Wednesday 19

13,00 -14,30 **LUNCH**

14,45 - 15,00 **WELCOME**

Session 1

15,00 - 15,30	David Jones	**S1**
15,30 - 16,00	Marcelo Catalan	**S2**
16,00 - 16,30	Diego Alvarez de la Rosa	**S3**

16,45 - 17,15 **Coffee break**

17,15 - 17,45	Carlos Flores	**S4**
17,45 - 18,15	Rodolfo Madrid	**S5**
18,15 - 18,45	Sebastián Brauchi	**S6**

Plenary Lecture I

19,00 - 19,45	Brian Harvey	**PL1**

DINNER, BBQ, DRINKS!

Thursday 20

Session 2

09,15 - 09,45	Rodolfo Briones	**S7**
09,45 - 10,15	Rocio Finol-Urdaneta	**S8**
10,15 - 10,45	Simon Scheuring	**S9**

10,45 - 11,20 **Coffee break**

Data Blitz

11,20 - 11,30	Daniel Bustos	**DB1**
11,30 - 11,40	Constanza Blanco	**DB2**
11,40 - 11,50	Deny Cabezas	**DB3**
11,50 - 12,00	Génesis Vega	**DB4**
12,00 - 12,10	Boris Lavanderos	**DB5**
12,10 - 12,20	Elia Matamala	**DB6**
12,20 - 12,30	José Rivas	**DB7**
12,30 - 12,40	Rodrigo Ceballos	**DB8**
12,40 - 12,50	Lisandra Flores	**DB9**
12,50 - 13,00	Patricio Cuevas	**DB10**

13.00 **LUNCH**

Session 3

15,00 - 15,30	Teresa Giraldez	**S10**
15,30 - 16,00	Brad Rothberg	**S11**
16,00 - 16,30	Andrea Meredith	**S12**
16,30 - 17,00	Juan Pablo Castillo	**S13**

16,30 - 17,00 **Coffee break**

Plenary Lecture II

17,00 - 17,45	Ramón Latorre	**PL2**

19,00 - 20,00 Concert at the Church

DINNER

Friday 21

Session 4

9,00 - 09,30	Jeffrey McArthur	**S14**
09,30 - 10,00	María Pertusa	**S15**
10,00 - 10,15	Dana Morales	**OP1**

10,15 - 10,45 **Coffee break**

10,45 - 11,15	Diego Varela	**S16**
11,15 - 11,30	Bastián Rivera	**OP2**

Plenary Lecture III

11,30 - 12,20	Gerard Zamponi	**PL3**

12,30 **LUNCH**

Session 5

14,30 - 15,00	Gail Robertson	**S17**
15,00 - 15,30	Robert Tarran	**S18**
15,30 - 15,45	Jimena Canales	**OP3**
15,45 - 16,00	Leandro Zúñiga	**OP4**
16,00 - 16,15	Claudio Coddou	**OP5**

16,15 - 16,45 **Coffee break**

Session 6

16,45 - 17,15	Scott Ramsey	**S19**
17,15 - 17,45	Crina Nimigean	**S20**
17,45 - 18,15	Wendy Gonzalez	**S21**
18,15 - 18,45	Chris Ahern	**S22**

Plenary Lecture V

19,00 - 19,45	Werner Treptow	**PL4**

Saturday 22

Session 7

09,30 - 10,00	Baron Chanda	**S23**
10,00 - 10,30	Robert French	**S24**
10,30 - 10,45	David Ramírez	**OP6**

10,45 - 11,15 **Coffee break**

Session 8

11,15 - 11,45	Alessio Accardi	**S25**
11,45 - 12,00	Iván Ruminot	**OP7**
12,00 - 12,15	Janin Riedelsberger	**OP8**
12,15 - 12,30	Amber Philp	**OP9**
12,30 - 12,45	Gaspar Peña-Muzenmeyer	**OP10**

What about the outreach?

12,45-13,00	Omar Porras	**EA**

Wednesday 19

Animal Models in Physiology and Disease.

S1

Targeting Intracellular hERG Domains as a Potential Therapeutic Approach for Impaired Repolarization.

David K. Jones[1], Carol Harley[2], Greg Starek[1], Tony Amolo[1], Andrei S. Fernandes[2], Joao Morais-Cabral[2], Gail A. Robertson[1].

1University of Wisconsin – Madison; 2Instituto de Biologia Molecular e Celular.

The human ether-à-go-go-related gene (hERG) potassium channel contributes to cardiac action potential repolarization. Reduced hERG current underlies long QT syndrome (LQTS) and is associated with cardiac arrhythmia and sudden cardiac death. In cardiomyocytes, hERG channels comprise at least two subunits, hERG 1a and hERG 1b. The hERG 1a contains an N-terminal Per-Arnt-Sim (PAS) domain that is missing in hERG 1b subunits. The PAS domain serves an inhibitory function, reducing current by slowing activation and promoting inactivation. In addition, the PAS domain slows deactivation. We developed two antibody fragments that recognize distinct epitopes on the PAS domain. In heteromeric hERG channels expressed in hEK293 cells, the antibody fragments accelerate deactivation but have different effects on channel activation, inactivation, and current hysteresis, a reporter of voltage sensor relaxation. Importantly, despite their differential actions on gating, both antibodies increase repolarizing current during a ventricular action potential and restore normal action potential duration in a model of acquired long QT syndrome in cardiomyocytes. These data demonstrate that PAS subdomains can be differentially manipulated, and represent a potential therapeutic target by which normal action potential duration may be restored in cases of impaired repolarization.

S2

K^+ secretion by the acinar epithelium in the mouse submandibular gland.

Marcelo A. Catalán[1,2], Yasna Jaramillo[1,2], Catherine E. Ovitt[3] & James E. Melvin[2].

1 Facultad de Ciencias de la Salud, Universidad Arturo Prat, Iquique, Chile, 2 Mechanisms and Dysfunction Section, Division of Intramural Research, National Institute of Dental and Craniofacial Research, National Institute of Health, Bethesda, MD 20892, 3 Center for Oral Biology, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, New York 14642

K^+ concentration in saliva is greater than in plasma, suggesting that there is a molecular mechanism by which K^+ ions are actively secreted. Classical micropuncture experiments performed on salivary glands have showed that K^+ secretion takes place in duct as well as acinar epithelium, but the later mechanism has received less attention. Here, using functional and immunolocalization studies performed on the mouse submandibular gland (SMG), we show that the amount of K^+ secreted is intimately linked to the fluid secretion process by the acinar epithelium. Interestingly, we found that KCa1.1, the K^+ channel involved in K^+ secretion, co-expresses with Nkcc1 in both acinar and a subset of ducts cells, raising the possibility that either acinar or duct KCa1.1 Nkcc1-expressing cells are responsible for K^+ secretion. In order to elucidate whether acinar and/or ductal KCa1.1 and Nkcc1 expressing-cells secrete K^+, we used a mouse model where KCa1.1- and Nkcc1-expressing duct cells were ablated by targeted expression of the diphtheria toxin. In this model, ex vivo SMG K^+ secretion was essentially unaffected. Together, our data strongly suggest that under stimulation, the acinar epithelium plays an essential role in the K^+ secretion process via KCa1.1 and Nkcc1.

Acknowledgments: FONDECYT 1171135.

S3

Mechanisms underlying decreased seizure susceptibility in SGK1.1 transgenic mice.

N. Armas[1], L. Pérez[2], A. Reboreda[3], Diego Alvarez de la Rosa[1], L.C. Barrio[2] and T. Giraldez[1].

1 . Facultad de Medicina. Universidad de La Laguna, Tenerife. 2 . Hospital Ramón y Cajal, Madrid. 3 . Facultad de Biología. Universidad de Vigo, Pontevedra.

SGK1.1 a neuronal isoform of the ubiquitous expressed serum- and glucocorticoid- regulated kinase 1 (SGK1) has been proposed by our group as a physiological regulator of M-channels, members of the Kv7/KCNQ gene family that control membrane resting potential and excitability (Miranda et al., 2013). We have found that SGK1.1 upregulates the Kv7.2/3 current in heterologous expressions systems. Superior cervical ganglion (SCG) neurons isolated from transgenic mice expressing a constitutively active form of SGK1.1 (Tg.sgk) showed a significant increase in M-current levels, paralleled by reduced excitability and more negative resting potentials. We have now assessed seizure susceptibility using the kainic acid (KA) model by either behavioral observation or EEG recordings. Seizure severity was evaluated according to previous scale (Racine 1972). Wild types mice showed higher rates of severe clonic seizures and 40% of mortality in contrast to reduced seizure activity and no mortality in Tg.sgk. Electroencephalographic studies were made to record changes in electrical activity in the hippocampus and cortex after systemic administration of KA. Analysis of the EEG data revealed that the Tg.sgk displayed a significant lower number of ictal episodes following KA application. The number of ictal discharges, and their total time spend in ictal seizure was significantly reduced when compared with wild type, with no difference in seizure onset. Ours finding reveals that SGK1.1 is involved in the maintenance and ending of seizures. Whole cell recordings in Tg.sgk hippocampal slices are underway to study the involvement of SGK1.1 in counteracting hyperexcitability.

Miranda P. et al. (2013).. J. Neurosci. 2013, 33:2684. Racine RJ (1972) Electroencephalogr Clin Neurophysiol 32:281–294.

Funding: Spanish Ministry of Economy and Competitivity, Grant BFU-2015-66490-R.

S4

Role of KCa3.1 potassium channels in airway epithelum.

Génesis Vega[1,2], Amber R. Philp[1,2], Ambra Gianotti[3], Karla Drogett[4], Mariana Rios[4], Paolo Scudieri[5], Luis J Galietta[5], O Zegarra-Moran[3], M Villalón[4], Carlos A. Flores[1].

1. Centro de Estudios Científicos (CECs). Valdivia, Chile; 2. Universidad Austral de Chile, Valdivia, Chile; 3 U.O.C. Genética Medica, Instituto G, Gaslini, Génova, Italy; 4 Universidad Católica de Chile, Facultad de Ciencias Biológicas, Santiago, Chile. 5 TIGEM, Puzzuoli, Italy. cflores@cecs.cl

Ubiquitously expressed in mammalian cells, the KCa3.1 channel regulates several functions including gene expression, migration, granules release, transepithelial transport and cell volume regulation. The channel has been found expressed in the basolateral membrane of airway epithelial cell from rat and its pharmacological inhibition proved to diminish lung inflamatory features as those observed in asthma in animal models, nevertheless the exact function of KCa3.1 in epithelial cells of the airways has not been explored in detail.

