
ION CHANNELS IN THE VALLEY
A JOURNEY THROUGH ATOMS AND LIVING SYSTEMS
APRIL 10-12, 2013
MONTEGRANDE CHILE
channel.in.the.valley.2013@gmail.com

Dear Friends,

We are pleased for your interest in participate in this Second Meeting of Ion Channel Science in the Valley.

In the following pages you will find all information regarding the scientific program and other related activities. The meeting will start on Wednesday 10 at 14:45 after lunch (covered by the inscription fee), so please arrive on time.

The accommodations are located in Cabañas el Galpón (http://www.elgalpon-elqui.cl) and Hostal Kiko (50 mts away from El Galpón). The Meeting Venue is at the "Francisco Varela Garcia" Town hall, just besides the School in Montegrande in front of the main square.

On Thursday 11, after the last session and before the dinner, the Orquesta de Niños del Valle del Elqui (Childs Orchestra) will offer us a selection of live Music.

We want to express our gratitude to all persons and institutions that help us in the making: The Ilustre Municipalidad de Paihuano and the Mayor Mr. Lorenzo Torres Medina and the Concejal de Educación Mr. Orlando Chelme Aliaga. Arquimed LTDA., PROLAB, Vicerrectoría de Investigación y Desarrollo, Universidad de Chile and Centro de Estudios Científicos (CECs). To Ms. Eliana Marín who will take care of the food preparation and Cavas de Valle Vineyards for provide us the wine.

Last but not least thanks to all of you for coming and participate. We hope you will spend a delightful time at the Valle.

See you there!

The Organizing Committee.-

Wednesday 10

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

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

Physiology & Regulation I

15,00 - 15,30	Norbert Weiss	**S1**
15,30 - 16,00	Patricio Orio	**S2**
16,00 - 16,30	William Kobertz	**S3**
16,30 - 16,45	Omar Porras	**OP1**
16,45 - 17,00	Jorge Parodi	**OP2**

16, 45 - 17,00 **Coffee break**

Physiology & Regulation II

17,15 - 17,45	Sebastián Brauchi	**S4**
17,45 - 18,15	Carlos Flores	**S5**
18,15 - 18,30	María Pertusa	**OP3**

Plenary Lecture I

19,00 - 19,45	Ramón Latorre	**PL1**

DINNER, BBQ, DRINKS!

Thursday 11

Channels & Disease I

9,30 -10,00	Andrés Stutzin	**S6**
10,00 -10,30	Brigitte van Zundert	**S7**
10,30 - 11,00	Anselm Zdebik	**S8**
11,00 - 11,15	Alejandro González	**OP4**

11,15 - 11,40 **Coffee break**

Data Blitz session

11,40 - 11,50	Texia Riquelme	**DB1**
11,50 - 12,00	Sandra Villanueva	**DB2**
12,00 - 12,10	Danilo Pezo	**DB3**
12,10 - 12,20	Imai Nesvara	**DB4**

Plenary Lecture II

12,20 - 13,10	Gerald Zamponi	**PL2**

LUNCH

Physiology & Regulation III

15,00 - 15,30	Diego Varela	**S9**
15,30 - 15,45	Ataulfo Martínez-Torres	**OP5**
15,45 - 16,00	Tamara Hermosilla	**OP6**
16,00 - 16,15	Agniezka Dyrda	**OP7**

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

Structure I

16,45 - 17,15	Carlos González	**S10**
17,15 - 17,45	Bob French	**S11**
17,45 - 18,00	Bárbara Arévalo	**OP8**

19,00.- **Welcome Reception. Ilustre Municipalidad de Paihuano**

DINNER

Friday 12

Structure II

9,30 - 10,00	Wendy González	**S12**
10,00 - 10,30	Brad Rothberg	**S13**
10,30 - 10,45	Rodolfo Briones	**OP9**

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

Physiology & Regulation IV

11,10 - 11,40	Carlos Restrepo	**S14**
11,40 - 12,10	Rocio Finol-Urdaneta	**S15**
12,10 - 12,40	Jeff McArthur	**S16**

Plenary Lecture III

12,40 - 13,30	Luis Galietta	**PL3**

Concluding remarks

LUNCH

S1

A $Ca_v3.2$/syntaxin-1A signaling complex controls T-type channel activity and low-threshold exocytosis.

Norbert Weiss
Department of Physiology and Pharmacology, Hotchkiss Brain Institute, University of Calgary, Calgary, Canada

Low-voltage-activated (LVA) T-type Ca^{2+} channels differ from their high-voltage-activated (HVA) homologues by unique biophysical properties. Hence, whereas HVA channels convert action potentials into intracellular Ca^{2+} elevations, T-type channels control Ca^{2+} entry during small depolarizations around the resting membrane potential. Several reports have uncovered an unrecognized feature of T-type channels in the control of vesicular neurotransmitter and hormone release, a process so far thought to be mediated exclusively by HVA channels. However, the underlying molecular mechanisms linking T-type channels to vesicular exocytosis have remained enigmatic.
Here, we report the existence of a syntaxin-1A/$Ca_v3.2$ T-type channel signaling complex that relies on molecular determinants that are distinct from the synaptic protein interaction site (*synprint*) found in synaptic high-voltage-activated calcium channels. This interaction potently modulates T-type channel activity, but was also found essential to support low-threshold exocytosis upon $Ca_v3.2$ channel expression in MPC 9/3L-AH chromaffin cells.

S2

Role of Ih and TRPM8 in cold thermotransduction: a modeling study.

Erick Olivares[1,2], Jean Paul Maidana[1], Rodolfo Madrid[3], Patricio Orio[1,2].
[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso. [2] Facultad de Ciencias, Universidad de Valparaíso. [3] Facultad de Química y Biología, Universidad de Santiago de Chile.

Cold thermotransduction occurs in specialized cold-sensitive (CS) nerve endings in the skin and peripheral tissues. Nerve endings that respond to non-noxious cold display an ongoing activity of single nerve impulses at regular intervals, and regular bursting activity at low temperatures, in what is known as the static response. In addition, a rapid drop in temperature causes a transient increase in the mean firing rate, usually in the form of bursts –the dynamic response–. As the nerve endings and fibers are accessible only to extracellular recordings, detailed electrophysiological characterization of ion currents involved in these responses has to be performed in cultured CS neurons from trigeminal and dorsal root ganglia. However, these neurons do not display ongoing regular nor bursting behavior, and mathematical models can be useful to understand how behavior can arise from a given set of conductances. Using this approach, we have been able to show how the HCN1 channel is critical in shaping the static firing patterns, helping to establish the correct burst timing. In mice lacking HCN1, the related HCN2 channel takes its place, with subtle changes in the firing patterns that nonetheless determine an altered cold perception. We are also studying how the TRPM8 channel originates the dynamic response, with its PIP_2-dependent sensitization that follows a similar temporal dynamics. By studying a set of possible solutions in the parameter space, we will be able to establish the requirements for a robust dynamic response and the role of the involved conductances.

Acknowledgments: The authors want to thank financial support from grants Anillo ACT-1113, Anillo ACT-1104, Fondecyt 1130862 and Fondecyt 1131064. CINV is funded by Grant P09-022-F of the Millennium Science Initiative (Ministerio de Economía, Chile).

S3

Fluorescent visualization and chemical manipulation of ion channel function.

William R. Kobertz, Ph. D.
Department of Biochemistry and Molecular Pharmacology, UMASS Medical School
364 Plantation Street LRB 804. Worcester, MA 01605

The fluorescent visualization of calcium ions entering the cytoplasm has reimaged our basic understanding of the inner workings of calcium channels in cells, tissues and living organisms. In contrast, there is a dearth of tools to fluorescently visualize ions exiting cells. Part of the challenge stems from the fact that cellular egress is contrary to the pervasive intracellular-centric experimental paradigm. Recently, we have been using glycan engineering to install chemical handles into the cell's glycocalyx that directly abuts the

plasma membrane in all cells. Subsequent chemical modfication of these unnatural sugars ideally positions molecular probes within nanometers of the extracellular vestibules of ion channels and transporters. My laboratory's efforts to manipulate ion channel function and fluorescently visualize ions exiting cells using this technology will be discussed.

