THE JUNIOR LEAGUE OF ION CHANNEL SCIENTISTS

STRUCTURE PHYSIOLOGY DISEASE

MARCH 30 - APRIL 1 2011

MONTE GRANDE CHILE

ARQUIMED.

Dear Friends,

We are pleased that you become interested to be a part of this First Meeting of Ion Channel Scientists organized by Chilean Young Investigators.

In the following pages you can find information about the venue and the scientific program. The Meeting is programmed to start on Wensday March 30 at 14:45, so we encourage all to arrive around Wensday noon. There will be no parallel sessions so we all are called to assist to all the scientific activities.

Valle del Elqui is located in the IV región of Chile, in the so called "Small North", and it's worldwide known for its clear sky that allow the almost non stop functioning of several of the biggest telescopes, that help Astrophycisists to find out about the origins and secrets of the Universe. For our delight, we will experience a couple of starry nights accompained with the local alcoholic potion "Pisco Sour" that if several glasses are to be drank, can help multiply the stars for your view.

The climate can range from 25ºC during the day to 7ºC in the night so bring clothes to be prepared for the changes in temperature.

The accomodations are located in Cabañas el Galpón (http://www.elgalpon-elqui.cl/) and located 3 Km away from the Venue. The swimming pool will be available so, bring your swim suits if you like!. The Meeting Venue is at the "Francisco Varela Garcia" Town Hall, just beside the school. (check out the map for more references).

Last but not least, we want to express our gratitude to the Institutions that help us in the making: The Ilustre Municipalidad de Paihuano, the Alcalde Mr. Lorenzo Torres Medina and the Consejal Mr Orlando Chelme Aliaga. Arquimed Ltda. and Centro de Estudios Científicos, Valdivia. Also a big "thank you" to our international participants, that made the big effort (physical and economic) to stay with us these days. We hope all of you to enjoy your time in the Valle!.

See you there!

Organizing Committe:

Dr Carlos A. Flores
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You can reach the Valle del Elqui from La Serena (Plaza de Abastos or Rodoviario) by bus that leave every 30 minutes or Taxis. The trip takes 1,5 – 2 hrs.

Cabañas el Galpón is located 100 Kms from La Serena in La Jariña, 3,5 Km from Montegrande and 2 Km before Pisco Elqui.

Mar-30

REGISTRATION

14:45-15:00 **WELCOME**

Structure I

15:00-15:30	Carlos Gonzalez	**S1**
15:30-16:00	Wendy Gonzalez	**S2**
16:00-16:15	Mariquez-Navarro	**OP1**
16:15-16:30	Burgos	**OP2**

16:30-16:45 **Coffee Break**

Physiology & Regulation I

16:45-17:15	Varas	**S3**
17:15:17:45	Rojas	**S4**
17:45-18:00	Añazco	**OP3**
18:00-18:15	Peña	**OP4**

Plenary Lecture

18:30-19:15	Prof. Ramón Latorre	**PL**

DINNER, BBQ, DRINKS!

Mar-31

Physiology & Regulation II

10:00-10:30	Varela	**S5**
10:30-11:00	Altier	**S6**
11:00-11:15	Parodi	**OP5**

11:15-11:30 **Coffee Break**

Data Blitz Session

11:30-11:40	Baez-Nieto	**DB1**
11:40-11:50	Salazar	**DB2**
11:50-12:00	Arias	**DB3**
12:00-12:10	Penna	**DB4**
12:10-12:20	Vivar	**DB5**
12:20-12:30	Sagredo	**DB6**

LUNCH

Structure II

15:00-15:30	P. Orio	**S7**
15:30-16:00	Finol-Urdaneta	**S8**

Channels & Disease I

16:00-16:30	Flores	**S9**
16:30-16:45	Simón	**OP6**
16:45-17:00	Sepúlveda	**OP7**

17:00-17:15 **Coffee Break**

Channels & Disease II

17:15-17:45	Van Zundert	**S10**
17:45-18:15	Zdebik	**S11**
18:15-18:30	Marcelain	**OP8**

DINNER

Apr-01

Physiology & Regulation III

10:00-10:30	Madrid	**S12**
10:30-11:00	Brauchi	**S13**
11:00-11:30	Armisen	**S14**
11:30-11:45	Pertusa	**OP9**
11:45-12:00	Toro	**OP10**
12:00-12:15	Ortiz	**OP11**

LUNCH

Plenary Lecture

ION CHANNELS AND THE MONTEMAR "CANTATA". LOOKING AT ION CHANNELS WITH FLUORESCENCE.