Using a KCa3.1-null animal (the *Kca3.1*$^{-/-}$ mouse) we observed that transepithelial voltage and sodium absorption were significantly reduced in freshly isolated tracheas mounted in Ussing chambers. Diminished sodium absorption was not due to changes in the expression of ENaC sodium channel subunits. We also observed that ciliary beating frequency is significantly increased in airway epithelial cells isolated from the *Kca3.1*$^{-/-}$ mouse leading us to hypothetize that the inhibition of the channel might favour the mucociliary clearence of the airways. Finally, we tested if the pharmacological inhibition of KCa3.1 in human bronchial epithelial cells (HBEC) can mimic some of the observed features that we described in the mouse model. Ussing chamber experiments demonstrated that HBEC exhibit a siginificant decrease of sodium absorption and reduced Il4-induced goblet cell metaplasia when incubated with TRAM-34 (300 nM).

These results suggest an important and novel role for KCa3.1 epithelial function. Our results demonstrate that KCa3.1 inhibition reduces sodium absorption in both mouse and human epithelium and might be explained by changes in cell membrane potential that do not favour the electrochemical gradient for sodium entry. In addition, the inhibition of KCa3.1 increased CBF, which could benefit MCC. The mechanistics of such increase are being explored. Inhibition of Il-4 induced goblet cell metaplasia indicates that KCa3.1 function is not exclusively restricted to maintenance of electrochemical gradients for movement of ions but is also part of signalling of relevant cytokines. In summary, inhibition of KCa3.1 could be suggested as a therapeutic strategy for the management of inflammatory lung diseases.

Funded by FONDECYT 1151142 and CONICYT BFP.

S5

Role of the excitability brake potassium current IKD in cold allodynia induced by chronic peripheral nerve injury

González, A.[1], Ugarte, G.[1], Restrepo, C.[1], Herrera, G.[2]; Gómez-Sánchez, J.[3], Piña, R.[1]; Pertusa, M.[1], Orio, P.[2] and Madrid, R.[1]

1Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile. 2Centro Interdisciplinario de Neurociencia de Valparaíso (CINV), Facultad de Ciencias, Universidad de Valparaíso. 3University College London, Cell and Biology Department, Gower Street, W1CE 6BT London, UK. E-mail: rodolfo.madrid@usach.cl

Cold allodynia is a common form of neuropathic pain induced by peripheral nerve injury. The mechanisms underlying this disabling sensory alteration are not entirely understood. In primary somatosensory neurons, cold-sensitivity is determined by a functional counterbalance between cold-activated TRPM8 channels and Shaker-like Kv1.1-1.2 channels underlying the excitability brake current IKD. This fast-activating and slow-inactivating outward voltage-dependent K+ current dampens the effect of the cold-induced depolarizing TRPM8-dependent current, shifting the cold threshold of the neuron to lower temperatures. IKD exerts its action at membrane potentials subthreshold to the action potential firing, reducing the excitability of the neuron and preventing an unspecific activation by cold of normally cold-insensitive neurons of other somatosensory modalities. We have studied the role of IKD in cold allodynia, using chronic constriction injury (CCI) of the sciatic nerve as a model of axonal damage. We found that the proportion of CSNs in CCI animals was larger compared to sham mice, and that their mean cold threshold was shifted ~2.0°C to higher temperatures. IKD density was reduced in high-threshold CSNs from CCI mice compared to sham animals, with no differences in TRPM8-dependent current density. The electrophysiological properties and neurochemical profile of CSNs revealed an increase of nociceptive-like phenotype among neurons from CCI animals compared to sham mice. Mathematical modeling of CSNs shows that a reduction in IKD density shifts the thermal threshold to higher temperatures, and that the reduction of this current induces cold-sensitivity in former cold-insensitive neurons expressing low levels of TRPM8-like current. Taken together, our results suggest that the transformation of high-threshold CSNs and nociceptors into neurons sensitive to innocuous and mild cold respectively, due to a reduction of IKD density induced by CCI in TRPM8-positive neurons, would be part of the main neural and molecular mechanisms that underpin this sensory alteration induced by peripheral nerve injury.

Supported by Grants FONDECYT 1161733 (RM), 1130862 (PO), 11130144 (MP), 3150431 (AG) and CONICYT ACT-1113 (RM, PO, MP, GU).

S6

Old dogs can't learn new tricks. Insights on the evolution of TRP channels.

Sebastian Brauchi.

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Sensory modalities are essential for navigation through an ever-changing environment. From insects to mammals and members of the Transient Receptor Potential (TRP) superfamily TRP channels are known

mediators for cellular sensing. TRP superfamily of polymodal cation channels contribute to the human genome with 28 known genes divided into six subfamilies based on amino acid sequence homology (TRPA1, TRPC1-7, TRPM1-8, TRPV1-6, TRPML1-3, and TRPP1-3). TRP channel proteins are widely expressed in many tissue and cell types, playing critical roles in cellular and environmental sensing. It has been proposed that unicellular organisms, including the eukaryotic unicells Chlamydomonas, Paramecium, Dictyostelium and Plasmodium may express evolutionary precursors of modern mammalian TRP channels. Unicellular organisms offer an invaluable tool for defining the function of proteins in vivo, and provide a model to study evolutionary origins and primordial design of proteins.

Here we present a proteome wide search of sequences sharing similarity with mammalian TRP channels using unicellular and multicellular organisms spanning bacteria, archaea, protazoa, algae, fungi, invertebrate and fish evolutionary branches. We have identified few homologues of mammalian TRP channels associated or related to members of A, C, M and V subfamilies in all but the prokaryotic lineage. Furthermore, phylogenetic and sequence similarity network analyses suggest that TRP channels diverged from a common ancestor and an early segregation originates the modern TRPML and TRPP subset. Although TRP channels seem to be absent in plants, *Chlamydomonas r.* possesses genomic sequences encoding for TRP proteins. We cloned and described CrTRP1, a TRP channel from *Chlamydomonas r.* displaying several functional and structural properties now segregated in different TRP channel families. Our studies suggest that basic TRP gating characteristics evolved early in the history of eukaryotes.

Plenary Lecture I.

PL1

Sexual dimorphism of ion channels in health and disease.

Brian Harvey.
Department of Molecular Medicine, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin 9, Ireland. bjpharvey@rcsi.ie

There is increasing interest in sexual and gender dimorphism in health, aging and disease. Sex differences are a known contributory factor in diseases affecting the cardiovascular, respiratory, renal, GI and immune systems. The sexual dimorphism (genetic and physiological factors) and gender differences (population, behaviour and environmental influences) can complicate physiological studies and clinical trials with compounding and opposing impacts. Over the past 25 years, our laboratory has been investigating the sexual dimorphism of estrogen actions outside the reproductive system to determine the molecular basis of sex differences in physiological conditions – hepatic cholesterol synthesis, glucose metabolism, intestinal and renal electrolyte transport, and under pathophysiological conditions where clear sex differences exist such as in colon and lung cancer and in cystic fibrosis.

Ion channels and transporters are among the major targets of estrogen to effect female sex-specific actions in diverse organs to modulate whole body electrolyte and fluid balance. These responses involve both rapid 'non-genomic' and latent genomic transcriptional events which inhibit chloride ion transport via CFTR in the lung and intestine to produce and anti-secretory response while stimulating sodium absorption via ENaC in the kidney and intestine to cause a pro-absorptive response. These effects of estrogen are observed in female tissues only and are most potent during the estrous cycle when circulating plasma estrogen is at its highest. The end result of the anti-secretory and pro-absorptive effects of estrogen is an increase in whole body extracellular fluid volume which is necessary for endometrial expansion and blastocyst implantation.

In the CF lung where secretion is already compromised through the lack of function of CFTR, the anti-secretory and pro-absorptive effects of estrogen only serve to exacerbate lung function in female CF patients – the CF Gender Gap. Estrogen causes the CF gender gap by lowering the height of the airway surface liquid, inhibiting mucociliary clearance and converting Pseudomonas aeruginosa to a mucoid phenotype.

The molecular target of estrogen's anti-secretory actions in the intestine is the KCNQ1:KCNE3 channel. We have recently discovered that this K^+ channel is complexed with β-catenin at the plasmamembrane of colonic epithelial cells and the bidirectional interactions between KCNQ1:β-catenin modulate colorectal cancer (CRC) cell differentiation, proliferation, growth and invasion. Moreover CRC patient survival is positively correlated with KCNQ1:KCNE3 expression independent of tumor stage, indicating a potential diagnostic and therapeutic role for this K^+ channel.

Thursday 20

New tools for ion channel research.

S7

Of channel conductances and lipid–protein interactions. A trip integrating electrophysiology, molecular dynamics simulations and electron crystallography.

Rodolfo Briones[1,*], Camilo Aponte-Santamaría[2], and Bert L. de Groot[1].
1Computational Biomolecular Dynamics Group, Max-Planck Institute for Biophysical Chemistry, Göttingen, Germany; 2Molecular Biomechanics Group, Heidelberg Institute for Theoretical Studies, Heidelberg, Germany. e-mail: rbrione@gwdg.de

Lipid sorting, hydrophobic matching, and protein oligomerization are some of the key principles by which lipids and proteins organize in biological membranes. How lipids modify membrane protein function and viceversa, can be studied with molecular dynamics (MD) simulations. Examples of a porin and a water channel are shown. The Voltage-Dependent Anion Channel 1 (VDAC-1) is an important porin of the outer mitochondrial membrane that is involved in the energy metabolism and apoptosis. We performed MD simulations and single-channel experiments of VDAC-1, which show agreement for the current-voltage relationships of an open channel. Within a range of ±400 mV, we observed the sub-microsecond voltage-dependent asymmetric distortions of the VDAC-1 whole structure and the motion of key gating amino acids. This evidence supports some VDAC gating hypotheses, where VDAC–membrane interaction might play a role (1).

The eye-lens specific Aquaporin-0 channel (AQP0) solved by electron crystallography (EC) is one of the few protein-lipid complexes available in atomic detail. AQP0 self assembles into a squared 2D lattice in native and model membranes. By simulating an AQP0-DMPC bilayer system under different protein and lipids mobility conditions, we found that the protein mobility strongly influenced the lipid localization. This lipid localization induced particular fluid- and gel-like areas around AQP0. Lipid stretching was the mechanism that compensated the lipid–protein hydrophobic mismatch. These protein induced lipid changes suggest a mechanism how the protein surface may recruit lipids and interact with other AQP0 copies.

The VDAC and AQP0 systems show how protein–lipid interfaces might play a role in regulating the voltage dependence or inducing specific fluid- or gel-phase prone lipid areas. The presence of these areas might guide the lipid sorting interactions with other membrane components, including protein oligomerization and lipid domains formation (2).