OP1

Between redox balance and endothelial function: is there room for ion channels?

Benitez J.P & Porras, O.

Laboratorio de Biología Celular, INTA-Universidad de Chile, Santiago, Chile.

Endothelial cells (ECs) belong to the vascular system which is heterogeneous in its geometry as well as in the nature of irrigated domains. In addition to this complexity, ECs face daily fluctuations in the nutrient content of blood after food intake, a phenomenon known as postprandial state. Due to crucial role of ECs in the control the vascular tone, internal homeostatic regulation must be matched to external fluctuations in order to ensure a proper nitric oxide (NO) production and bioavailability. Especially critical is the reduction-oxidation (redox) balance, because NO synthesis depends of reducing cofactors and NO itself is feasible to be oxidized by superoxide anion, O2-. Therefore, when the metabolic machinery is working during postprandial state, a new redox balance can be predicted. In this physiological context, FoxO1, appears as a metabolic integrator that regulates the expression of redox active proteins thanks to its transcriptional activity.

In this study, EOMA cells, a murine endothelial cell line, were exposed to an in vitro postprandial state by treating serum-deprived cells with 10% FBS. This experimental maneuver allows us to observe a rapid decrease of FoxO1 in the cellular nuclear zone. The dynamic for this phenomenon was tracked in GFP-FoxO1 transfected add-HEK cells resulting in a t1/2 for nuclear extrusion of 10.5 ± 2.5 min (17 cells, 4 exps). In addition, nuclear FoxO1 migration was abolished by the treatment of cells with SB 352080 (10 µM), a p38MAPK inhibitor, which suggests a role for this kinase in metabolic FoxO1 regulation of ECs.

Funded by Fondecyt Grant 1120201 & U-Inicia Grant 11/17.

OP2

Crude extracts from *Ugni molinae* potentiate endogenous ion currents of *Xenopus laevis* oocytes

[1]Jorge Parodi, [3]Ignacio Jofre & [2]Patricia Navarrete.

[1]Laboratorio de Fisiología de la Reproducción, Universidad Católica de Temuco. [2]Laboratorio de Neurobiología Molecular y Celular, Instituto de Neurobiología, Campus Juriquilla-Querétaro, UNAM. [3]Centro de Neurociencia y biología de péptidos-BIOREN-UFRO.

Introduction: Chemical extracts of the Chilean shrub *U molinae* possess diverse molecules, with antioxidant properties. To test effects of crude extracts from this plant we used the oocytes of *X. laevis*. These oocytes present a series of endogenous currents, such as the calcium-dependent chloride-current generated by TMEM16A, as well as other conductances. Thus, the aim of this study was to determine whether the extract of *U. molinae* induces or modulates the responses generated by endogenous ion-channels from frog oocytes. Material and Methods: Oocytes were collected from several frogs (n=4) and kept at 15-16oC in Barth's solution. Recordings were performed by TEVC in oocytes superfused with Ringer at room temperature (20-25ºC). For Tout current, the membrane was stepped from the holding potential of -60 mV to -100 mV for 1 sec, then held to +20, to + 60 for 1 sec,. The hyperpolarization activated current was evidenced by stepping the membrane from -60 mV to -160 mV (1 sec). Results: Application of crude extract did not generate any evident endogenous ion-current. The Tout current was potentiated by 150% (±15%) when the crude extract 1.5 µg/ml was present in the bathing solution during the activation protocol. On the other

hand, when the oocytes were exposed to the extract (1.5 µg/ml) for 24 hrs hours, the resting membrane potential was stable for up to 10 days (-24 ± 2 mV as compared to control -31 ± 2 mV). Finally, the hyperpolarization-activated current was also potentiated by 200 % ± 25 %. Discussion: *X. laevis* oocytes have been used to screen for novel bioactive molecules; our results show that the crude extract of *U. molinae* executes a series of effects easily assayed in oocytes: 1: induction stability of resting membrane potential, 2: potentiation of the *Tout* current, and 3: potentiation of the hyperpolarization activated current.

Physiology and regulation II

S4

The cloning of a TRPM ion channel from the single-celled algae *Chlamydomonas reinhardtii*.

Sebastian Brauchi, PhD.
Physiology Department, Faculty of Medicine, Universidad Austral de Chile, Campus Isla Teja, Valdivia, 511-0566, Chile. Email: sbrauchi@uach.cl

Sensory modalities are essential for navigation through an ever changing environment. From insects to mammals, TRP channels are known mediators for cellular sensing. Chlamydomonas reinhardtii is a motile single-celled freshwater green algae, guided by photo-, mechano-, and chemo-sensory cues. In this type of algae, sensory input is first detected by membrane receptors located in the cell body, and then transduced to the beating cilia by membrane depolarization. Although TRP channels seem to be absent in plants, chlamydomonas possess genomic sequences encoding for TRP proteins. Here we described a C. reinhardtii version of a TRP channel sharing key features present in mammalian TRPM channels. Electrophysiological experiments conducted on intact algae and HEK-293T cells expressing the crTRPM clone were combined with behavioral studies. Our results suggest that crTRPM is associated with the repolarization that follows a depolarizing receptor potential, highlighting a primitive function of TRPM proteins, and the robustness of TRP channel architecture.

Funding: FONDECYT 1110906.

S5

Role of KCNN4 potassium channel in neutrophil chemotactic response.

Vera, D. [1,2], Henríquez, C.[2], Riquelme, T.T.[1], Figueroa C.D[2]., Ehrenfeld, I[2]., Sarmiento, J[2] & Flores, C.A.[1]
[1]Centro de Estudios Científicos(CECs), Valdivia, Chile and [2]Universidad Austral de Chile, Valdivia, Chile.

Arrival of neutrophils to the sites of inflammation or infection is one fundamental mechanism that sustains an adequate innate immune response. Diseases that are characterized by impaired neutrophils migration coincide with repeated and often lethal infections. On the other hand an overamplified response of neutrophils can lead to severe host tissue damage. The latter scenario includes patients affected by cystic fibrosis, a disease distinguished by severe destruction of lung parenchyma due to an aggressive neutrophil response during infections. The KCNN4 channel has been described participating in the mechanism of migration of several cells, but their presence in neutrophils has not yet been described. We aim to explore if KCNN4 is present in neutrophils and by means of genetic and pharmacological silencing to test if the channel participates in neutrophil migration. RT-PCR analysis demonstrated the presence of mRNA for KCNN4 in human and mouse neutrophils. Human cells responded with a 3-fold increase in fMLP-induced migration, an effect that was blocked when the cells were incubated with the KCNN4 specific inhibitor TRAM-34. Same results were obtained in neutrophils isolated from wild type mice (WT). But when the cells tested were isolated from a *Kcnn4*$^{-/-}$ animal, fMLP was unable to induce chemotaxis. Nasal inoculation of LPS (*Pseudomonas aeruginosa*) into mice

demonstrated that the percentage of neutrophils arriving to the lung was lowered in the *Kcnn4*$^{-/-}$ animals when compared to WT. At 8hr post-LPS neutrophils in broncoalveolar lavage (BAL) was 58 ± 14% and 24 ± 13% in WT and *Kcnn4*$^{-/-}$ respectively. 20hr post-LPS the values obtained were 80 ± 4% and 55 ± 14% in WT and *Kcnn4*$^{-/-}$ respectively. Control animals of both genotypes inoculated with saline gave values between 3 to 10% of neutrophils in BAL. Histological analysis of lung samples from WT animals showed a notorious neutrophil infiltration that was not observed in the *Kcnn4*$^{-/-}$ mice. Literature reports that when mice with CF are challenged with nasal LPS they have a greater percentage of neutrophils in BAL than their WT littermates. Preliminary results on animals carrying the F508 CFTR deletion showed 82 ± 2% of neutrophils in BAL 8hr post-LPS, and when these animals were breed into the *Kcnn4*$^{-/-}$ background and challenged, the value obtained was lowered to 41 ± 5%.

