNBhD interaction on I_{Kr} currents.

69. Is the inactivation of $K_v1.2$ channels similar to C-type inactivation? **ESTEBAN SUÁREZ-DELGADO,* TERIWS G. RANGEL-SANDIN,* GISELA E. RANGEL-YESCAS,* TAMARA ROSENBAUM,[$] LEÓN D. ISLAS*** *[$]Instituto de Fisiologia Celular, UNAM; *Department of Physiology, School of Medicine, UNAM, Circuito Escolar S/N, CDMX, Mexico, 04510, tel: +52 55 56232132, leon.islas@gmail.com*

C-type inactivation has been described in multiple voltage-gated K^+ channels and in great detail in the *Drosophila Shaker* channel with fast inactivation removed. As channels have moved into the structural era atomic details of this and other gating mechanisms have started to be better understood. To date, the only voltage-gated channels whose structure has

been solved are K_VAP (x-ray diffraction), the K_V1.2- K_V2.1 "paddle" chimera (x-ray diffraction) and K_V1.2 (Cryo-EM); however, the characteristics and mechanisms of slow inactivation in these channels are unknown or poorly characterized. Here we present a detailed study of slow inactivation in the rat K_V1.2 and show that it has some properties consistent with the C-type inactivation described in Shaker. We also study the effects of some mutations that are known to modulate C-type inactivation in *Shaker* and show that qualitative and quantitative differences exist in their functional effects, possibly underscoring subtle but important structural differences between the C-inactivated states in *Shaker* and K_V1.2.

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70. Developmental changes in subunit stoichiometry alter hERG channel kinetics and magnitude **CHIAMAKA U. UKACHUKWU,[1] ABHILASHA JAIN,[1] ANDRÉ MONTEIRO DA ROCHA,[2] DAVID K. JONES[1]** *[1]Department of Pharmacology, University of Michigan, Ann Arbor, MI; [2]University of Michigan Frankel Cardiovascular Regeneration Core, Ann Arbor, MI*

At least two subunits combine to form a functional hERG potassium channel, hERG 1a and hERG 1b. Mutations in hERG are linked to sudden infant death syndrome (SIDS) and intrauterine fetal death. In human cardiac tissue, hERG subunit mRNAs are developmentally regulated, suggesting that hERG current (IKr) may similarly affected. However, little is known about the relationship between hERG subunits, IKr, and cardiac excitability in the developing human heart. Human induced pluripotent stem cell-derived cardiomyoctes (hiPSC-CMs) are an increasingly useful model of developmental cardiac physiology. The University of Michigan Frankel Cardiovascular Regeneration Core (UMFCRC) recently demonstrated that culturing the hiPSC-CMs on polydimethylsiloxane (PDMS) substrate enhances hiPSC-CM maturation compared with glass cultured hiPSC-CMs. We partnered with the UMFCRC and used PDMS maturation of hiPSC-CMs to characterize changes in hERG subunit stoichiometry during cardiac maturation. We differentiated hiPS cells into CMs and measured hERG subunit mRNA, hERG subunit fluorescence, and IKr from hiPSC-CMs cultured on either PDMS (matured) or glass (immature). We differentiated hiPS cells into CMs and measured hERG subunit mRNA, hERG subunit fluorescence, and IKr from hiPSC-CMs cultured on either PDMS (matured) or glass (immature). hERG 1b mRNA, measured by qRT-PCR, was significantly reduced in matured cardiomyocytes compared with immature cells. Conversely, hERG 1a mRNA was elevated in matured cells, although this effect was not statistically significant (P = 0.06). These data recapitulate a previous report from human tissue, and further validate PDMS-driven maturation as a model of cardiac maturation. We also observed the same trend on hERG subunit protein levels using subunit-specific immunofluorescence. In matured hiPSC-CMs hERG 1a fluorescence was significantly increased whereas hERG 1b fluorescence was significantly decreased. To test whether the changes in hERG 1a and 1b fluorescence corresponded with altered IKr kinetics we recorded E-4031-sensitive currents, indicative of IKr, from mature and immature hiPSC-CMs. Maturation on PDMS substrate significantly slowed IKr kinetics and increased peak tail IKr density compared with IKr in immature cells. Additional recordings using a voltage command that mimics a human ventricular action potential showed that the slowed IKr kinetics in matured cells reduced the repolarizing charge conducted during a cardiac action potential. These data clearly demonstrate that hERG subunit stoichiometry and IKr behavior are developmentally regulated in human cardiomyocytes.