Professor Ramón Latorre.
Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaiso, Chile.

Structure I

S1

GATING CHARGES INVOLVED IN Hv CHANNELS ACTIVATION

Carlos Gonzalez*+, Marta Perez*, Ramon Latorre+ & Peter Larsson*
*University of Miami, Physiology & Biophysics Department,USA +Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaiso, Chile

Voltage-gated proton (H_V) channels have been shown to play an essential role in immune system function. They are homologous to the voltage-sensing domain (VSD) of voltage-gated potassium (Kv) channels. In contrast to the tetramer structure of Kv channels, we found that Hv channels are dimers with just two S4 segments. Recently, we showed that the total effective gating charges are $5.9\pm0.4e_0$ in the dimer and $2.7\pm0.1e_0$ in the monomer and that S4 movement (containing 3 arginines) precedes channel opening. However, which specific arginine from S4 segment contributes to the effective gating charge is still unknown. To answer this question, we replaced each arginine residue with asparagines separately in *Ciona intestinalis* Hv channels. Patch-clamp recordings in *Xenopus* oocytes showed a dramatic decrease of total gating charges when measured by the limiting slope method: 1.9, 2.9 and 2.2 e_0 for R255, R258 and R261, respectively. In addition, our cysteine accessibility measurements are consistent with an outward movement of these three S4 charges during channel opening. According to our findings, we conclude that the S4 segment moves and functions as the voltage sensor and all S4 charges contribute to voltage gating in Hv channels.

S2

ROLE OF THE ELECTROSTATIC POTENTIAL IN THE COOPERATIVITY BETWEEN PHO-SENSORS IN K2P CHANNEL TASK-3

Wendy González Díaz1, Leandro Zúñiga2, María Isabel Niemeyer2, Pablo Cid2, Francisco Sepúlveda2.
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Two-pore domain potassium (K2P) channels are membrane proteins identified in mammals and other organisms. The functional channel is a dimer and each subunit has two pore-forming loops and four transmembrane domains. In mammals, fifteen KCNK genes have been identified, and their codified proteins (K2P potassium channels) are related with several pathologies in humans.

Among K2P channels, those belonging to the TASK subfamily such as TASK-1 and TASK-3 are sensitive to extracellular proton concentration. TASK-3 is closed at acid extracellular pH (pHo) but it is open when pHo increased. The pHo-sensor is a histidine residue (H98) placed above the selectivity filter (sequence GYGH). When one H98 is protonated, the potassium ion (K+) placed in the S0 site of the selectivity filter is rapidly destabilized. The loss of K+ placed on S0, diminishes the positive electrostatic potential around the second H98 requiring less energy for its protonation. This mechanism is in agreement with experimental measurements of pKa reporting that protonation of the sensor in one subunit enhances the propensity of that in the second to become protonated.

Acknowledgments: Fondecyt 11100373

OP 1

F380 INFLUENCE THE UNITARY CONDUCTANCE IN BK CHANNEL

Paula Manríquez-Navarro (1), Juan Pablo Castillo(2), Eduardo Rossenman(3), Tomas Pérez-Acle(4), Osvaldo Alvarez(2), Ramón Latorre(3).
(1) Facultad de Medicina, Universidad de Chile. (2) Facultad de Ciencias, Universidad de Chile. (3) Centro Interdisciplinario de Neurociencias de Valparaíso, Universidad de Valparaíso. (4) Centro de Modelamiento Matemático, Universidad de Chile.

The amino acid organization related to BK high conductance is a puzzle from which only a few pieces have been obtained. The conserved amino acids in the lining of the internal vestibule are considered relevant because of their possible contribution in channel conductance, such is the case of phenylalanine at position 380 (F380). In silico substitution of this amino acid suggest alterations in the internal vestibule radii and the number of water molecules that surround the ion, that can affect unitary conductance.

In our work, we asses the F380 π-cation and volumetric effect contribution on BK unitary conductance. we evaluated this contribution through mutants with amino acid of different sizes and hydrophobicity. Long-chain mutants, leucine and tyrosine had unitary conductances similar to native channel (221 and 216 pS) and differences between radius were of up to 1.1 Å. In short-chain mutants, isoleucine and valine, the unitary conductance were significantly diminished, nearly half (134 and 144 pS) with bigger radii of 1.6 and 1.7 Å respectively.