Taken together these data suggests that KCNN4 is participating in the migration of neutrophils. Experiments in the whole animal demonstrated that the impairment in migration due to KCNN4 silencing is not exclusively observed for fMLP stimulation, is also occurring in a more pathophysiological signalling.

FONDECYT 11100408 and Conicyt BFP.

OP3

N-Terminus domain contributes to thermal and chemical sensitivity of TRPM8 ion channel.

Hardy P.[1], González A.[1], Rivera B.[1], Piña R.[1], Madrid, R.[1] and Pertusa, M.[1]

E-mail: maria.pertusa@usach.cl

[1]Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile.

TRPM8, a calcium-permeable cation channel activated by cold, cooling compounds such as menthol, and voltage, is the main molecular entity responsible for detection of cold in the somatosensory system. This channel is a homotetramer and each subunit contains six transmembrane segments flanked by N- and C-terminal cytoplasmatic domains. The N-terminal domain of TRPM8 consists of 693 aminoacids and little is known about the contribution of this domain to the function and regulation of this channel. In order to evaluate the role of N-terminal domain, we performed different N-terminus TRPM8 chimeras with other members of the TRP superfamily and evaluated the activity of these mutants using calcium imaging technique in transfected HEK293 cells. This strategy let us to identify different regions in the N-terminal domain that contribute to the assembly and activity of this channel. The most relevant result showed that deletion (□40TRPM8) or the substitution of the first forty aminoacids of mouse TRPM8 by the first 40 residues of mouse TRPV1 (1-40TRPV1TRPM8) yield ion channels with higher responses to cold and menthol. This increase in the activity includes a mean shift of two Celsius degrees in the temperature threshold of activation towards higher temperatures, and a shift in the dose-response curve to menthol to lower concentrations of this chemical agonist than those observed in the wild-type channel. Our results suggest an important contribution of the initial region of the N-terminus domain of TRPM8 in the thermal and chemical sensibility of this polymodal ion channel, which could modulate cold-thermoreceptor function.

ACKNOWLEDGEMENTS:
Supported by FONDECYT grants 3110128 (MP) and 1100983 (RM), and CONICYT grant ACT-1113 (RM, MP). RP is recipient of a CONICYT PhD Fellowship.

Plenary Lecture I

Modulation of the BK Channel by β subunits

G. F. Contreras, A. Neely, O. Alvarez, C. Gonzalez, and R. Latorre

Centro Interdisciplinario de Neurociencia de Valparaíso (CINV), Universidad de Valparaíso, Valparaíso, Chile.

Despite that the Ca2+- and voltage-activated K+ (BK) channel protein is coded by a single gene (slowpoke1), channel diversity is large. This diversity is produced by splicing, metabolic modulation (e.g., phosphorylation) and/or auxiliary β subunits (β1-β4). We have been interested in understanding the mechanisms allowing β subunits to increase the apparent Ca2+ sensitivity and to slow down both the activation and deactivation kinetics in the case of β1, β2 and β4. Also, using chimeras between the different β subunits, we have searched for the structural determinants that mediate the functional coupling between α (the pore-forming subunit and the β subunits). Measuring gating currents in macropatches of Xenopus laevis membrane, we found that the apparent increase in Ca2+ sensitivity mediated by β1 and β2 is largely due to a stabilization of the active conformation of the voltage sensor. The behavior of β4 is more complex since at low Ca2+, β4 produces a decrease in the apparent BK Ca2+ sensitivity whereas at internal [Ca2+] ≥ 1 μM, β4 increases Ca2+ sensitivity. Gating currents measurements indicate that these results can be explained by a stabilization of the active configuration of the voltage sensor and a decrease in the number of effective gating charges. The β3 subunit does not modify the BK Ca2+ sensitivity or the channel gating kinetics. The gating current results forced us to modify the two-tiered allosteric model to a three-tiered allosteric model. The fact that β1 does not affect the kinetics of the gating currents strongly suggest that the slowing of BK channel activation and deactivation is a process related to the transition between closed and open states. In terms of the three-tiered allosteric model this is equivalent to increase the energy barrier that communicates the intermediate closed state with the open state. Chimeric exchange of the different region of β1, β2 and β3 subunits demonstrate that the N and C termini and the external loop are the most relevant regions in defining the behavior of the subunits.

Supported by Fondecyt Grants 1110430(RL) 1120802 (CG) and 1120864 (AN). CONICYT doctoral fellowships (to G.F.C.); and Doctoral Thesis Support Fellowship AT-24110157 (to G.F.C.).CINV is a Millennium Institute supported by the Millennium Scientific Initiative of the Ministerio de Economía, Fomento y Turismo.

Channels and Disease I

S6

TRPM4, hydrogen peroxide and necrotic cell death.

Felipe Simón, Elías Leiva-Salcedo, Ricardo Armisén, Ana Riveros, Oscar Cerda, Diego Varela, Ana Luisa Eguiguren, Pablo Olivero and Andrés Stutzin.

Centro de Estudios Moleculares de la Célula and Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Av. Independencia 1027, 838-0543, Independencia, Santiago, Chile.

Necrosis is associated to an increase in plasma membrane permeability, cell swelling and loss of membrane integrity with subsequent release of cytoplasmic constituents. Severe redox imbalance by overproduction of reactive oxygen species (ROS) is one of the main causes of necrosis. Here we demonstrate that H_2O_2 induces a sustained activity of TRPM4, a Ca^{2+}-activated, Ca^{2+}-impermeant nonselective cation channel resulting in an increased vulnerability to cell death. In HEK 293 cells overexpressing TRPM4, H_2O_2 was found to eliminate in a dose-dependent manner TRPM4 desensitization. Site-directed mutagenesis experiments revealed that the Cys1093 residue is crucial for the H_2O_2-mediated loss of desensitization. In HeLa cells which endogenously express TRPM4, H_2O_2 elicited necrosis as well as

apoptosis. H_2O_2-mediated necrosis but not apoptosis was abolished by replacement of external Na^+ ions with sucrose or the non-permeant cation N-methyl-D-glucamine ($NMDG^+$) and by knocking down TRPM4 with a shRNA directed against TRPM4. Conversely, transient overexpression of TRPM4 in HeLa cells in which TRPM4 was previously silenced reestablished vulnerability to H_2O_2-induced necrotic cell death. In addition, HeLa cells exposed to H_2O_2 displayed an irreversible loss of membrane potential which was prevented by TRPM4 knock-down.

S7

Mutant SOD1 expressing astrocytes release toxic factors that trigger motor neuron death by inducing hyper-excitability.

Elsa Fritz[1,2†], Pamela Izaurieta[1,2†], Alexandra Weiss[3], Franco R. Mir[4], Patricio Rojas[5], Rodolfo Madrid[5], Robert H. Brown Jr[3], and Brigitte van Zundert[1*]

[1]Center for Biomedical Research, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andres Bello, Avenida Republica 217, Santiago, Chile. [2]Faculty of Biological Science, University of Concepción, Concepción, Chile. [3]Department of Neurology, University of Massachusetts Medical Center, Worcester, Massachusetts, USA. [4]Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMEC-CONICET, Córdoba, Argentina. [5]Department of Biology, Faculty of Chemistry and Biology, University of Santiago de Chile, Chile. †E.F. and P.I. contributed equally to this work.