1. Briones, R., C. Weichbrodt, L. Paltrinieri, I. Mey, S. Villinger, K. Giller, A. Lange, M. Zweckstetter, C. Griesinger, S. Becker, C. Steinem, and B. deGroot, 2016. Voltage Dependence of Conformational Dynamics and Subconducting States of VDAC-1. Biophys J 111:1223–1234. 2. Briones, R., C. Aponte-Santamar´ıa, and B. L. de Groot, 2017. Localization and ordering of lipids around Aquaporin-0: Protein and lipid mobilityeffects. Frontiers in Membrane Biophysics In Review.

S8

Reverse engineering of heteromeric Kv channels.

Rocio K. Finol-Urdaneta[1], Jeffrey R. McArthur[1], Heinrich Terlau[2], Robert J. French[3], Baldomero M. Olivera[4].

1University of Wollongong, Australia; 2Christian-Albrechts-Universität, Germany; 3University of Calgary, Canada; 4University of Utah, USA.

It has long been recognized that voltage-gated potassium channels (Kvs) play a fundamental role in cell signaling by modulating the rate of return to the resting membrane potential after action potential firing in excitable cells. Kv channels form heteromeric complexes with nuanced electrophysiological properties. The vast array of Kv channel alpha and accessory subunits described to date, and the wide range of expression profiles among them, have rendered nearly impossible the identification of cell-specific pharmacological targets. Conotoxins are invaluable pharmacological tools for studying ion channels. However, traditional screenings have focused on homotetrameric Kv channels in heterologous systems, which frequently diverge functionally from the physiologically relevant counterparts. Thus, different strategies are required to exploit the potential that natural venoms represent. Reverse transcription -Linear after the exponential (LATE)- quantitative PCR (RT-LATE-qPCR) is a form of asymmetric PCR developed in the Wangh lab (Brandeis University) for specific, super-sensitive molecular diagnostics. Using RT-LATE-qPCR it is possible to detect low (1-2 copies) and high abundance (>106) transcripts in the same amplification reaction. Detection using highly specific fluorogenic probes of different melting temperatures allows a greater number of transcripts to be identified. RT-LATE-qPCR constitutes a powerful and relatively inexpensive strategy to identify and quantify multiple targets within a single cell. In this work, we coupled conotoxin sensitivity of rat dorsal root ganglion neurons from whole-cell patch clamp experiments, and single cell RT-LATE-qPCR, to identify the target of various Kv1 channel modulating conotoxins. The preferential target is composed of defined Kv1 channel isoforms in stoichiometries that corresponds to the conotoxin affinities of heterologously tested tandem constructs. This work constitutes a proof of principle that can be expanded to medium-throughput screening technologies such as constellation pharmacology as well as higher throughput platforms like automated multi-cell electrophysiology recordings.

Acknowledgments: Lawrence Wangh, Kenneth Pierce, Jesus A. Sanchez (Brandeis University); Russell W. Teichert, Tosifa A. Memon, Lee Leavitt, (University of Utah); Mahsa Sadeghi, David J. Adams (University of Wollongong). Contact info: rfinolu@uow.edu.au, jeffreym@uow.edu.au, h.terlau@physiologie.uni-kiel.de, french@ucalgary.ca, olivera@biology.utah.edu

S9

High-Speed Atomic Force Microscopy (HS-AFM): A new tool for the direct study of conformational changes in gated ion channels.

Martina RANGL[a,b], Yi Ruana, Arin MARCHESI[a], Pierre-Jean CORRINGER[c], Crina NIMIGEAN[d], Simon SCHEURING[a,b].

a Weill Cornell Medicine, Departments of Anesthesiology and Physiology & Biophysics, 1300 York Avenue, New York, NY 10065, USA; b U1006 INSERM / Aix-Marseille Université, Campus Scientifique de Luminy, 13009 Marseille, France; c Channel-Receptors Unit, Institut Pasteur, CNRS UMR 3571, 75015 Paris, France; d Weill Cornell Medicine, Departments of Anesthesiology, Physiology and Biophysics, and Biochemistry, 1300 York Avenue, New York, NY 10065, USA.

The advent of high-speed atomic force microscopy (HS-AFM[1]) has opened a novel research field for the dynamic analysis of single bio-molecules: Molecular motor dynamics[2,3] membrane protein diffusion[4], assembly[5] and conformational changes of transporters[6] could be directly visualized. Further developments for buffer exchange[7] and temperature control[8] during HS-AFM operation provide breakthroughs towards the performance of

dynamic structural biochemistry using HS-AFM. Here, we show the direct visualization of conformational changes of the cyclic nucleotide gated potassium channels upon ligand binding[9], and of a pentameric receptor ion channel in pH-gating[10].

1) Ando et al., Chemical Reviews, 2014, 114(6):3120-88.; 2) Kodera et al., Nature, 2010, 468(7320):72-6.; 3) Uchihashi et al., Science, 2011, 333(6043):755-8.; 4) Casuso et al., Nature Nanotechnology, 2012, 7(8):525-9.; 5) Chiaruttini et al., Cell, 2015,163(4):866-79.; 6) Ruan et al., PNAS, 2017, in press; 7) Miyagi et al., Nature Nanotechnology 2016, 11: 783-790; 8) Takahashi et al., Small, 2016, 12(44):6106-6113; 9) Rangl et al., Nature Communications, 2016, 7: doi:10.1038/ncomms12789; 10) Ruan et al., in preparation.

Data Blitz:

DB1

A new prominent TASK-3 activator: a molecular study.

Bustos Daniel[1], Decher Niels[2], Dominik Oliver[2], González-Díaz Wendy[1].

1Center for Bioinformatics and Molecular Simulations, Universidad de Talca, Chile.; 2University of Marburg, Germany. Contact: dbustos@alumnos.utalca.cl; wgonzalez@utalca.cl.

The two-pore domain potassium (K2P) channels contribute to the background leak potassium currents in nearly all cells regulating the resting membrane potential. K2P channels represent important clinical targets for the treatment of cardiovascular disease and several neurological disorders. These channels are regulated by natural ligands including polyunsaturated fatty acids such as arachidonic acid (AA); by physical factors such as mechanical stretch, by voltage, temperature and intra- and extracellular pH. Their activity can also be modulated by diverse pharmacological agents such as volatile anesthetics, neuroprotective drugs and antidepressants. However, the mechanisms that control channel gating are unclear to date. The recent elucidation of crystallographic structures has help us to clarify how natural ligands, physical factors and drugs modulate the K2P channels at molecular level. Through the 3D structures of K2P channels has been identify the so called “fenestrations” that corresponds to pockets localized near to C-terminal site, connecting the lipid membrane core and the central cavity in the intracellular part of the ion conduction pathway. These fenestrations allow the entrance of some lipids and compounds that, in theory, converts the K2P channels in druggables via these lateral intermembrane pockets. Dr. Dominik Oliver and Dr. Niels Decher from University of Marburg (Germany) have been working with a compound called R59022. This compound blocks TASK-3 channel. However, in the mutant TASK-3 L239A the compound acts as an activator of the channel. Employing the predictive and descriptive power of computational methods, we are focused in the structural characterization of the binding site of R59022 with the purpose to explain how this compound can modulate the activity of TASK-3 and TASK-3 L239A.

Funded by FONDECYT 1151142 and CONICYT BFP.

DB2

Generating competitive peptides against TRPM4 and End Binding proteins interaction and their use as trafficking regulators.

Constanza Blanco, Ignacio Mogollones, Anibal Romero, Jimena Canales, José Rivas, Ismael Aldunate, Mónica Cáceres, Oscar Cerda.

Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, Santiago 8380453, Chile. Contact: oscarcerda@uchile.cl

Abstract: TRPM4 is a Ca2+ activated non-selective cationic channel expressed in various human tissues. This channel is involved in several physiological processes such as the generation and transmission of electrical signals in cardiac cells, myogenic vasoconstriction, cell death, cell proliferation and migration. Interestingly, increased TRPM4 expression is related to several pathophysiological states such as cancer, spinal injury, cardiovascular and neurodegenerative diseases. As such, the mechanisms of regulation of TRPM4 expression and activity constitute an interesting area for drug development. We have found that the microtubule-associated End Binding (EB) proteins interact with a ‘SxIP’ motif in the amino terminal region (N-terminal) of TRPM4. Moreover, TRPM4-EB interaction regulates the exportation and activity of the channel. As such, we hypothesized that the interference of this interaction might constitute a mechanism to diminish the TRPM4 expression at the plasma membrane. We generate TRPM4-EB uncoupling peptides based on the N-terminal region of TRPM4 to reduce the trafficking and surface expression of the channel. The interaction between these peptides and EB proteins was determined by pull down assays. The subcellular localization of TRPM4 in presence of competitive peptides was evaluated by immunofluorescence assays and confocal microscopy. In addition, the functional effect of the peptides was determined through three dimensional contractility assays and cell migration assays. We observed that N-TRPM4WT-EGFP, but not the ‘SxIP’ mutants, binds EB proteins. Moreover, N-TRPM4WT-EGFP expression diminishes the plasma membrane localization of TRPM4 in COS-7 cells and a loss of localization of endogenous TRPM4 at focal adhesions. Also, the peptides with the 'SxIP' motif decrease the contractility and cell invasion. These data suggest that N-terminal region might constitute a blocker for TRPM4 trafficking and localization, presumably by competing for EB protein binding. Thus, TRPM4 and EB interaction might represent a potential therapeutic target for TRPM4 gain-of-function associated diseases.

We are thankful to Mr. Nicanor Villarroel for technical support. We thank to Drs. Pierre Launay and Anna Akhmanova for kindly providing the pcDNA4/TO-FLAG-hTRPM4 and GST-EBs plasmids, respectively. This research was funded by Fondecyt #1160518 to OC.

DB3

TRP domain function on integration of stimuli to the gate in TRPV1 channels.

Deny Cabezas-Bratesco, Christopher Ahern, Sebastián Brauchi.

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TRP channels constitute a family of non-selective, polymodal cation channels. They can sense chemical, electric, thermic and mechanic stimulus. As distant relatives to the voltage-gated cation channels superfamily, TRP channels have a tetrameric structure, composed by four protomers each having six transmembrane domains (TMD) together with large N- and C-terminal intracellular domains. 1-4 TMD region is considered the ligand binding domain, and the pore of the channel is shaped by the 5-6 TMD and a loop between them. TRP channels are conceived as modular proteins, with sensors allosterically coupled to gate. The recently released high-resolution structures from different members of TRPV subfamily allow us visualize distinct features at atomistic level. The structures reveal a rich network of interactions between the N- and C-terminal domains and the intracellular loops. The TRP domain, which is an alpha-helix continued almost immediately from the sixth TMD, is localized in the core of this interaction network, suggesting that this domain integrate and communicate to the gate signals from the different sensors. Using a system that allow us replace a particular residue in the channel by a cross-linkable, non-canonical aminoacid, we try to trap the channel in different conformations in response to some stimulus and so describe state-dependent interactions. We find a position able to keep the low gate open, allowing us to test the existence of a second independent gate.