71. Molecular determinants of the TRPC6 channel association with VAPA endoplasmic reticulum contact proteins **ARIELA VERGARA-JAQUE,[1,3] ALHEJANDRA ÁLVAREZ,[2,3] JAVIER DÍAZ,[1] HORACIO POBLETE,[1,3] WENDY GONZÁLEZ,[1,3] IAN SILVA,[2,3] BORIS LAVANDEROS,[2,3] OSCAR CERDA[2,3]** *[1]Center for Bioinformatics and Molecular Simulation (CBSM), Universidad de Talca, Talca, Chile; [2]Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, Santiago, Chile; [3]Millennium Nucleus of Ion Channels-Associated Diseases (MiNICAD). E-mail:arvergara@utalca.cl*

TRPC channels are broadly distributed in human tissues and involved in diverse pathological conditions, such as neurological and cardiovascular disorders, kidney function, and cancer. Important advances have been performed to understand the mechanisms that regulate the activity of these channels. In this regard, protein-protein interactions have recently emerged as promising pharmacological targets. Here, we evaluate the association of the vesicle-associated membrane protein-associated protein A (VAPA) and the transient receptor potential cation 6 channel (TRPC6). VAPA is an endoplasmic reticulum resident protein involved in vesicle trafficking, membrane fusion, protein complex assembly, and cell motility. Through a mass spectrometry-based proteomics approach, we identified VAPA as a novel TRPC6-interacting protein. In addition, TRPC6-VAPA association was validated by coimmunoprecipitation assays in HEK293 cells. In humans, VAPA has shown to bind proteins containing a FFAT motif essential for its function. Interestingly, TRPC6 exhibits a putative FFAT motif in the N-terminal domain that might mediate the binding with VAPA. To characterize this particular interaction, we refined the recently revealed TRPC6 crystal structure adding, by ab initio modeling, the missing residues surrounding the putative FFAT motif. Proteinprotein docking

were then performed with the VAPA structure, identifying as contact region a set of residues previously found in the binding interface between VAPA and other FFAT-containing proteins. Molecular dynamics simulation of the TRPC6:VAPA complex were carried to describe the specific interactions modulating that association. The trajectories reveal polar contacts between negative charged residues surrounding the putative FFAT motif in TRPC6 and positively charged residues in VAPA. We hypothesize that this interaction constitutes a mechanism to regulate the TRPC6 trafficking to plasma membrane, serving VAPA as a novel TRPC6-regulatory protein. To our knowledge, this is the first study reporting TRPC channels to be modulated by endoplasmic reticulum contact proteins.

72. Neurodegeneration in Niemann-Pick Type C Disease is Associated with Reduced KCNQ2/3 Current and Hyper-Excitability of Sympathetic Neurons **OSCAR VIVAS,[1,2] SCOTT A. TISCIONE,[1] ROSIE E. DIXON,[1] DANIEL S. ORY,[3] EAMONN J. DICKSON[1]** *[1]Department of Physiology and Membrane Biology, University of California, Davis, California, 95616; [2]Department of Physiology and Biophysics, University of Washington, Seattle,Washington, 9819, email: vivas@uw.edu, phone: +1 (206) 454–9324; [3]Department of Internal Medicine, Washington University School of Medicine BJC, St. Louis, Missouri,63110.*