Amyotrophic lateral sclerosis (ALS) is a devastating paralytic disorder caused by dysfunction and degeneration of motor neurons starting in adulthood. Recent investigations with primary and stem-cell-derived motor neurons have documented that motor neuron death in ALS is partially non-cell autonomous and that astrocytes expressing mutant superoxide dismutase (hSOD1) contribute to the pathogenesis of ALS by releasing a neurotoxic factor(s). The mechanism by how this neurotoxic factor induces motor neuron death nor its cellular site of action have been elucidated. Here we show that acute exposure of primary wild-type spinal cord cultures to conditioned medium derived from astrocytes expressing mutant SOD1 (ACM-hSOD1G93A) increases persistent sodium currents (PCNa), repetitive firing and intracellular calcium transients, leading to specific motor neuron death days later. Reduction of hyper-excitability by specific (mexiletine) and non-specific (spermidine and riluzole) blockers of Nav channels restored basal calcium transients and prevented motor neuron death induced by ACM-hSOD1G93A. By contrast, TTX paradoxically increases calcium transient amplitudes and was not beneficial for motor neuron cell survival. As with riluzole treatment, application of mexiletine starting at postnatal day 40-50 significantly prolongs survival of hSOD1G93A mice. These findings suggest that riluzole, the only FDA-approved drug with known benefits for ALS patients, acts by inhibiting hyper-excitability. Together, our data document that a critical element mediating the cell non-autonomous toxicity of ACM-hSOD1G93A on motor neurons is increased excitability, an observation with direct implications for therapy of ALS.

Acknowledgements
This work was supported by ALS Therapy Alliance-CVS Pharmacy (B.v.Z), Fondecyt 1101012 (B.v.Z), Conicyt 24090204 (P.I), Fondecyt 1100983 (R.M.). Additionally, R.H.B. is supported the NIH/NINDS (1R01NS050557 and RC2NS070-342), the ALS Therapy Alliance, Project ALS, the Angel Fund, the Pierre L. de Bourgknecht ALS Research Foundation, the Al-Athel ALS Research Foundation, and the ALS Family Charitable Foundation.

S8

EAST syndrome in fish and EAT syndrome in humans.

Anselm A. Zdebik, Fahad Mahmood, Detlef Bockenhauer, Robert Kleta, Claire Russell.

University College London (UCL), Neuroscience, Physiology and Pharmacology, London

Part I: We aimed to develop and validate a reliable method for stable long-term recordings of EEG activity in zebrafish, which is less prone to artifacts than current invasive techniques. EEG activity was recorded with a blunt electrolyte-filled glass pipette placed on the zebrafish head mimicking surface EEG technology in man.

In addition, paralysis of agarose-embedded fish using D-tubocurarine excluded movement artifacts associated with epileptic activity. This non-invasive recording technique allowed recordings up to one hour and produced less artifacts than impaling the zebrafish optic tectum with a patch pipette. Paralyzed fish survived and normal heartbeat could be monitored for over 1h. Our technique allowed the demonstration of specific epileptic activity in kcnj10a morphant fish (a model for EAST syndrome) closely resembling epileptic activity induced by pentylenetetrazol. To demonstrate its usefulness, we showed that this new method could document that seizures in the zebrafish EAST model were ameliorated by pentobarbitone but not diazepam. In conclusion, non-invasive recordings in paralyzed EAST syndrome zebrafish proved stable, reliable and robust and showed qualitatively similar frequency spectra to those obtained from pentylenetetrazol-treated fish. This technique may prove particularly useful in zebrafish epilepsy models which show infrequent seizure activity.

Part II: We investigated a novel mutation in KCNJ10 found in a Dutch and an unrelated Italian family, presenting with magnesium wasting. Neurological symptoms were incomplete, and epilepsy was atypical. We hypothesized that co-expression of KCNJ16 in some glial cells and the distal tubule, but not in all cells expressing KCNJ10 modifies the phenotype conferred by this mutation. Indeed, expression of KCNJ10 alone in oocytes showed 65% current in the negative voltage range, not enough to explain symptoms in these patients (parents carrying a heterozygous loss-of-function mutation are asymptomatic). However, co-expression with KCNJ16 in various stoichiometries reduced currents to 10…25% of WT+KCNJ16 current levels, suggesting that heteromeric channels reach a loss of function which is fully compatible with impairment of renal tubular function. We predict that these patients will also show ERG abnormalities, as both channels are co-expressed in retinal Müller cells.

OP4

Insulin hypersecretion in islets from diet-induced hyperinsulinemic obese mice is associated to several functional adaptations in individual beta-cells.

González A.[1,3], Merino B.[1], Alonso-Magdalena P.[1], Ñeco P.[1], Marroquí L.[1], Caballero-Garrido E.[1], Soriano S.[2], Nadal A.[1], Quesada I.[1]

[1] Bioengineering Institute. Miguel Hernández University, Elche (Spain), [2] Department of Physiology, Genetics and Microbiology. University of Alicante, Alicante (Spain), [3]Department of Biology, University of Santiago de Chile, Santiago de Chile (Chile)

Characteristic hyperinsulinemia associated with obesity arises from a compensatory enhancement in the pancreatic insulin release. If this compensatory response fails to match the increased insulin requirements, obese insulin-resistant individuals can progress to impaired glucose tolerance and, eventually, to type 2 diabetes. Augmented insulin output has been associated with changes in pancreatic beta-cell mass, but little is known about the functional mechanisms allowing for beta-cell adaptation to insulin-resistance.

Here, we have established a diet-induced obese model in mice which displays typical pre-type 2 diabetes symptoms. Using electrophysiological and Ca^{2+} imaging techniques together with high resolution capacitance measurements, we performed a detailed characterization about the modifications regarding key steps in the stimulus-secretion coupling in these obese mice and his control counterparts.

Our structural analysis showed that obese mice exhibited higher beta-cell mass and cell size. However, several functional changes were observed at different steps downstream of KATP channels closure. Obese mice beta-cells exposed to 8 mM glucose showed an increased action potential height, which could be the basis to explain the increased Ca^{2+} signals observed during dual-wavelength microfluorimetry recordings. Simultaneous measurement of depolarization evoked Ca^{2+} currents and capacitance showed a cell size-related increase in the exocytotic

response within the obese group without major changes in Ca^{2+} current density.
These results demonstrate that compensatory adaptation to insulin resistance in obesity involves not only structural but functional changes in individual pancreatic beta-cells.

Acknowledgement: We thank the Science and Innovation Ministry of Spain (Grant: BFU2007-67607) for the financial support.

Data Blitz Session

DB1

KCNN4 inactivation impairs mast cell migration. Role of mast cells in cystic fibrosis intestinal disease.

Riquelme TT. [1], González, R.[1,2], Millar, P.[1,2], Cid, L.P.[1], Sepúlveda, F.V.[1] & Flores, C.A.[1].
[1]Centro de Estudios Científicos, [2]Universidad Austral de Chile.

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the *Cftr* gene that produces malfunction of the chloride channel CFTR. In addition, CF disease concurs with alterations in the immune system whose origin is currently unknown.
We observed that the CF mouse model, the ΔF508 animal, presented with mast cell hyperplasia in the intestinal tissue and 43% of lethality due to intestinal obstructions at 60 days old. When the KCNN4 channel was genetically inactivated in the ΔF508 mice (double mutant mice) the lethality decreased to 3% and the number of intestinal mast cells lowered to normal values.
To study the role of KCNN4 in mast cells functions we isolated and differentiate mast cells from the bone marrow. We found that the chemotactic response of mast cells was completely abolished when KCNN4 was inactivated both genetically and pharmacologically. Serum samples taken from the animals demonstrated that IgE levels (canonical immunoglobulin that triggers the activation of mast cells) were increased in the ΔF508 animals but also in the double mutant mice.
Our data suggest that the mast cell hyperplasia observed in the the ΔF508 can be due to the increased IgE release. Blocking KCNN4 impair mast cell chemotactic response and migration to the intestine. Mast cells are known to participate as enhancers of the inflammatory response, thus the impairment in their recruiting can explain the decrease in lethal intestinal obstruction in the ΔF508 mice.

FONDECYT 11100408 and Conicyt BFP.

DB2

Effect of genetic inactivation of KCNQ1/KCNE3 potassium channel in the intestinal chloride secretion.