Proyecto Fondecyt 1151430

DB4

Increased mucociliary clearence in the trachea of the *KCa3.1*$^{-/-}$ mice.

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Mucociliary clereance (MCC) is pivotal in lung/airway homeostasis and a funcdamental function in the innate immune system. MCC is tightly controlled by volume of the airway surface liquid (ASL) that depends on the activity of ion channels present in the airway epithelium. We have observed that when the KCa3.1 potassium channel is absent genetically silenced in the mouse, the sodium absorption is significantly decreased and ciliary beating frequency increased. We aim to understand if those changes in airway function affect MCC in freshly isolated mouse tracheas.

We built a custom perfusion chamber and using video microscopy recorded MCC of poliestirene beads (5µ) in trachea from wild type and Kca3.1-/- mice. We observed a significant increase in MCC in the absence of KCa3.1 channels (VALUES).

This result confirms that inhibition of KCa3.1 channels favours MCC by increasing ASL due to the reduction in sodium absorption in airway epithelium. Inhibition of KCa3.1 might be an useful pharmacological traget for human disesases that course with ASL reduction as cystic fibrosis and chronic obstructive pulmonary disease.

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DB5

TRPM8 modulation by basal phosphorylation.

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TRPM8 is the main molecular entity responsible for detection of cold temperatures in the somatosensory system. This calcium-permeable cation channel is activated by cold, cooling compounds such as menthol, and voltage. It has been suggested that TRPM8 function could be regulated by several kinases that phosphorylate the channel in basal conditions, in both recombinant systems and cold thermoreceptor neurons. In order to explore the mechanism underlying this regulation, we evaluated the contribution of this posttranslational modification in TRPM8 function. To this aim, we have analyzed the phosphorylation state of immunoprecipitated TRPM8 channels in basal conditions, identified the residues where this modification takes place, and assessed channel function using calcium imaging in both HEK293 and F11 cells. We found that TRPM8 is phosphorylated in several residues within the N-terminal domain. The inhibition of the basal kinase activity using staurosporine enhances TRPM8 responses to cold and menthol, and causes a shift of 2ºC in its temperature threshold to warmer temperatures in both cell lines. Altogether, these results indicate that basal kinase activity acts as a negative modulator of TRPM8 function, and suggest that constitutive phosphorylated residues in TRPM8 channels tune their responses to cold and menthol.

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DB6

Imaging electrical activity of lysosomes in living cells.

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Lysosomes are acid subcellular organelles responsible for the digestion of macromolecules for nutrient recycling, cell clearance and pathogen degradation. They play a key role in cellular homeostasis affecting different processes like secretion, signaling, membrane repair and energy metabolism (deDuve 2005; Saftig&Haas 2016). To perform its catabolic function, it's required the

expression of a wide range of membrane proteins such as channels, pumps and transporters, which allows the regulated flow of ions into and out of the lysosomal compartment, separating the electric charge and establishing a voltage gradient through the membrane of the organelle (Xu & Ren 2015; Mindell 2012). Lysosomes are key in adapting to nutrient availability, coupling nutritional status to global cellular responses through signaling pathways under the control of the master cell growth regulator, mTOR. It has been described through direct electrophysiology on endolysosomal membranes that deprivation of nutrients or decrease in ATP levels triggers the inactivation of mTOR and the apearance of a sodium conductance in lysosomes (LysoNaATP), generated by a protein complex formed by channels TPC1,TPC2 and mTORC1 (Cang et al., 2013; Cang, Bekele, & Ren, 2014). Then, a decrease in ATP levels generates a drop in the potential of the lysosomal membrane with triggers the flow of calcium to the cytosol via TRPML1 as a pulse signaling towards proteins in the cytosolic side of the compartment. We adapted a non-invasive method to evaluate changes in the membrane potential ($\Delta\Psi$) of subcellular structures in intact cells (Chanda et al., 2005). The method takes advantage of a FRET pair consisting of a GFP-Lamp1 fusion acting as a donor and a colorless hydrophobic anion as an acceptor that rapidly moves across the membrane in response to changes in membrane polarity, altering GFP emission. We tested this approach by reporting $\Delta\Psi$ of lysosomal membranes in HEK-293T cells in response a pharmacologic inactivation of mTORC1/TRPML1 pathway with rapamacyn and torin1 (mtorc1 inhibitors). Additionally we evaluated changes in luminal pH and calcium permeability of lysosomes by multiplexed fluorescence microscopy using genetically encoded sensors, directed to the lysosomal membrane.

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DB7

KCTD5 is a novel protein that regulates TRPM4 activity.

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TRPM4 is a Ca^{2+}-activated non-selective cationic channel that conducts monovalent but not divalent ions. We previously demonstrated that TRPM4 channels regulate cell migration, contractility and is required for focal adhesion disassembly. Moreover, increased TRPM4 expression has been related to pathologies in which cytoskeletal rearrangement and cell migration are altered, such as fibrosis and cancer. Thus, the elucidation of the mechanisms that regulate TRPM4 activity might contribute important information for therapeutic strategies. We used a mass spectrometry-based proteomics approach to identify TRPM4-associated proteins. These studies revealed that K^+ Channel Tetramerization Domain 5 (KCTD5), a putative adaptor of cullin-3 E3 ubiquitin ligase involved in the process of ubiquitination, is a novel TRPM4-interacting protein. Therefore, we hypothesized that KCTD5 is a novel interacting and regulatory protein of TRPM4. TRPM4-KCTD5 interaction was validated by co-inmunoprecipitation assays in HEK293 cells and we evaluated its co-localization by inmunofluorescence. The effect of KCTD5 on TRPM4 activity was determined by patch clamp recordings in HEK293 cells. Ubiquitination of TRPM4 via KCTD5 was evaluated by Ni-NTA affinity chromatography in HEK293 cells. We evaluate membrane expression by biotinylation assay in HEK293 cells. Here, we demonstrate that KCTD5 interacts with TRPM4, inducing the ubiquitination of the channel. In addition, we show that KCTD5 silencing diminishes maximal TRPM4 currents without changing TRPM4 membrane expression. In conclusion, KCTD5 interacts with TRPM4, promoting its ubiquitination and regulating its activity.

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DB8

TBA.

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TBA

Fondecyt #1160900 funds to DV.

DB9

A new piece to understand the calcium-dependent inactivation of TRPV5 and TRPV6 channels.

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TRPV5 and TRPV6 are Ca^{2+} selective channels belonging to the family of Transient Receptor Potential (TRP) channels. TRPV5 and TRPV6 are viewed as the gatekeepers of epithelial calcium transport, being key elements in the regulation of the intracellular and systemic calcium homeostasis. It has been described that intracellular Ca^{2+} exerts a negative control over the activity of these channels. In this context, it has been described both a fast and a slow calcium-dependent phase. The latter is associated to the binding of the Ca^{2+}-Calmodulin Complex to the channel and is common to

both channels. On the contrary, the fast phase of this inactivation allows to differentiate both channels from a functional point of view as TRPV6 show a faster calcium-dependent inactivation when compared to TRPV5. To this moment, it is has been evidenced that the intracellular loop located between the transmembrane segments S2-S3 and residues downstream the transmembrane segment S6 are involved in the mechanism of fast inactivation, but the location of the associated calcium-binding site and the details regarding ion coordination are unknown. In the present study we establish a structural-functional correlation of this process. By means of molecular dynamics simulations and electrophysiological analysis, we propose that residues belonging to the Helix-Loop-Helix domain are involved in this mechanism, forming part of a putative calcium binding pocket that directly modulates inactivation of the channels.

DB10

Electrophysiological characterization of subunits of chloride channel glutamate receptor GluCl from Caligus rogercresseyi.

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The sea lice Caligus rogercresseyi is the most important parasite affecting Atlantic salmon and rainbow trout sea water farming in Chile. The most widely used drug against Caligus is the macrocyclic lactone emamectin, an avermectin thought to act by irreversible activation of the chloride channel glutamate receptor (GluCl) leading to neuronal inhibition, paralysis and death of the parasite. However, resistance to emamectin has emerged and become a major source of economic loss. Little is known about the mechanism of action of the drug and we are lacking assays to evaluate new compounds affecting the same pharmacological target We have previously reported the cloning and electrophysiological characterization in Xenopus laevis oocytes of a glutamate gated chloride channel subunit from C. rogercresseyi (CrGluClα), that however could not be functionally expressed in mammalian or fish cells. One possible explanation of this failure is that CrGluClα requires of further subunits to form a functional, normally pentameric, functional unit. We have now cloned four new putative subunits C. rogercresseyi GluCls, called CrGluClB-D, which share different homologies to CrGluClα. Assay of a CrGluClB subunit on its own in HEK-293 cells generates ion currents elicited by glutamate and inhibited by the anion channel blocker picrotoxin. Although the amino acids that form the glutamate binding sites are conserved in CrGluClB, there was a large difference in the glutamate EC50 of CrGluClα, 7 μM measured in oocytes, and that of CrGluClBG4, 331 μM measured in HEK-293 cells. We are in the process of assaying the other subunits, which are the product of separate genes, and attempting to ascertain whether functional heteromeric assemblies can be formed.

Proyecto Fondecyt 1151430.

Voltage and Calcium Activated Potassium Channels.

S10

Molecular rearrangements underlying BK channel function.

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In neurons, sites of Ca^{2+} influx and Ca^{2+} sensors are located within 20-50 nm, in subcellular "Ca^{2+} nanodomains". Such tight coupling is crucial for the functional properties of synapses and neuronal excitability. Two key players act together in nanodomains, coupling Ca^{2+} signal to membrane potential: the voltage-dependent Ca^{2+} channels (Cav) and the large conductance Ca^{2+} and voltage-gated K^+ channels (BK, hslo or KCa1.1). BK channels are characterized by synergistic activation by Ca^{2+} and membrane depolarization, but the complex molecular mechanism underlying channel function is not adequately understood. Information about the pore region, voltage sensing domain or isolated intracellular domains has been obtained separately using electrophysiology, biochemistry and crystallography. Nevertheless, the specialized behavior of this channel must be studied in the whole protein complex at the membrane in order to determine the complete range of structures and movements critical to its in vivo function. In our laboratory we use a combination of genetics, biochemistry, electrophysiology and spectroscopy, which we correlate with protein structural analysis, to investigate the real time structural dynamics underlying the molecular coupling of Ca^{2+}, voltage and activation of BK channels in the membrane environment, its regulation by accessory subunits and channel effectors (Miranda et al., 2013; Miranda et al., 2016). BK subcellular localization and role in Ca^{2+} neuronal nanodomains make these channels perfect candidates as reporters of local changes in [Ca^{2+}] restricted to specific subcellular regions close to the neuronal membrane. We have created fluorescent variants of the channel that report BK activity induced by Ca^{2+} binding, or Ca^{2+} binding and voltage (Giraldez et al., 2005). We aim to optimize and deploy these novel optoelectrical reporters to study physiologically relevant Ca^{2+}-induced processes both in cellular and animal models. Overall, optically-active BK channels with spectrally-separate photoactivation and FRET modules offer many possibilities for the study of activation in mammalian cells.