KCNQ2/3 channels control the intrinsic electrical excitability. In peripheral sympathetic neurons, the opening of KCNQ2/3 channels leads to a phasic firing, and their closing leads to a tonic firing of action potentials. Neurotransmitters and neuromodulators control the opening and closing of KCNQ2/3 channels through the activation of G-protein coupled receptors that bind Gαq proteins, resulting in the hydrolysis of PtdIns(4,5)P2. Hence, the amount of PtdIns(4,5)*P2* is critical to determine the level of excitability of sympathetic neurons. In this project, we tested the hypothesis that reduced levels of PtdIns(4,5)*P2*, observed in Niemann-Pick type C disease, leads to the over-activation of sympathetic neurons via a reduction of KCNQ2/3 current. We used three models to mimic Niemann-Pick type C disease (NPC1): pharmacological inhibition of NPC1 protein, deletion of NPC1 gene, and mutation of NPC1 gene, which leads to degradation of 80% of NPC1 protein. We found that neurons from all three NPC1 models exhibit 50% of KCNQ2/3 current density. Less current correlated only with less PtdIns(4,5)*P2* and not with an alteration in the abundance of channels measured by Westernblot. Current density was rescued by the application of exogenous PtdIns(4,5)*P2*, supporting the hypothesis that the less PtdIns(4,5)*P2* underlies a reduction of KCNQ2/3 current in NPC1 disease. Moreover, these neurons exhibited a more depolarized resting membrane potential, were more prone to fire action potentials, and showed a tonic firing pattern. We propose that neurodegeneration in Niemann-Pick type C disease is caused by a reduction of PtdIns(4,5)*P2* leading to an alteration in the intrinsic electrical excitability of neurons expressing KCNQ2/3 channels.

73. Conformational Changes of the Na^+-pump Alpha Subunit's First External Loop **VICTORIA YOUNG AND PABLO ARTIGAS** *Department of Cell Physiology and Molecular Biophysics, Texas Tech University Health Sciences Center 3601 fourth St. Lubbock, TX 79430. pablo.artigas@ttuhsc.edu (806)368-1901*

The Na^+-pump builds the Na^+ and K^+ gradients that are essential for excitability. It is formed by a 10 transmembrane segments (TM1-TM10) catalytic α-subunit and an auxiliary, single-TM β-subunit. During its cycle, the pump alternates between phosphorylated and dephosphorylated forms of two major conformations: E1 and E2. The conformation-dependent movement of the external-most part of the α-subunit's TM1-TM2 region was studied with voltage-clamp fluorometry (VCF). One at a time, each residue from TM1's Q116 to TM2's D126 was mutated to cysteine, expressed in *Xenopus* oocytes, tagged with tetramethylrhodamine-6-maleimide (TMRM), and functionally evaluated with two-electrode VCF. All TMRM-tagged residues showed conformation-dependent fluorescence changes. In the presence of Na^+_o, without K^+, the pump produces voltage-dependent transient currents as it transits from ion-free E2 states at positive voltages to Na^+-occluded E1 states at negative potentials. Under these conditions, all but A117C-TMRM showed large voltage-dependent fluorescence signals (ΔF/F~2–15%) that were both, slower than the transient currents, and inhibited by the specific inhibitor ouabain. The centers of the Boltzmann distributions describing DF/F-voltage and charge-voltage curves matched only for some residues. Additionally, application of K^+ or ouabain altered the steady-state fluorescence at -50 mV. Taken together, these results suggest complex conformational changes in TM1-TM2. To determine the extent of TM1-TM2 movement, TMRM was attached to the static residue R977C between TM9 and TM10, and concomitantly, a tryptophan residue was introduced, one at a time, from Q116 to L134 within TM2. When located within 5.5 Å of the TMRM fluorophore Trp quenches its fluorescence. In Na^+_o-only conditions ΔF/F was observed only in TM2, from Q124W to V132W, with quenching happening at positive voltages (E2-states) and unquenching at negative voltages (E1 states), indicating that TM1-TM2 moves significantly in the inward direction w6hen moving to E1. Fluorescence changes under other conditions are under way. NSF-MCB1515434.