Villanueva S[1]., Bustos V[1, 2]., Flores CA[1], Roa-Rojas HA.[1,2]., Cid L.P[1]. and Sepúlveda F.V[1].
Centro de Estudios Científicos[1] and Universidad Austral de Chile[2]. svillanueva@cecs.cl

KCNQ1 is a voltage-gated potassium channel, this pore forming unit associates with different auxiliary β- subunits, which modulate its activity. In the small and large intestinal epithelium the subunit KCNE3 allows voltage independent opening of the channel. Its activation allow potassium efflux across the basolateral membrane setting a membrane potential compatible with cAMP- stimulated chloride and hence intestinal fluid secretion. We aim to determine the effect of KCNQ1/KCNE3 inactivation in the bowel. First we study in whole cell patch clamp in CHO cells the effect of the D90N KCNE3 mutation. The cotransfection generates a marked reduction in KCNQ1/KCNE3-mediated current (1635 ± 670 pA for HA-KCNE3 and 309± 34 pA for HA-D90N KCNE3 (n=4) measurements at 0 mV). The addition of the HA epitope did not changes the channel activity nor subcellular localization. Afterward transgenic mice were generated using the villin promoter to express the D90N KCNE3 in the intestinal epithelium.

Short circuit current experiments of these mice showed impaired chloride secretory response to cAMP and virtually abolished C293B-sensitive Cl^- currents in jejunum and distal colon. The calcium-activated secretion elicited by muscarinic activation was conserved. These results suggest that the D90N KCNE3 is a dominant negative mutation enough to abolish cAMP-dependent intestinal chloride secretion in transgenic mice; however it is not essential for fluid secretion in the intestine. KCNQ1/KCNE3 function is probably compensated by the potassium channel activated by calcium.

FONDECYT 1100859 and Conicyt BFP.

DB3

An Integrative Model of a Motion Direction-Selective Neural Network: Preliminary Results.

Danilo Pezo[1], Patricio Orio[1,2]

[1]Centro Interdisciplinario de Neurociencia de Valparaíso and [2]Facultad de Ciencias, Universidad de Valparaíso.

In the retina of some mammals, the Direction Selective Ganglion Cells (DSGCs) respond selectively to the motion direction of stimuli. Starburst Amacrine Cells (SACs) and DSGCs form a network where SACs interact with themselves and with DSGCs mainly through inhibitory synapses. To understand the role of each of these connections, as well as the particular morphology of SACs and DSGCs, in silico models are of invaluable help. Although some authors have proposed different computational models to give account of specific aspects of DS mechanisms, there is a lack of an integrative model of the SAC-DSGC network. Using the NEURON simulation environment we are setting up a model which will be able to mimic some of the biophysical features associated to the retinal mechanism of DS condensed on a unique Directional Selective Index (DSI), considering the interaction of the cells in the network. The stimuli used in our simulations consist of moving bars (right-left or vice versa) and annuli (centripetal and centrifugal movement). Here we present preliminary stages of the model in which we have accomplished the building of an unique SAC with inputs from Bipolar Cells (BCs), and we present findings of DS at this level. We are now working on the correct biophysical connections between SACs and looking for DS at a network level measuring just one cell. Further work will be to connect this BC-SAC network to one DSGC and examine in this case the spiking produced in presence of different moving stimuli.

Support: ANR-47 CONICYT. CINV is funded by Grant P09-022-F of the Millennium Science Initiative (Ministerio de Economía, Chile).

DB4

Role of TMEM16A in cell migration in prostate cancer cells.

Imai Nesvara, Diego Varela and Andrés Stutzin.

Centro de Estudios Moleculares de la Célula and Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Av. Independencia 1027, 838-0543, Independencia, Santiago, Chile.

The calcium-activated chloride cannel TMEM16A has been associated to multiple types of cancer, including breast cancer, SCCNH, prostate cancer and stromal gastrointestinal tumors. In breast cancer, it associates to neoplastic progression through CAMK and EFGR, whilst in SCCNH its overexpression correlates to MAPK activation and decreased overall patient survival. In prostate cancer, it is found overexpressed in tumoral cell lines but also in human prostate cancer tissues. The experiments designed in order to knockdown the channel show inhibition of cell migration and the suppression of tumoral growth in vivo.

However, there are no studies showing whether the role of TMEM16A in neoplastic progression relates to its ion conducting activity. Furthermore, the TMEM16A-dependent molecular mechanisms that promote migration are unknown. In my thesis, using different prostate cancer cell lines as experimental models, I'll perform an electrophysiological and biophysical

description of endogenous TMEM16A channels, as well as the study of cell migration and the participation of TMEM16A channel in epithelial to mesenchymal transition by expressing a mutant non-conductive TMEM16A protein.

Plenary Lecture II

Voltage gated calcium channels as targets for pain therapeutics.

Gerald Zamponi
Hotchkiss Brain Institute, University of Calgary. Canada.

Physiology and regulation III

S9

$Ca_V\beta_2$ variants and calcium homeostasis in newborn rat cardiomyocytes.

Moreno C[1], Hermosilla T[1], Sarmiento D[2], Simon F[2], Varela D[1]
[1]CEMC & ICBM, Facultad de Medicina, Universidad de Chile, 838-0453, Santiago, Chile. [2]MIII & Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas and Facultad de Medicina, Universidad Andrés Bello.

In the heart, L-type calcium currents are carried by a multi-subunit membrane complex that includes CaV1.2 as the pore-forming subunit that co-assembles with the auxiliary $Ca_V\alpha_2\delta_1$ and, preferentially, with the $Ca_V\beta_2$ subunits. To date, five distinct $Ca_V\beta_2$ transcriptional start site (TSS) variants ($Ca_V\beta_2$a-e) differing only in the composition and length of the N-terminal domain has been described, each of them conferring distinct biophysical proprieties to the L-type current. However the impact of $Ca_V\beta_2$ TSS variants over the calcium handling in cardiomyocytes has not been explored. In this work we show that only four of these variants are present in neonatal rat cardiomyocytes. The cloning of these variants and the creation of adenoviral particles carrying three of the four variants allow us to infect newborn rat cardiomyocytes and by standard electrophysiological techniques, we characterize the endogenous L-type calcium currents in cardiomyocytes transduced with each of these variants; the impact of these auxiliary subunits over calcium transients, induced by extracellular pulses, was studied.

FONDECYT 1120240 (DV) and 1121078 (FS)

OP5

An outwardly rectifying chloride current of Xenopus tropicalis oocytes.

Ataúlfo Martinez-Torres, Ángeles Edith Espino-Saldaña, Juan Pablo Reyes, Lenin David Ochoa-de la Paz.
Laboratorio de Neurobiología Molecular y Celular. Instituto de Neurobiología. Universidad Nacional Autónoma de México. Campus UNAM-Juriquilla. CP 76230. Email: ataulfo@unam.mx

Oocytes of *X. tropicalis* present an outward rectifying current that has two components: 1) one voltage activated and independent of intracellular or extracellular Ca^{2+}, and 2) a smaller component that is Ca^{2+} dependent. The properties of the Ca^{2+}-independent current, such as voltage dependence and outward rectification, resemble those of ClC anion channels/transporters. This current is sensitive to NPPB and NFA, insensitive to 9AC and DIDS, and showed a whole-cell conductance sequence of $SCN^- > I^- > Br^- > Cl^-$. RT-PCR revealed the expression in oocytes of ClC-2 to ClC-7, and major reductions of current amplitudes were observed when a ClC-5 antisense oligonucleotide was injected into oocytes. The Ca^{2+}-dependent component was abated after injection of BAPTA or EGTA, whereas Mg^{2+} inhibited the current. This component was blocked by 9-AC, NFA, and NPPB, whereas DIDS did not elicit any evident effect. The ion sequence selectivity was $SCN^- = I^- > Br^- > Cl^-$. To try to determine the molecular identity that gives rise to this component we assessed by RT-PCR the expression of the Ca2+-dependent Cl- channel TMEM16A, which was found to be present in the oocytes. However, injection of antisense TMEM16A oligonucleotides did not inhibit the transient outward current. This result fits well with the

electrophysiological data, since the observed currents do not correspond to those of either TMEM16A or Bestrophins. Together, these results suggest that ClC-5 is a major, but not the sole channel responsible for this outwardly rectifying Cl^- current.