Giraldez et al., JGP (2005) 126(5), 429; Miranda et al., PNAS (2013) 110(13), 5217; Miranda et al., PNAS (2016) 113(49), 14055. Funding: European Research Council ERC-CoG-2014-648936 (NANOPDICS).

S11

Discovery of novel probes of BK channel gating by a fluorescence-based high throughput screen.

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Large-conductance Ca^{+2}-and voltage-activated K^+ channels (BK channels) control electrical activity in nerve and muscle cells. Mutations in BK channels are linked to human diseases that include epilepsy and asthma, and BK channel activity has been linked to cell proliferation in some prostate cancers. Thus it is of interest to expand the arsenal of BK channel gating modifiers both as potential treatments for disease, and as research tools to better understand the molecular basis of BK channel gating. To facilitate discovery of BK ligands, we have optimized an assay for high-throughput screen in a 384-well plate format, using a fluorescent thallium indicator to detect BK channel activity in cultured cells. We have validated the assay using the known BK activators NS1619 and NS11021. From an initial pilot screen of 1280 small molecules, we have discovered previously unknown BK agonist activity in six compounds (hit rate of 0.5%), and we determine their mechanism of action using patch-clamp electrophysiology. These results provide evidence for a robust discovery strategy that should lead to identification of sites for effective BK channel modulation.

S12

Big time for BK: molecular mechanisms of BK current rhythmicity in the circadian clock.

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BK Ca^{2+}- and voltage-activated K^+ channels (KCa1.1) regulate excitability in a variety of cell types, tuned by several molecular mechanisms including alternative splicing, post-translational modifications, and protein partnering (accessory subunits and Ca^{2+} channels). We investigated the integration of these mechanisms for regulation of BK current properties in the suprachiasmatic nucleus (SCN), the brain's intrinsic 'clock.' The molecular components that translate the clock mechanism into specific firing patterns during distinct time windows are just beginning to emerge. In the SCN circuit, BK channels play a unique role in dynamic regulation of excitability as part of the mechanism that produces daily oscillations in action potential firing. Our studies probe the clock-linked regulation of BK channel activity. The circadian difference in BK current magnitude is generated by daily changeovers in BK's Ca^{2+} source, alternative splicing of the α subunit, and N-type inactivation mediated by the β2 subunit. These mechanisms work in concert to establish BK channel gating as a biophysical switch, toggling membranes between day and night states, to contribute to the daily variation in SCN excitability that underlies circadian rhythm.

S13

An ion channel and a molecular ruler - a little journey.

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Calcium and voltage -activated potassium (BK) channels are involved in a large variety of physiological processes. Because there is only a single gene encoding the pore forming α subunit, regulatory β and (recently discovered) γ subunits are one of the mechanisms responsible for creating BK channel diversity, fundamental to confer the adequate function in different tissues. We used lanthanide-based resonance energy transfer (LRET) as molecular ruler to probe the external architecture of BK channels. As LRET donor we used the fluorescent ion terbium bound to a genetically encoded lanthanide binding tag (LBT). As LRET acceptor a fluorophore-labeled iberiotoxin was used for measurement of intramolecular distances within the BK channel structure. By introducing LBTs in the extracellular region of the α or β1 subunits we determined (i) a basic extracellular map of the BK channel, (ii) β1 subunit-induced rearrangements of the voltage sensor in α subunits, and (iii) the relative position of the β1 subunit within the α/β1 complex.

Plenary Lecture II.

PL2

Modulation of the BK channel by the β1 subunit and hormones.

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BK channels are modulated by β subunits (β1- β4) in a tissue-specific manner. The β1 and β2 increase in the apparent Ca2+ sensitivity of BK can be explained by a stabilization of the voltage sensor domain (VSD) in its active configuration[1,2]. However, whether β1 modifies the number of charges associated with the voltage sensor activation is still a matter of controversy. Using gating current measurements, we found that the total number of charges per channel was 4.4 and 3.0 e0 for BKα and BKα/β1 channels respectively. Increasing intracellular [Ca2+] in BKα promotes a significant leftward shift) of the charge-voltage (Q-V) curve. The calcium effect on voltage sensor in BKα becomes apparent at [Ca2+] ≥ 1

μM. However, the leftward shift of the Q-V curve in BKα/β1 channels becomes evident in the nanomolar [Ca2+] range. We conclude that: a) β1 subunit not only modifies the resting-active equilibrium of the voltage sensor but also decreases the total number of apparent gating charges; and b) there is a strong coupling between voltage and Ca2+ sensors, this coupling is increased in the presence of β1.

In arterial smooth muscle cells (SMC) the physiological importance of β1 is underscore by its role in modulating arterial tone and blood pressure. In SMC, 17β-estradiol (E2) increases the BK channel probability of opening, triggering a modulatory effect β1 subunit-dependent[3]. The mechanism of action of this hormone or the location and molecular nature of the E2 binding site in BK channels is not clear at present. We found that the β1 subunit can reach the membrane in the absence of the BK pore-forming α subunit and that E2 is able to bind to β1 or to α/β1 complex but not to the α subunit alone. Using molecular dynamics simulations and docking we identified a hydrophobic surface in the second transmembrane (TM2) domain of β1 as a potential binding site for E2. Mutation of a tryptophan (W163I) in the β1 TM2 obliterates the E2 binding and the functional effect of the estrogen.

1Contreras, GF et al. 2012. Proc. Natl. Acad. Sci. (USA). 109:18577-18582; 2Castillo, K., et al. 2015. Proc. Natl. Acad. Sci. (USA). 112(15):4809-4814; 3Valverde, MA et al. 1999. Science. 285:1929-1931 (491). FONDECYT 1150273

Friday 21

Moving calcium is what we love!.

S14

Old toys, new games: Using Conus venom-derived peptides to isolate the physiological roles of T-type calcium channels.

Jeffrey R. McArthur[1], Rocio K. Finol-Urdaneta[1], Thomas Durek[2] and David J. Adams[1].

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T-type calcium (CaV3s) channels are low voltage-activated calcium selective proteins represented by three closely related members CaV3.1, 3.2 and 3.3. These channels are composed of 4 domains, each containing six transmembrane segments similar to eukaryotic voltage-gated sodium channels (NaV). T-type calcium channel currents contribute to a large number of physiological and pathophysiological functions including, sleep, neuropathic pain, cardiovascular homeostasis and epilepsies. The lack of selective CaV3.2 modulators has hindered their functional study in native cells. Recently, we discovered that the well-studied peptides isolated from cone snail venoms, the μ-conotoxins previously described as sodium channel inhibitors, are effectively selective and potent blockers of the T-type human (h)CaV3.2 channels over hCaV3.1 and hCaV3.3. Examining representative members of the μ-conotoxin family including, GIIIA/B/C, PIIIA and KIIIA, we found that not all members inhibit hCaV3.2. GIIIA, the skeletal muscle specific sodium channel blocker (IC50 for hNaV1.4 ~1.5 μM) has an IC50 of ~700 nM for hCaV3.2 without any apparent inhibitory effects on hCaV3.1 or hCaV3.3 (up to 10 μM). PIIIA exhibits similar potency against hCaV3.2, whereas GIIIB, GIIIC and KIIIA at concentration as high as 10 μM, do not block this T-type channel isoform. Our results thus highlight the following unrecognized aspects: 1) despite having similar structures and sequences, the μ-conotoxin family of peptides have widely different activity on non-canonical targets; 2) these peptides show promise as tools in the study of T-type calcium channel physiology in native tissues; and 3) GIIIA may serve as a scaffold for the development of drugs aimed to treat or ameliorate T-type calcium channelopathies. Furthermore, using our knowledge of the molecular determinants of μ-conotoxin interactions with NaV channels, we can potentially design μ-conotoxin derivatives with increased selectivity and potency against CaV3.2 over NaV channels while retaining the selectivity differences within the T-type calcium channel family.

S15

Molecular determinants involved in TRPM8 cold response.

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TRPM8, a calcium-permeable cation channel activated by cold, menthol and voltage, is the main molecular entity responsible for detection of cold temperatures in the somatosensory system. Several molecular determinants involved in TRPM8 responses to chemical agonists such as menthol have been described; however, the residues or regions involved in cold and voltage activation remain more elusive. In order to identify structural domains related to TRPM8 temperature sensitivity, we took advantage of the differences in cold response displayed by mouse (mTRPM8) and chicken (cTRPM8) TRPM8 channels, and constructed chimeras using these two orthologs. Cold and menthol responses of these mutants were evaluated using calcium imaging and patch clamp techniques in transfected HEK293 cells. This approach allows us to identify different regions into the N-terminus and the pore loop that contribute to channel function. The transference of the proximal N-terminus region from cTRPM8 to mTRPM8 caused an increase in the responses to cold and menthol, a 3ºC shift in the temperature threshold to warmer temperatures, and a reduced EC50 to menthol. On the other hand, chimeric channels also revealed that the pore loop contains amino acids that tune the cold responses of these two orthologs, without an apparent alteration of the menthol response. Altogether, these results suggest an important

contribution of the N-terminal and the pore loop domain in cold responsiveness of this polymodal ion channel.

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OP1

L-type Ca2+ channels inactivation kinetics during an action potential from neonatal rat cardiomyocytes.

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L-type Ca2+ channels (LTCC) are the primary route for Ca2+ entry into cardiac myocytes during the cardiac action potential (AP), Ca2+ influx through these channels promotes opening of ryanodine receptors and the subsequent calcium release from sarcoplasmic reticulum initiating cardiomyocyte contraction. This channel is activated by plasma membrane depolarization and is inactivated through voltage- and Ca2+-dependent mechanisms. However, how these LTCC inactivation processes affect the AP is not fully understood. In this work, a dynamic AP-clamp protocol was used to determine the LTCC activity during the cardiac action potential, this protocol is characterized by the acquisition of the cell-specific AP under current-clamp mode and the subsequence injection of this AP as a command potential instead of a square-pulse protocol. To study both inactivation processes independently, the CaVβ2a subunit, known to abolish the voltage-dependent inactivation of CaVα subunit was overexpressed in neonatal rat cardiac myocytes, while Ba2+ was used to prevent the calcium-dependent inactivation process; APs evoked at different frequencies (1 and 3 Hz) were used to promote channels inactivation. Here we show that the time course of L-type current during dynamic AP-clamps activated rapidly to a peak value, to decline after with a kinetic similar to the AP repolarization, Ba2+ replacement does not change L-type current kinetic, suggesting that calcium-dependent inactivation is absent in this condition. In contrast, in cardiomyocytes overexpressing the CaVβ2a subunit, L-type current activate slower and is abruptly interrupted, suggesting that in absence of voltage-dependent inactivation, Ca2+-dependent inactivation become preponderant.