74. Rational design of Hv1 inhibitors reveals a new druggable pocket in a voltage-sensing domain **CHANG ZHAO,[1] LIANG HONG,[1] SALEH RIAHI,[2] JASON D. GALPIN,[3] CHRISTOPHER A. AHERN,[3] DOUGLAS J. TOBIAS,[2] FRANCESCO TOMBOLA[1]** *[1]Department of Physiology and Biophysics, University of California, Irvine, CA 92697; [2]Department of Chemistry, University of California, Irvine, CA 92697; [3]Department of Molecular Physiology and Biophysics, University of Iowa, Iowa City, IA 52242*

Voltage-gated sodium, potassium, and calcium channels play critical roles in excitable tissues, from the generation and propagation of action potentials to synaptic transmission and muscle contraction. As a result, they are pharmacological targets for the treatment of a variety of diseases. They consist of four voltage-sensing domains (VSDs) surrounding a central pore domain. While many types of organic molecules bind pore domains, the number of molecules known to bind VSDs is limited. The Hv1 channel is made of two VSDs and does not contain a pore domain, providing a simplified model for studying how small ligands interact with VSDs. We previously identified a binding site for arginine-mimic compounds in the center of the Hv1 VSD, which is accessible only when the proton-conduction pathway is open. Based on a rational-design approach, we have developed a new generation of arginine mimics, named HIFs, able to interact with an additional binding pocket within the VSD intracellular vestibule. HIFs can reach the binding pocket even when the proton-conduction pathway is closed. Once inside the pocket, they become trapped and can only be released over extended periods of time (several minutes). We used electrophysiological measurements, combined with kinetic modeling, molecular docking, and atomistic simulations to determine the location and composition of the pocket. Our findings suggest that similar binding sites could be found in the VSDs of other channels and exploited for drug development.

75. The Molecular Mechanisms of TRMP3 Channel Regulation **SIYUAN ZHAO[1], TIBOR ROHACS[1]** *[1]Department of Pharmacology, Physiology & Neuroscience, School of Graduate Studies, Rutgers University*

Transient Receptor Potential Melastain 3 (TRPM3) ion channel belongs to the Transient Receptor Potential (TRP) superfamily. As a member of thermoTRP family, TRPM3 channel has been identified as a thermosensitive nociceptor channel which can detect noxious heat. TRPM3 knockout mice show impairment in avoiding noxious heat and have defects in developing inflammatory heat hyperalgesia. Therefore, understanding the regulation of TRPM3 channels are crucial and meaningful. Recently published papers show TRPM3 channels can be regulated by G**ßγ** subunits but the mechanism of this regulation hasn't been clarified yet. In the first part of our research, we will focus on the molecular mechanisms of TRPM3 regulation by G**ßγ** subunits. We will use Xenopus oocytes system and HEK cells system to identify molecular determinants of TRPM3 regulation. In addition to G**ßγ** subunits, the activity of TRPM3 channels is also dependent on Phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P2). Therefore, we want to explore how PtdIns(4,5) P2 could regulate TRPM3 activities. Since PtdIns(4,5)P2 is also a downstream target of GPCR signaling, we also intend to test whether there is interplay between the G**ßγ** subunits regulation and Phosphoinositides regulation of TRPM3 channels. Our research has identified that Gβ1-I80, W99, M101 ,L117, D228 are very important for TRPM3 regulation of G**ßγ** subunits. Also TRPM3-N991, K992 AND R1131 are possible interacting residues of PtdIns(4,5)P2 and mutating either of them could make channels become more sensitivity to PtdIns(4,5)P2 depletion. Besides of it, interrupting putative binding sites could also make TRPM3 channels more susceptible to the inhibition caused by G**ßγ** subunits. The long-term goal of this project is to clarify the molecular mechanisms of TRPM3 regulation and provide more insights about TRPM3 regulations and GPCR signaling.