Acknowledgments. Supported by CONACYT 101851 & PAPIIT-UNAM.

OP6

Characterization of the $Ca_V1.2$/AT 1 macro-complex in newborn rat cardiomyocytes.

Hermosilla T, Salamanca E, Moreno C, Linsambarth S and Varela D.
CEMC & ICBM, Facultad de Medicina, Universidad de Chile, 838-0453, Santiago, Chile.

The cardiac isoform of L-type Ca2+ channel is formed by the association of the pore subunit CaV1.2 and the accessory subunits CaVβ and CaVα2δ1. It has been shown that the magnitude of Angiotensin II (AngII) inhibition of L-type Ca2+ currents in neonatal rat cardiomiocytes is dependent on the CaVβ subunits expressed, however, the molecular mechanism for this inhibition is unknown. Here we show that the angiotenisn II receptor type I, AT1, forms a macro-complex with $Ca_V1.2$ and we provide evidence for a direct interaction between the receptor and the channel. The impact of this complex is demonstrated in heterologous system by AB feeding experiments showing that $Ca_V1.2$ is internalized after AT1 activation, in a $Ca_V\beta$ subunit-dependent manner.

FONDECYT 1120240

OP7

Gardos and anionic channels activation induced by local membrane deformation in intact human red blood cells.

Dyrda[1,5] Agnieszka, Cytlak[1,2] Urszula, Ciuraszkiewicz[1,2] Anna, Cueff[1] Anne, Lipińska[1]Agnieszka, Bouyer[1] Guillaume, Egée[1] Stéphane, Bennekou[3] Poul, Virgilio[4] L. Lew, Thomas[1] Serge.
(1) CNRS-UPMC, Station Biologique, Roscoff, France, (2) Institute of Physics, Wroclaw University of Technology, Poland, (3) Institute of Molecular Biology and Physiology, University of Copenhagen, Denmark (4) Department of Physiology Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom, (5) Center for Biomedical Research, Faculty of Biological Sciences and Faculty of Medicine, University Andrés Bello, AvenidaRepública 239-Interior, Santiago, Chile.

Evolutionary red blood cells (RBCs) optimised they mechanical, rheological and shape properties to provide dynamic deformability required for flow through capillaries much narrower than the cell's diameter. Despite RBCs simplicity and decades of intensive studies, their composition, intracellular machinery as well as conductive pathways remain still largely elucidative. Electrophysiological studies, however, have focussed mostly on the function of ion channels present in the membrane of RBCs under pathological conditions.
Objective: To test to what extent membrane deformation of healthy human RBC could induce changes in ionic channel activity.
Methods: We used the cell-attached configuration of the patch-clamp technique. Deformation was obtained by depression of 10 mm Hg applied for less than 10 seconds through glass micropipettes brought in contact with the cell's surface.
Results: We present evidence that Ca2+-sensitive K+ channels are transiently activated when seal formation induces membrane deformation and that this phenomenon can result only from activation of a permeability pathway with a finite Ca2+ conductance. This transient activity generates secondary transient anionic channel activity. Using molecular approaches we identified this anionic current to be mediated by voltage-dependent anionic channels (VDACs).
Conclusions: These findings expose unexpected RBC responses, with participation of anion permeability changes never seen before in experiments with RBCs suspensions. Our results are of potential relevance to RBC behaviour in the circulation in vivo and an important new contributing factor for consideration in the normal mechanism of densification of aging

red blood cells and in the pathophysiology of sickle cell dehydration.

Acknowledgments: AD was supported by an EU 'Marie Curie' grant for young researchers (EST/6th PCRDT/661/06/DR17). dyrda.agnieszka@gmail.com

Structure I

S10

Permeation pathway in Hv channels.

Carlos Gonzalez[1], Ester Otarola[1], Feng Qui[3], Santiago Rebolledo[3], David Baez[1], Osvaldo Alvarez[2], Alan Neely[1], Ramón Latorre[1] , Peter Larsson[3]

[1]Centro Interdisciplinario de Neurociencias Valparaíso, Facultad de Ciencias Universidad de Valparaíso, [2]Departamento de Biología, Facultad de Ciencias. Universidad de Chile, [3]Physiology and Biophysics Department, University of Miami, USA.

Voltage-gated proton (HV) channels are found in the plasma membrane and intracellular compartments, such as phagosomes and in many mammalian cells. In other voltage-gated cation channels, the pore-forming domains are formed by S5, S6, and the P loop connecting S5 and S6. Since Hv channels do not contain a S5-S6 region, some other part of the channel must form the pore-forming domain. Mutations in S4 of Na and K channels have been shown to allow for a proton or cation permeation through the VSD of these mutated channels independent of the pore domain. We hypothesize that the S4 is an important part during proton conduction. We tested whether this is due to an increase in open probability or an increase in single channel conductance, which would suggest that the asparagine forms part of the proton conduction pathway. Using noise analysis on the Ciona Intestinalis wt HV channels, we determined that the single channel conductance of wt and N264Q HV channels was 200 fS. Similar single-channel conductance has been reported for endogenous HV channels in macrophages. In contrast, the single channel conductance for the N264C mutation in S4 was 330 fS, suggesting that N264 forms part of the proton conduction pathway. The mechanism for this change in single-channel conductance is not clear, but we hypothesize that the smaller cysteine residue sterically allows for more proton flux than the larger asparagine side chain. In contrast to the N264C mutation, introduction of an arginine or lysine at position 264 drastically reduced or abolished the proton currents. Combining N264R with S242C and fluorescently labeling 242C with Alexa488-maleimide results in voltage-dependent fluorescence changes similar to 242C channels, showing that the N264R channels are expressed in the plasma membrane and that S4 moves in response to voltage steps. This suggests that introducing a positive charged amino acid at N264 reduce the conductance significantly. We also detect two conformational changes reported by a fluorophore attached to the voltage sensor S4 in Hv1 channels. The first is voltage dependent and precedes channel opening, with properties consistent with reporting on independent S4 charge movements in the two subunits. The second is less voltage dependent and closely correlates with channel opening. Mutations that reduce dimerization or alter the intersubunit interface affect both the second conformational change and channel opening. These observations suggest that, following an initial S4 charge movement in the two subunits, there is a second, cooperative conformational change, involving interactions between subunits, that opens both pathways in Hv1 channels.

Supported by Fondecyt grants ACT 1104 and Fondecyt 1120802 to CG 1110430 to R.L, 1120864 to AN, and HL-095920 and American Heart Association Grant 10GRNT4150069 to PL. The Centro Interdisciplinario de Neurociencia de Valparaíso is a Millennium Science Institute.

S11

Prokaryotic sodium channels: What can they tell us about molecular mechanisms in voltage-gated sodium channels of multi-cellular organisms?.

Rocio K. Finol-Urdaneta[1], Yibo Wang[2], Ahmed Al-Sabi[1], Chunfeng Zhao[2],

Robert Glavica[1], Jeff R. McArthur[1], Sergei Yu. Noskov[2], Robert J. French[1]*

[1]Department of Physiology and Pharmacology, and the Hotchkiss Brain Institute. [2]Institute for Biocomplexity and Informatics, Department of Biological Sciences. University of Calgary, Calgary, Alberta, Canada

Voltage-gated sodium (Nav) channels from prokaryotes (e.g. NaChBac and NavAb) are homo-tetrameric proteins whose monomeric units are homologous with the repeat domains of eukaryotic Nav channels. Although prokaryotic Nav channels, such as NaChBac and NavAb, show voltage-dependent activation and inactivation gating, and selectivity among monovalent cations, which are qualitatively similar to properties of eukaryotic Nav channels, the prokaryotic channels possess neither structures resembling the fast inactivation gate, nor the asymmetric, DEKA selectivity filter typical of eukaryotes. Based on experimental studies using NaChBac, and molecular dynamics simulations using the crystal structure of NavAb (Payandeh et al., 2011, Nature) we have found evidence that the molecular basis of ion selectivity in these channels may have more in common with the multi-ion mechanisms which occur in voltage-gated potassium and calcium channels of multi-cellular organisms. Also noteworthy is that μ-conotoxins (μCTXs), which are potent and highly specific blockers of particular Nav1 isoforms, block NaChBac with high affinity, consistent with recent predictions of Chen & Chung (2012, Biophys J). For wildtype PIIIA, dose-response data (0.001-10,000nM) yielded an IC50=0.005nM, maximal fraction of current blocked = 0.95, and Hill coefficient = 0.7.