Fondecyt 1160900.

S16

Prolonged AT1R activation induces β-arrestin1 recruitment to L-type channel complex and CaV1.2 internalization in rat cardiomyocytes.

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The cardiac L-type calcium channel is a multi-subunit complex that includes the poreforming subunit, CaV1.2, co-assembled with the auxiliary subunits CaV□2□ and CaV□. It is widely accepted that these subunits differentially alter channel behavior, including open probability, gating kinetics activation and inactivation over L-type Ca2+-current, moreover, these subunits regulates the expression of functional channels at the plasma membrane. Similarly, trafficking of many ion channels from and to the plasma membrane has been shown to be controlled either by direct interaction with G-protein coupled receptors (GPCR) or by the activation of this type of receptors, among them, the angiotensin receptor type 1 (AT1R). Thus, we decide to explore the consequences of the activation of AT1R over CaV1.2 trafficking. Here we show that 1-hr AngII stimulation (1 μM) of adult rat cardiomyocytes causes a decrease in L-type Ca2+ current, Ca2+ transients amplitude and myocytes contractility, together with a faster repolarization phase of action potentials; immunofluorescence of adult rat cardiomyocytes shown an apparent loss of CaV1.2 immunostaining specifically at the T-tubules, while Bioluminescence Resonance Energy Transfer (BRET) assay between β-arrestin and L-type channel in AngII-stimulated cells demonstrate that AngII exposure results in β-arrestin1 (but not β-arrestin2) recruitment to the channel complex. Altogether, our results demonstrate that AT1R activation induces β-arrestin1 recruitment and the subsequent internalization of CaV1.2 channels. This novel AT1R-dependent CaV1.2-trafficking modulation likely contributes to AngII-mediated cardiac remodeling.

Fondecyt 1160900.

OP2

Modulation of TRPM8 by protein kinase C.

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TRPM8 is a member of the transient receptor potential (TRP) ion channel family, which is expressed in sensory neurons and is activated by cold and cooling compounds, such as menthol. This channel is the major component of the cold transduction machinery in cold thermoreceptor neurons of the somatosensory system. In cold thermoreceptors and in recombinant systems, it has been found that that TRPM8 function could be regulated by several kinases; among them, PKC could play a relevant role in inflammatory conditions. In order to further explore the mechanism underlying this regulation, we evaluated the effect of PKC activation induced by either phorbol esters (PMA) or the proinflammatory mediator bradykinin, on TRPM8 function, using calcium imaging in native and recombinant systems and extracellular recording in corneal cold thermoreceptors. Our results show that the activation of PKC reduces the maximal response of TRPM8 to cold and menthol, and causes a shift of 2ºC in the temperature threshold activation to lower temperatures. In corneal cold thermoreceptors, PMA produces a reduction of the ongoing activity and maximal response to cold. These

results indicate that PKC activity, acts as a negative modulator of TRPM8 channels, suggesting a relevant role of this kinase in cold sensing in inflammatory conditions.

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Plenary Lecture III.

PL3

Molecular mechanisms of pain – from optogenetics to calcium channel dysregulation.

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Cellular and animal models for ion channel studies.

S17

Coherent mRNA Assemblies of Ion Channel Transcripts Underlying the Cardiac Action Potential.

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How excitable cells regulate the precise balance of different ion channels mediating electrical signaling is poorly understood. In the heart, perturbation of this balance can lead to dangerous arrhythmias and sudden cardiac death. We previously reported that alternate transcripts of the hERG (KCNH2) gene, which encode two subunits of repolarizing IKr channels, are physically associated. The proximity of the transcripts promotes cotranslational assembly and favors the proper subunit constituency required for normal cardiac repolarization. We now report that the hERG transcripts also associate with transcripts encoding other ion channels whose coordinate expression underlies the cardiac ventricular action potential. Using an antibody specifically directed against the nascent hERG 1a subunit, we co-purified hERG 1a, hERG 1b, SCN5A, CACNA1C and KCNQ1 but not KCNE1, RYR2 or KCNJ2 mRNA transcripts from human cardiomyocytes derived from induced pluripotent stem cells (iPSC-CMs) and from human heart tissue. Using fluorescent in situ hybridization (FISH), we have so far observed co-localization of single hERG and SCN5A transcripts in iPSC-CMs. The same transcripts shown to interact with hERG transcripts exhibited a co-knockdown effect in RT-qPCR assays when either hERG 1a or hERG 1b was silenced, while those that did not interact were unaffected. Whole-cell patch-clamp recordings performed in iPSC-CMs revealed that hERG 1b-specific silencing reduced the magnitude of corresponding ion currents involved in the ventricular action potential including IKr, INa and ICa, but not IK1, as predicted from the RT-qPCR results. This coherent assembly of transcripts, or microtranslatome, represents a novel mechanism coordinating the activities of a subset of channel types underlying the ventricular action potential.

S18

The secreted protein SPLUNC1 is diverse regulator of plasma membrane cation channels.

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Short palate lung and nasal epithelial clone 1 (SPLUNC1) is a secreted protein that is highly abundant in airway surface liquid, where it is thought to play a role in innate defense. We have previously identified SPLUNC1 as a regulator of the epithelial Na+ channel. Our new data indicate that SPLUNC1's N-terminal "S18" region binds extracellularly to ENaC and induces ENaC ubiquitination in a NEDD4.2-dependent fashion. When we label ENaC subunits C-terminally with eGFP and mCherry, we can detect changes in intracellular FRET upon extracellular SPLUNC1 binding, suggesting that SPLUNC1 induces allostery and that this is how the information is transmitted. Our data also suggest that SPLUNC1 induces ENaC trafficking to lysosomes. Consistent with this observation, SPLUNC1(-/-) mice have reduced airways mucus clearance, suggesting a role for SPLUNC1 in regulating Na^+ absorption and mucus clearance in vivo. Surprisingly, SPLUNC1(-/-) mice also have spontaneous airway hyperreactivity and bronchospasm. Further analysis revealed that SPLUNC1 is also secreted basolaterally from airway epithelia and that it negatively regulates Orai1 Ca^{2+} channels in the plasma membrane of airway smooth muscle. Although SPLUNC1's mode of regulation of Orai1 is not yet understood, we have found that SPLUNC1 also internalizes Orai1 and sends it to lysosomes. Furthermore, recombinant SPLUNC1 inhibited both Ca^{2+} influx and contraction in airway smooth muscle. However, whilst SPLUNC1's disordered N-terminus binds to ENaC, we have found that SPLUNC1's C-terminal alpha helix is required for Orai1 inhibition. Of note, SPLUNC1 expression is massively diminished by IL-13 exposure and asthma patients are unable to secrete SPLUNC1. Thus, we propose that a lack of SPLUNC1 in asthma patients contributes to their airway hyperreactivity. In conclusion, despite being a relatively small protein (~25 kDa), SPLUNC1 is able to regulate different classes of plasma membrane cation channels through distinct regulatory subdomains to serve different physiological functions.

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OP3

TRPM4 channels regulate cell migration, invasion and metastasis of melanoma cells.

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Cell migration is a fundamental process for cancer progression and mestastasis and it is regulated by diverse mechanisms involving protein kinase/phosphatase activities, Ca^{2+} oscillations and cytoskeleton rearrangements. TRPM4 is a Ca^{2+}-activated non-selective cationic channel that participates in actin cytoskeleton rearrangement, focal adhesions disassembly and cell migration. Moreover, TRPM4 overexpression has been observed in prostate cancer, B-cell non-Hodgkin lymphoma, and in human cervical-uterine tumor samples, suggesting a role of these channels in cancer development. Thus, the understanding of the signaling pathways dependent on TRPM4 activity might contribute to the design of novel therapeutic strategies against those diseases. In this work we proposed to evaluate the effect of TRPM4 in migration, invasion and metastasis and the cell signaling by which these channels would promote those proccesses. For assay this, B16F10 melanoma cells were used in in vivo and in vitro approaches and there the TRPM4 activity was impaired by pharmacological inhibition and shRNA-based silencing strategies. We demonstrate that TRPM4 modulates cell migration and invasion in vitro. Accordingly, we observe that TRPM4 regulates the tumor growth and metastasis of B16-F10 cells in vivo. Moreover, we show that suppression of TRPM4 activity impacts on intracellular Ca^{2+} levels and cofilin activity, a protein that play an important role in actin cytoskeletal polymerization, and whose activity may depend on intracellular calcium. Together, these findings suggest that TRPM4 might constitute a novel therapeutic target against cancer development and metastasis.

We are thankful to Mr. Nicanor Villarroel for technical support. We thank to Dr. James Bamburg for kindly providing the EGFP-cofilin and EGFP-cofilin(S3A) plasmids, respectively. This research was is funded by Fondecyt #1160518 to OC. Fondecyt #11140064 and Fondecyt #11160900 fund to MC and ELS, respectively.

OP4

TASK-3 knockdown effect in breast cancer cells.

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TASK-3 potassium channel is overexpressed in several types of human tumors, including those originating in the breast and lungs. However, the understanding of their regulation and function during cancer progression is incomplete. Here, we corroborate the overexpression of TASK-3 in human breast cancer cell lines using qPCR, Immunofluorescence and Western blot analysis. We also evaluated the effect of reducing the expression of TASK-3 in MCF-10F and MDA-MB-231 cell lines. Our results show that TASK-3 knockdown, in both cell lines, inhibits cell proliferation process. While knocking down the expression of TASK-3 in fully transformed MDA-MB-231 cells led to a cell cycle arrest characterized by pRB activation and a senescent phenotype, non-transformed MCF-10F cells with reduced levels of TASK-3 generate a cytotoxicity induction. Our observations implicate to TASK-3 as critical factor in tumor cell proliferation and corroborate their potential as therapeutic target in breast cancer treatment.

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OP5

Role of purinergic signaling on the pathophysiology of gastric cancer.

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Gastric Cancer (GC) is the one of the most prevalent cancer and one of the leading causes of cancer-induced deaths. Previously, we have found that the mRNA for the purinergic P2Y2 receptor is significantly increased in GC samples as compared to adjacent healthy mucosa taken from patients diagnosed with GC. In this work, we studied the purinergic signaling in biopsies from healthy and tumoral gastric mucosa and in the gastric adenocarcinoma-derived AGS cell line. In tumor-derived biopsies, we found that the expression of P2Y2 is significantly increased, the expression of ATP-gated ion channel P2X4 is significantly decreased, as compared to healthy tissues. In AGS cells, we also found an altered expression of purinergic receptors as compared to the non-tumoral GES-1 cell line. In functional studies we found a strong contribution of P2Y2-mediated increases in intracellular calcium, elicited by ATP, UTP and the specific agonist MRS 2768. The responses were preserved in the absence of extracellular calcium and inhibited by U73122, a PLC inhibitor and by the purinergic antagonists suramin and AC-R.