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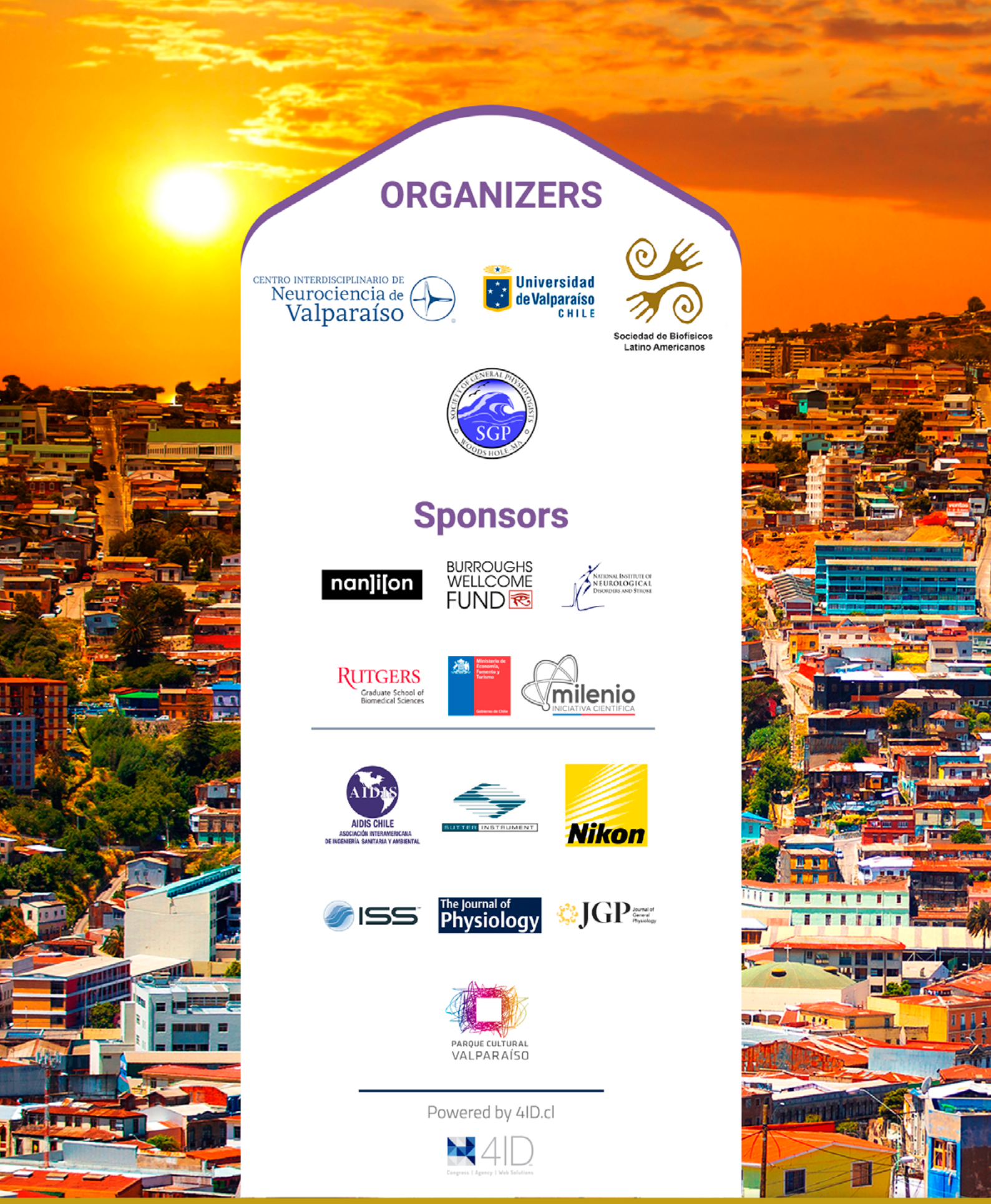

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