OP8

Role of the electrostatic potential in the cooperativity between pHo-sensors in K_2P channel TASK-3.

Bárbara Arévalo[1], Leandro Zúñiga[2], María Isabel Niemeyer[2], Pablo Cid[2], Francisco Sepúlveda[2],Wendy González Díaz[1]

1Centro de Bioinformática y Simulación Molecular, Universidad de Talca. 2Centro de Estudios Científicos, Valdivia. barevaloramos@gmail.com

Potassium channels are part of one of the most abundant family of transmembrane proteins. These channels cross the cell membrane and allow the selective transfer of K+ ions inside or outside the cell. Into this family we find K2P channels, which have four transmembrane segments and two pore domains per subunit to form a functional dimer. K2P channels have an implication in diverse cellular functions such as the maintenance of resting potential, regulation of cell excitability, transport of ions and metabolic regulation.

Between K2P channels, we find TASK-3, belonging to the subfamily of TASK. TASK channels are sensitive to the extracellular proton concentration. TASK-3 is closed when extracelullar pH is acidic (PH0) but opens while PH0 increases. The PH0 sensor is a histidine residue (H98) located in the vicinity of the selectivity filter.

A recently published crystallographic structure of the K2P channel TWIK-1 has served as template to build the homology model of TASK-3 channel and to analyze the opening mechanism and the contribution of the H98 residue to the PH0 sensing.

Starting from the model of TASK-3 potassium channel, 6 different systems were constructed; wildtype channel (TASK3-E70), mutated channel (TASK3-E70K) in both protonation states of the PH0 sensor residue H98.

The obtained results show dependency between the protonation state of H98 residue and the occupancy of potassium ions in the selectivity filter. The in-silico results agree with experimental measurements of pKa of H98, where the neutralization of H98 sensor in one subunit increases the propensity of the second H98 to neutralize.

Acknowledgements: Fondecyt 11100373.

S12

Molecular modeling and structural analysis of TASK-1 potassium channel interacting with the blocker A1899.

Wendy González*, Gonzalo Martínez, Tomás Poblete, Jans Alzate-Morales, Francisco Sepúlveda.

Centro de Bioinformática y Simulación Molecular, Universidad de Talca, Talca, Chile & Centro de Estudios Científicos (CECs), Valdivia, Chile. Address correspondence to: wgonzalez@utalca.cl

Two-pore domain potassium (K2P) channels are expressed as functional dimers in the central nervous system, cardiovascular system, genitourinary system and gastrointestinal system. They are related with several pathologies in humans. Thus, members of this family have emerged as molecular candidates for the action of pharmacological agents. The K2P channel TASK-1 is an important modulator of multiple sclerosis and in 2011 a highly-selective blocker of TASK-1, named A1899, was discovered. It was suggested that A1899 acts as an open-channel blocker and binds to residues forming the wall of the central cavity. In 2012 the first crystal structures of K2P channels were published. Electron density maps revealed two open lipid cavities or fenestrations, one on each side of the dimer, that expose the central cavity to the membrane. We constructed a homology model of TASK-1, based on the crystal structures of the recently crystallized K2P channels and studied the specific binding site of A1899. Our results suggest that A1899 travels through the fenestrations to the center of the pore to block the currents of TASK-1.

Acknowledgments: Fondecyt 11100373

S13

Crystal structures of a K^+ channel RCK domain reveal allosteric interactions among Ca^{2+} binding sites.

Brad S. Rothberg, Ph.D.

Department of Biochemistry. Temple University School of Medicine. 3440 N. Broad St. Philadelphia, PA 19140 email: rothberg@temple.edu

Regulator of K^+ conductance (RCK) domains form a conserved class of modulatory domains that undergo conformational changes with binding of metal cations and other ligands, to control gating of channels and transporters. In MthK, a prototypical RCK-containing K^+ channel, each of the channel's RCK domains binds multiple Ca^{2+} ions to reach the fully-activated state; however, it is yet unclear whether individual Ca^{2+} binding sites contribute to channel activation independently of one another, or whether they might interact and thus modulate each other's binding properties, to affect channel gating. To test for these possible allosteric interactions, we determined crystal structures of wild-type and mutant MthK RCK domains bound with Ca^{2+} in a series of singly-, doubly-, and triply-liganded states. Crystals formed at low to moderate [Ca^{2+}] show Ca^{2+} bound only at a single site, termed C1, determined by residues D184, E210, and E212. In contrast, higher [Ca^{2+}] (in otherwise identical conditions) results in a new crystal form, with Ca^{2+} bound at sites C1, C2 (near residues E248 and E266), and C3 (residues D305 and E326). The mutation D184N, which abolishes Ca2+ binding at C1, permits Ca^{2+} binding at C3 with moderate [Ca^{2+}], suggesting that Ca^{2+} binding at C1 allosterically inhibits binding at C3. This apparent negative coupling between sites C1 and C3 can be alleviated by the mutation E212Q, which permits Ca^{2+} binding at both C1 and C3 and facilitates Ca^{2+} dependent activation, as observed in single-channel recordings of reconstituted MthK channels. These results suggest a structural basis for allosteric interactions in the RCK domain that, in turn, modulate Ca^{2+}-dependent channel gating.

OP9

Lipid-protein interactions in channels and porins by molecular dynamics simulations.

Rodolfo Briones[1,*] and Bert L. de Groot[1]

[1]Computational Biomolecular Dynamics Group, Max-Planck Institute for Biophysical Chemistry, Göttingen, Germany.

Lipid-Protein interactions are poorly understood at atomistic level. How lipids modify membrane protein function and vice versa, can be studied with molecular dynamics (MD) simulations. In this work examples of a porin and a water channel are shown.

The mitochondrial porin VDAC (Voltage Dependent Anion Channel) is involved in ATP transport and also related to apoptosis and cancer. Recent X-ray and NMR studies revealed VDAC as a unique barrel structure. The protonation state and mutations of certain amino acids change the experimental and simulated dynamics of the protein. Local lipid properties like thickness are also affected by the mutations (1).

The human aquaporin-1 (hAQP1) is a tetrameric water channel ubiquitously expressed in the cell membranes. Here, we address the question if we could find a global structural model able to explain the changes observed in the the water permeability (pf). By using a methodology based in partial least-squares and cross-validation, we were able to correlate pf with structural changes around the aromatic/arginine region of the pore (2).

1. Villinger, S., R. Briones, K. Giller, U. Zachariae, A. Lange, B. de Groot, C. Griesinger, S. Becker, and M. Zweckstetter, 2010. Functional dynamics in the voltage-dependent anion channel. Proc. Natl. Acad. Sci. U.S.A. 107:22546-22551.

2. Krivobokova, T., R. Briones, J. Hub, A. Munk, and B. deGroot, 2012. Partial Least-Squares Functional Mode Analysis: Application to the Membrane Proteins AQP1, Aqy1, and CLC-ec1. Biophys. J. 103:786-796.

*To whom correspondence should be addressed. Postdoctoral Researcher at Max-Pl

S14

Role of I_{KD} current in painful hypersensitivity to cold induced by chronic peripheral nerve injury.

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

[1]Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, 9160000 Santiago, Chile. [2]Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, 03550 San Juan de Alicante, Spain.