Proliferation studies showed that ATP regulates AGS cell proliferation in a biphasic manner, slightly increasing cell proliferation at 10 and 100 µM, but inhibiting at 300 µM ATP. On the other hand, 1-300 µM UTP, a selective P2Y2 agonist, increased concentration-dependently cell proliferation. The effects of UTP and were prevented by wide range and specific purinergic antagonists. In contrast in GES-1 cells ATP only decreased cell-proliferation in a concentration dependent manner and UTP had no effect. Taken together, these results demonstrate the involvement of different purinergic receptors and signaling in GC, and the pattern of expression changes in tumoral cells, and this change

probably directs ATP and nucleotide signaling from an anti-proliferative effect in healthy cells to a proliferative effect in tumoral cells.

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Structure 1

S19

Gating Currents in the Hv1 Proton Channel.

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Similar to tetrameric voltage-cation cation channels (tVGCs), membrane depolarization is sufficient to activate the voltage sensor (VS) domain in Hv1 proton channels. Using the limiting slope method, the effective gating charge valence (QG) in H+ channels was previously estimated to be ~2-2.5 elementary charges (e0) per VS domain, or 4-5 e0 per dimeric Hv1 channel complex. One limitation of the limiting slope method is that it does not reveal the voltage dependence of gating charge movement, and it remains unclear whether the Hv1 VS domain exhibits gating hysteresis (i.e., mode shift or 'relaxation') like tVGCs and the voltage sensitive phosphatase from Ciona intestinalis (Ci VSP). Direct measurement of gating currents in Hv1 has been impeded by the inability to remove the permeant ion (H+) and possibly also low-level expression of mutant proteins. We show that a Hv1 mutations which simultaneously accelerate activation kinetics and block outward current carried by GAQ are robustly expressed in a tetracycline-inducible mammalian cell line, yielding unambiguous inward tail current (ITAIL) and transient outward currents gating current. QG is directly proportional to ITAIL and exhibits a saturable dependence on voltage that is well-fit to a single Boltzmann function. Furthermore, the positions of both ITAIL-V and QG-V relations depend on the duration of a voltage prepulse, resulting in gating hysteresis that is similar to previous reports from other VS domain proteins. Our method for QG measurement in Hv1 paves the way for incisive investigation of voltage- and pH-dependent activation mechanisms in Hv1.

S20

Activation Gating at the Selectivity Filter in K^+ Channels.

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Potassium channels are opened by ligands and/or membrane potential. In voltage-gated K^+ channels and the prokaryotic KcsA channel, conduction is believed to result from opening of an intracellular constriction that prevents ion entry into the channel. On the other hand, numerous ligand-gated K^+ channels may open by a smaller change within the selectivity filter, a narrow region at the extracellular side of the pore. We investigated the location of the gates in the prokaryotic Ca^{2+}-activated K^+ channel MthK, homolog with eukaryotic BK channels, and we found that both the voltage and the Ca^{2+} gates are located within the selectivity filter. In both cases, closure of the MthK pore at the selectivity filter is accompanied by a coupled movement of the pore-lining helices, similar to the movement undergone by the pore-lining helices in K^+ channels proposed to gate at the bundle crossing, albeit in the MthK case, the helices do not approach each other enough to form a steric barrier. We thus asked the question whether the selectivity filter may be a universal gate for K^+ channels and revisited the location of the gate in KcsA channels. Using molecular dynamics simulations and electrophysiology, we found that ligand-induced conformational changes at the intracellular entryway had the effect of removing steric restraints at the selectivity filter, thus resulting in structural fluctuations, reduced K^+ affinity, and increased ion permeation at this location. Such activation of the selectivity filter may be the universal gating mechanism within K^+ channels.

S21

Structure-based Discovery of Potential Two-Pore-Domain Potassium Channel TASK-3 ($K_{2P}9.1$) modulators.

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TASK-3 is a two-pore domain potassium (K2P) channel highly expressed in hippocampus, cerebellum, and cortex. TASK-3 regulates neurotransmitter functions and has been identified as an oncogenic potassium channel and it is overexpressed in different cancer types, for this reason the development of new selective TASK-3 modulators could influence the pharmacological treatment of cancer and several neurological conditions. In the present work we search for potential TASK-3 modulators using a virtual screening (VS) protocol that includes pharmacophore modeling, molecular docking, and free energy calculations (MM/GBSA). At the end, 18 hits were identified, these hits were screened against TASK-3 using Fluorometric imaging plate reader – Membrane potential assay (FLIPR–MPA), and two lead ligands showing inhibition of 40.6 μM and 43.1 μM were obtained as potential modulators.

S22

Divergence in domain IV of an electric fish Nav channel

tunes its fast inactivation to support rapid firing rates by electro-motorneurons.

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In some neuronal cell types, persistent or resurgent current through voltage-gated sodium channels enables the regular firing of “spontaneous” action potentials from a few to a hundred Hz. However, the neurons with the fastest spontaneous firing rates known are found in the nocturnal ghost knifefish (family apteronotidae), which accomplish active electrolocation via a unique neuronal electrical organ that spontaneously fires action potentials at rates exceeding 1000 Hz. Voltage-clamping of electro-motorneurons from apteronotidae revealed voltage-gated sodium currents with incomplete fast inactivation (i.e., the existence of a persistent sodium current). Unexpectedly, RT-PCR revealed that the Nav 1.4b (skeletal muscle) isoform is expressed in the apteronotid spinal cord. We found that this gene contains five apteronotid-specific substitutions in the S4-S5 cytoplasmic linker of Domain IV, a region that has been implicated in the process of fast inactivation of mammalian voltage gated sodium channels. Using two electrode voltage clamp electrophysiology, we assayed the effects of making combinations of these substitutions in the human cardiac sodium channel Nav 1.5 (R1644W, L1647W, M1651R, I1660F, G1661S). Interestingly, when these apteronotid substitutions are incorporated into DIV S4-5 of hNav 1.5, persistent sodium currents and rapid recovery from inactivation are observed that are qualitatively similar to that of the apterotontid Nav current in native cells. Finally, these effects were moderated by the presence of conservative substitutions in the inactivation particle that naturally occur in fish, (I1485L, M1487L). Taken together, these results suggest that the evolution of rapid electrical organ discharge in knifefish was driven by the tissue-specific expression of and sequence divergence within the voltage gated sodium channel Nav 1.4b. Moreover, they help define the structural requirements within the DIV S4-S5 linker for normal inactivation in mammalian sodium channels.

Plenary Lecture IV.

PL4

Structural Basis for Activation and Modulation of Ion Channels.

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For over 60 years, ion channels have been the subject of intense investigations due mostly to their remarkable role in electrical cells signaling. Ultimately, the prospective implications of such studies imparts the understanding of channel molecular properties involved in consciousness, anesthesia and diseases. Our talk aims to sum-up the knowledge that we have produced on the subject so far, meandering from early electrophysiolgy experiments to the most recent atomistically detailed structural data.

Also, we outline major future challenges for the field, such as ion channel modulation by ligands and the need to improve our understanding of molecular origins for channels dysfunctions.

Saturday 22

Structure 2

S23

Single Molecule ligand binding dynamics in pacemaker ion channels.

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Molecular recognition in conjunction with signal transduction is a cornerstone of cellular signaling process. Ensemble measurements of the biophysical properties of these ion channels may not reveal the underlying complexity and disentangle the heterogeneities associated with conformational fluctuations. Single channel recordings measure the activity of single ion channel but single binding events, which are the main drivers of conformational change in ligand activated ion channels, have not been directly observed. Here, we observe the binding of a single fluorescent cyclic nucleotide derivative (fcAMP) to cyclic nucleotide binding domains (CNBDs) of the hyperpolarization and cyclic-nucleotide gated ion channel (HCN) which is found in the pacemaking cells in the heart and brain. To overcome limitations in diffraction-limited microscopy that hamper single-molecule resolution at the micromolar concentrations necessary to drive fcAMP binding, we used a combination of zero-mode waveguides and fluorescence resonance energy transfer (FRET). Together with a novel X-ray crystal structure of the unliganded CNBD, our approach reveals the underlying dynamic events during fcAMP binding: an initial encounter complex when the binding site is in its receptive state, followed by an isomerization of the bound complex that traps the ligand and places the C-linker in an active conformation. This work lays the groundwork for further studies to deconstruct the mechanisms of channel regulation by cAMP.

S24

Allosteric and direct gating modulation of prokaryotic, voltage-gated sodium channels by agonists and antagonists.

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Drugs and biological toxins, affecting nerve and muscle excitability, take advantage of the multiple conformational states of voltage-gated sodium channel (Nav) proteins to achieve specific targeting and functional actions. Prokaryotic Nav channels like NaChBac, are homotrameric. In contrast, eukaryotic channels of the Nav1 family, possess a single, major structural α-subunit, consisting of 4 homologous non-identical domains (D1-D4), which show specific functional specializations. We compare the actions of batrachotoxin (BTX), the most dramatic of known Nav1 channel agonists, with actions of inhibitory local anaesthetics (LAs), which block conduction by binding within the inner cavity, cytoplasmic to the selectivity filter (SF), at a receptor overlapping that of BTX. We can begin to understand these diverse actions in the context of homology models based on recently determined, high-resolution molecular structures of prokaryotic Nav channels. Both LAs and BTX exert apparently allosteric influences on channel gating. For LAs, the dominant effect is a “use-dependent” inhibition based on pore occlusion. In contrast, BTX is a powerful agonist, yielding a negative shift in activation voltage, and partial occlusion of the pore by the bound BTX molecule. For both LAs and BTX, the dominant allosteric actions are “use-dependent” (favored by repetitive activation). LAs inhibit, while BTX predominantly activates. In common with BTX activation of eukaryotic Nav1 channels, modification of NaChBac gives:

(1) a negative shift (~ -20mV) of the half-activation voltage;

(2) a progressive decrease in maximal conductance, consistent with partial block of single-channel conductance as reported previously for eukaryotic channels;

(3) diminished effects after mutation of NaChBac residues homologous to those important for BTX modification of eukaryotic channels.

Thus, key actions of BTX in eukaryotic Nav channels are re-capitulated in prokaryotic Navs. We interpret them using a NaChBac homology model, where the rigid BTX molecule binds to, and stiffens, the pore, inhibiting closure of the channel’s activation gate.