Painful hypersensitivity to innocuous cold is a common form of neuropathic pain after axonal damage. The molecular and cellular bases of this often disabling sensory alteration are not entirely understood. Cold-sensitivity of primary somatosensory neurons is mainly determined by a balance between the activity of TRPM8 channels, the main molecular entity responsible for the cold-activated excitatory current, and Shaker-like Kv1.1 and Kv1.2 channels, the molecular counterpart of IKD, a current that acts as an excitability brake in primary sensory neurons. We studied the role of IKD in painful hypersensitivity to cold after peripheral nerve damage, using chronic constriction injury (CCI) of the sciatic nerve as a model of axonal damage developing cold allodynia, in combination with calcium imaging and patch clamp technique in dissociated primary somatosensory neurons. We found that the percentage of cold-sensitive neurons (CSNs) was larger in CCI group, and that the mean cold threshold of low- and high-threshold CSNs was shifted to higher temperatures. In control animals, the pharmacological suppression of IKD shifted the threshold of CSNs to higher temperatures, a shift that was not observed in CSNs from CCI animals. Mean IKD current density was significantly reduced in CSNs from CCI animals compared to sham mice, and the electrophysiological properties of these neurons revealed an increase of a nociceptive-like phenotype among the CSNs involved in this phenomenon. Our

results suggest an important role of IKD in the painful sensitivity to cold in response to axonal damage in primary sensory neurons, providing a mechanism for cold allodynia.

Supported by Grants FONDECYT 1100983 (RM), CONICYT ACT-1113 (RM) and FONDECYT 3110128 (MP). CR is recipient of a Colombian CES University MSc Fellowship. RP is recipient of a Chilean CONICYT PhD Fellowship.

S15

Block of Kv1.7 potassium currents increases glucose-stimulated insulin secretion.

R.K. Finol-Urdaneta, M.S. Remedi, W. Raasch, S. Becker, R.B. Clark, N. Strüver, E. Pavlov, C.G. Nichols, R.J. French, & H. Terlau.

OBJECTIVE - Pancreatic glucose-stimulated insulin secretion (GSIS) relies on repetitive, electrical spiking activity of the β-cell membrane. Several voltage-gated potassium (Kv) channels contribute to the outward, delayed-rectifier current which speeds membrane repolarization, thus limiting insulin release. We used the inhibitory Conus peptide, Conkunitzin-S1 (Conk-S1), to determine the previously unconsidered role of Kv1.7 channels in regulation of GSIS.
RESEARCH DESIGN AND METHODS - We evaluated the specificity of Conk-S1's action by whole-cell voltage clamp screening of a variety of potassium channels. Single-cell PCR identified rat islet cells containing transcripts encoding insulin and Kv1.7 (kcna7). Pools of isolated rat islets, exposed to different concentrations of glucose, allowed assay of the Conk-S1 sensitive, Kv-channel contribution to Rb86 efflux, and the effect of Conk-S1 on insulin secretion. Current clamp of islet cells was used to test the effect of Conk-S1 on action potential firing, and Ca2+ imaging allowed monitoring of changes in intracellular calcium. Oral glucose tolerance tests and glucose "clamp" in conscious or pithed rats, respectively, revealed the in vivo effects of Kv1.7 inhibition on insulin secretion and plasma glucose levels.
RESULTS - Conk-S1 specifically blocked expressed Kv1.7 channels and caused a significant decrease (~20%) of the delayed rectifier current in insulin- and kcna7-positive rat islet β-cells. Also, Conk-S1 increased action potential firing and augmented islet-cell cytoplasmic free calcium. In live rats, Conk-S1 increased glucose-dependent insulin secretion without decreasing basal glucose.
CONCLUSIONS - Kv1.7 contributes significantly to β-cell repolarizing currents. Block of this current may offer a novel strategy to enhance GSIS with minimal risk of hypoglycemia during metabolic disorders such as Type 2 diabetes.

S16

Modulation of Cav2.1 and Cav2.3 channels by GABAB receptor agonists.

Jeff R McArthur, Géza Berecki, David J Adams.
Health Innovations Research Institute, RMIT University, Melbourne, Victoria 3083

Cav2 channels (Cav2.1 (P/Q-tpe), Cav2.2 (N-type) and Cav2.3 (R-type)) are modulated by pathways involving G protein-coupled receptors (GPCRs) which modulates their signalling and synaptic transmission in the nervous system. Inhibition of calcium channel currents via the GABAB receptor, a GPCR, has been classically described for its native ligand, GABA, as well as a synthetic derivative, baclofen. The inhibition is described classically as either voltage-dependent, i.e. through the Gβγ subunit, or voltage-independent, which doesn't involve the classical Gβγ inhibition of calcium channels. α-Conotoxin Vc1.1 from the cone snail, Conus victorae, has been shown to be an inhibitor of Cav2.2 channels by activating GABAB receptors. We investigated the modulation of stably expressed human Cav2.1 and Cav2.3 channels by baclofen, GABA, and cyclized-Vc1.1 (cVc1.1) in HEK293 cells transiently expressing human GABAB receptors. Depolarization-activated barium currents through Cav2.1 and Cav2.3 were inhibited by both baclofen and GABA,

with a maximal inhibition of <50% and IC50 values of ~400 nM. Unlike baclofen and GABA, cVc1.1 did not inhibit Cav2.1 channels but potently inhibited Cav2.3 channels (IC50 = 300 pM). To examine the role of the voltage-dependent/independent pathways in Cav2.3 inhibition, depolarizing paired pulses were used, which relieves any voltage-dependent component of G protein-mediated inhibition. Under these conditions, inhibition by baclofen of Cav2.1 was ~90% voltage-dependent, whereas, baclofen and cVc1.1 inhibition of Cav2.3 was almost exclusively via a voltage-independent pathway. The cVc1.1 inhibition of Cav2.3 was pertussis toxin-sensitive and was abolished by intracellular application of pp60c-src tyrosine kinase (c-src) inhibitor peptide or by coexpression of a dominant negative mutant R295/F527 c-src protein. Alternatively, over expression of wild type c-src significantly increased the magnitude of cVc1.1 inhibition of Cav2.3 channels. These results suggest that GABAB receptors can couple to both Cav2.1, and Cav2.3, and identify Cav2.3, through a voltage-independent pathway, as a new target for cVc1.1.

Plenary Lecture III

PL3

TMEM16A and TMEM16B proteins as calcium-activated chloride channels.

Luis J.V. Galietta.
U.O.C. Genetica Medica, Gaslini Institute, Genova, ITALY.

TMEM16A and TMEM16B, also known as anoctamin-1 and anoctamin-2 (ANO1 and ANO2), respectively, are major components of Ca^{2+}-activated Cl^- channels (CaCCs), a class of membrane proteins involved in various physiological roles, including epithelial secretion, sensory transduction, nociception, control of neuronal excitability, and regulation of smooth muscle contraction. Although the detailed structure of TMEM16A protein is unknown, predictive tools and experimental work suggest the presence of eight transmembrane domains with the N- and C-termini protruding in the cytosolic environment.

CaCCs generated by TMEM16A expression have slower kinetics and nearly ten-fold higher calcium sensitivity than those associated with TMEM16B. To investigate the basis of these differences, we have generated chimeric channels. Our results indicate that most regions of TMEM16A, including various transmembrane domains and the N-terminus, can be replaced by equivalent regions of TMEM16B without an alteration in CaCC properties. However, significant changes were observed when the third intracellular loop or the carboxy-terminus of TMEM16A were substituted.

We have also investigated the physiological role of TMEM16A protein in the airway epithelium. TMEM16A protein expression was particularly abundant when cultured bronchial epithelial cells were stimulated with interleukin-4, a Th2 cytokine associated with asthmatic inflammation. After IL-4 treatment, TMEM16A was strongly expressed in non-ciliated cells that were positive for MUC5AC staining. Therefore, our results indicate that TMEM16A expression in the bronchial epithelium is associated with goblet cell metaplasia, a condition observed in asthma and other chronic respiratory diseases. The expression of TMEM16A, by providing a pathway for chloride and bicarbonate secretion, may be important to favor mucus secretion and expansion.