OP6

Side fenestrations provide an `anchor´ for a stable binding of A1899 to the pore of TASK-1 potassium channels.

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A1899 is a potent and selective antagonist of the two-pore domain potassium (K2P) channel TASK-1. It was previously reported that A1899 acts as an open-channel blocker and binds to residues of the P1, P2 regions, the M2, M4 segments and the halothane response element. The recently described crystal structures of K2P channels together with the newly identified side-fenestrations, indicate that residues relevant for TASK-1 inhibition are not purely facing the central cavity as initially proposed. Accordingly, the TASK-1 binding site and the mechanism of inhibition might need a re-evaluation. We have used TASK-1 homology models based on recently crystalized K2P channels and molecular dynamics simulation to demonstrate that the highly potent TASK-1 blocker A1899 requires binding to residues located in the side fenestrations. Unexpectedly, most of the previously described residues, which interfere with TASK-1 block by A1899, project their side chains towards the fenestration lumina, underlining the relevance of these structures for drug binding in K2P channels. Despite its hydrophobicity, A1899 does not seem to use the fenestrations to gain access to the central cavity from the lipid bilayer. In contrast, binding of A1899 to residues of the side fenestrations might provide a physical `anchor´, reflecting an energetically favorable binding mode that after pore occlusion stabilizes the closed state of the channels.

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Ion exchangers, cotransporters and scramblases.

S25

Molecular mechanism of ion and lipid transport by TMEM16 ion channels and scramblases.

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The TMEM16 family is comprised of Ca^{2+}-gated ion channels and/or scramblases, proteins that collapse the lipid asymmetry of the membrane causing the

externalization of phosphatidylserine, a trigger for blood coagulation and for macrophage engulfment during apoptosis. The structure of a fungal TMEM16 homologue revealed that these proteins have a hydrophilic cavity ~11Å wide at the protein/lipid interface that could accommodate phospholipids headgroups. This led to the proposals that phospholipid scrambling by the TMEM16s occurs via a mechanism in which the lipid headgroups traverse the membrane through the cavity while their tails remain within the hydrocarbon core and that ion permeation occurs through this protein-lipid interface. We investigated how the size of the phospholipid headgroups affects scrambling and how transport of ions and lipids is affected by mutating residues facing the cavity to tryptophan (Trp). We generated NBD-labelled phosphatidylethanolamine (PE) lipids conjugated to PEG molecules of increasing size (PEG2000-5000) and tested whether they are scrambled. We found that these lipids are efficiently scrambled, despite that the PEG molecules are 4-6 times larger than the cavity. Through our mutagenic approach we found that while mutation of most cavity-facing residues has small impact activity a small number of key residues what when mutated nearly inhibit scrambling and ion transport. Interestingly, three of these high-impact residues define a constriction in the groove suggesting that this is a key region for scrambling. Unexpectedly however, mutation of these residues to Alanine (Ala) also adversely impact scrambling, suggesting that side-chain size is not a key factor. Taken together our results suggests that lipid scrambling does not require that the headgroups enter and permeate through the entirety of the lipid cavity. Rather, we proposed that a narrow region of the cavity is important for catalyzing lipid transport, but that scrambling can also occur outside of the cavity proper.

OP7

The NBCe1 pathway mediates fast neurometabolic coupling in mouse organotypic hippocampal slices.

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The potassium ion, K^+, a neuronal signal that is released during excitatory synaptic activity, produces acute activation of glucose consumption in cultured astrocytes, a phenomenon mediated by the sodium bicarbonate cotransporter NBCe1 (SLC4A4). We have explored here the relevance of this mechanism in brain tissue by imaging the effect of neuronal activity on CA1 astrocytic pH, glucose and pyruvate dynamics using BCECF and FRET nanosensors. Electrical stimulation of Schaffer collaterals produced fast activation of glucose consumption with a parallel increase in intracellular pyruvate. These responses were blocked by TTX and were absent in tissue slices prepared from NBCe1-KO mice. Direct depolarization of astrocytes with elevated extracellular K^+ or Ba^{2+} mimicked the metabolic effects of electrical stimulation. We conclude that the glycolytic pathway of astrocytes in situ is acutely sensitive to neuronal activity, and that extracellular K^+ and the NBCe1 cotransporter are involved in metabolic crosstalk between neurons and astrocytes. Glycolytic activation of astrocytes in response to neuronal K^+ helps to provide an adequate supply of lactate, a metabolite that is released by astrocytes and which acts as neuronal fuel and an intercellular signal.

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OP8

Investigating the transport mechanism of HKT Na^+/K^+ transport proteins.

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An appropriate balance of the sodium (Na^+)/potassium (K^+) ratio, among others, is essential for regular plant growth and to generate high crop yield in agriculture. A lack of K^+ causes systemic damages within plants that manifest in growth retardation and reduced crop yield. Similar effects are observed when plants are exposed to higher Na^+ concentrations. Due to common physico-chemical properties transport proteins cannot always discriminate sufficiently between the twin-ions Na^+ and K^+. Effects of Na^+ influx can be boon and bane at the same time. At low concentrations Na^+ complements K^+ functions (albeit incompletely), whereas at higher concentrations it causes symptoms of K^+ deficiency and becomes toxic by putting plants under salt stress. To cope with Na^+ adequate management systems are required. Plants are featured with these systems how various examples of salt tolerant plant species demonstrate. One comprehensive family that is involved in Na^+ usage and detoxification are HKT proteins. They are divided into two subclasses – (i) proteins transporting only Na^+ and (ii) proteins allowing Na^+ and K^+ symport. The selectivity of HKT proteins is influenced by external ion compositions. The presence of one ion species can stimulate the transport of other ion species. The process behind is not understood yet.

To investigate the selectivity and conduction mechanism in more detail we built homology models of the subclass II member HKT2;2 from rice. Characterization of the ion conduction pathway during molecular dynamics simulations revealed conformational differences dependent on the N-terminus position. Additionally, possible ion binding sites were predicted. Our data will gain insight into the conduction and selectivity mechanisms of HKT proteins and may contribute to a better understanding of their permeation mechanism.

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OP9

The *Slca4a4*$^{-/-}$ mouse display a muco-obstructing lung phenotype resembling human cystic fibrosis.

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A failure in optimal bicarbonate supply, as seen in the airways of cystic fibrosis patients, produces severe lung disease due to abnormal mucus maturation and acidic pH of airway surface liquid in the airways. The membrane proteins known to be responsible for bicarbonate movement across airway epithelium include CFTR and TMEM16A channels in the apical membrane and a series of transporters of the SLC4 family in both apical and basolateral membranes. While most of these exchangers work in an electroneutral fashion, the SCLA4A4 or NBCe1 Sodium/Bicarbonate cotransporter moves a ratio of 2-3 bicarbonate and 1 sodium ion into the cell. We aim to test if the NBCe1 cotransporter is necessary for electrogenic bicarbonate transport in mouse airways.

Ussing chamber experiments of freshly isolated mouse trachea showed that roughly half of the UTP-evoked anionic current is lost when bicarbonate is replaced with HEPES in the bath solution (-105±11 vs -47±3 $\mu A \cdot cm^{-2}$ respectively). To further determine if UTP induces $HCO3^-$ exit, acutely isolated airway cells from mouse trachea were loaded with BCECF to track pH changes. UTP induced rapid acidification of airway cells that was fully abolished in CO2/HCO3- free bath solution but not in low chloride solution (ΔpH -0.21 ± 0.003 vs -0.016 ± 0.005, respectively). Using a specific NBCe1 inhibitor (S0859) we observed that both UTP-evoked short-circuit current and intracellular acidification were significantly decreased, supporting the involvement of NBCe1 in epithelial anionic secretion. Nevertheless, Ussing chamber experiments in the *Slca4a4*$^{-/-}$ mice were not possible to perform due to low tissue resistance (32.7±8.7Ωcm^2). Preliminary observations of histological tissue samples obtained from 3 week old *Slca4a4*$^{-/-}$ mice showed signs of mucus accumulation in bronchi and lung emphysema. In summary, our electrophysiological and imaging data demonstrate that bicarbonate secretion is occurring in mouse airways and seems to be dependent on NBCe1 cotransporter function. Genetic silencing of NBCe1 produces a lung phenotype with features often observed in cystic fibrosis human patients that could be due to restricted bicarbonate delivery and acidification of airways.

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OP10

Participation of Ae4 anion exchanger in Cl^--dependent saliva secretion.

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Transcellular Cl^- movement across acinar cells is the rate-limiting step for salivary gland fluid secretion. Recently, we demonstrated that Ae4 (Slc4a9) anion exchangers are expressed in mouse submandibular acinar cells where they contribute to Cl^--dependent fluid secretion. Ae4 null mice show reduced $HCO3^-$-dependent Cl^- uptake, in keeping with $Cl^-/HCO3^-$ exchanger activity. However, the functional properties of Ae4 remains controversial. It has been proposed that Ae4 mediates $Cl^-/HCO3^-$ exchange or Na^+-$HCO3^-$ cotransport. We studied the biophysical proprieties of Ae4 to better understand how it promotes saliva secretion. We find that native Ae4 activity in mouse salivary gland acinar cells supports Na^+-dependent $Cl^-/HCO3^-$ exchange that is comparable with that obtained upon heterologous expression of Ae4 in CHO-K1 cells. Additionally, whole cell recordings and ion concentration measurements demonstrate that Na^+ is transported by Ae4 in the same direction as $HCO3^-$ (and opposite to that of Cl^-) and that ion transport is not associated with changes in membrane potential. We also find that Ae4 can mediate Na^+-$HCO3^-$ cotransport–like activity under Cl^--free conditions. However, whole cell recordings show that this apparent Na^+-$HCO3^-$ cotransport activity is in fact electroneutral $HCO3^-/Na^+$-$HCO3^-$ exchange. Although the Ae4 anion exchanger is thought to regulate intracellular Cl^- concentration in exocrine gland acinar cells, our thermodynamic calculations predict that the intracellular Na^+ concentrations required for Ae4-mediated Cl^- influx differ markedly from those reported for acinar secretory cells at rest or under sustained stimulation. Given that K^+ ions reach intracellular concentrations of 140–150 mM (essentially the same as extracellular [Na^+]), we hypothesize that Ae4 could mediate K^+-dependent $Cl^-/HCO3^-$ exchange. Indeed, we find that Ae4 mediates $Cl^-/HCO3^-$ exchange activity in the presence of K^+ as well as Cs^+, Li^+ and Rb^+. In summary, our results strongly suggest that Ae4 is an electroneutral Cl^-/nonselective cation–$HCO3^-$ exchanger. We postulate that the physiological role of Ae4 in secretory cells is to promote Cl^- influx in exchange for $K^+(Na^+)$ and $HCO3^-$ ions.

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