The different pore radii generated by the mutants also affected the Po with a shift to the left on the voltage axis, suggesting a possible role of F380 stabilizing the closed channel state.

The F380 plays a role in the removing of water molecules that surround the permeating ion, and that this process is largely regulated by the

volume and hydrophobicity of phenylalanine and not by electrostatic interactions.
These results together with a theoretical study of molecular modeling, allow us to suggest that F380 are close correlated to internal vestibule radii, therefor, to unitary BK conductance.

OP 2

GATING OF THE ClC-2 CHLORIDE CHANNEL, BY EXTRACELLULAR PROTONS.

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ClC-2 is a broadly expressed Cl^- channel member of the CLC family of membrane proteins which comprises both Cl^- ion channel and H^+/Cl^- and H^+/NO^{-3} exchanger members. ClC-2 has a biphasic response to extracellular pH with activation by moderate acidification followed by abrupt channel closure at pH values lower than ~7. In our laboratory an extracellularly-facing histidine 532 at the N-terminus of transmembrane helix Q as a pH-sensor involved in this process was identified previously. Protonation of H532 leads to cooperative channel closure. We now explore the mechanism of acidification-induced inactivation of ClC-2 using molecular dynamics simulations and site-directed mutagenesis combined with electrophysiological experiments. The evidence presented is consistent with a model in which the state of protonation of H532 regulates a distant intracellular gate of the ClC-2 channel to mediate inactivation at strongly acid pH. In molecular dynamics, the H532 sensor appears to interact with the extracellular loop I-J, In which the mutation of three phenylalanine residues F308, F312 and F316, putative interacting to H532, affects the acidification-induced channel closure. Mutating pore residue Y555, which resides in helix R and forms part of a central Cl-binding site in the selectivity filter, abolishes inactivation of ClC-2-Y555F by acidification. We propose that H532 is an allosteric modulator of ClC-2 exerting its effect through a long-range action involving α-helices Q and R. The interaction of H532 with extracellular loop I-J is crucial in a conformational change leading to a final effect on Y555 which would act as an intracellular gate.

Physiology and Regulation I

S3

THE ROLE OF THE TASK-LIKE BACKGROUND POTASSIUM CHANNEL IN CAROTID BODY FUNCTION.

Rodrigo Varas and Fernando C Ortiz
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The carotid body is the main peripheral arterial chemoreceptor and it is essential to initiate the respiratory compensatory reflex responses to changes in arterial O_2 and pH. All of these stimuli act in a similar manner, by inhibiting a background TASK-like potassium channel they induce membrane depolarization, voltage-gated Ca^{2+} entry and neurosecretion. Several evidences suggest that oxygen is sensed through changes in energy metabolism therefore we tested if TASK-like channel activity can be regulated by different intracellular factors.
In excised inside-out patches we find that TASK-like channels are strongly activated by "intracellular" Mg-ATP by MgATP (but not ATP4-) within the physiological range (EC50 = 2.3 mM). This effect was mimicked by other Mg-nucleotides including GTP, UTP, AMP-PCP and ATP-gamma-S, but not by PP(i) or AMP, suggesting that channel activity is regulated by a Mg-nucleotide sensor. Channel activation by MgATP was not antagonized by either 1 mM AMP or 500 μM ADP. Thus MgATP is probably the principal nucleotide regulating channel activity in the intact cell.
In cell-attached patch recordings from oxygen-sensitive (chemoreceptor) cells channel activity was reduced by approximately 50% during muscarinic receptor activation and by application of the diacylglycerol analogue oleoylacetylglycerol (20 μM). The co-application of both metacholine and OAG do not further inhibit channel activity. In addition, inhibition of protein kinase C increases channel activity and suppresses the muscarinic inhibition.
Our results suggest that the TASK-like channel activity is regulated by cell metabolism, providing a link between excitability and energetic status of the carotid body chemoreceptor cells.

Funded by Proyecto Limite VRAID-PUC 11/2009

S4

GABAA RECEPTORS AT THE AXON INITIAL SEGMENT CONTROL EXCITABILITY OF DENTATE GYRUS NEURONS.

Patricio Rojas(1), Lawrence N. Eisenman(2), Steven Mennerick(2).
1Departamento de Química y Biología, Universidad de Santiago de Chile and Centro de Estudios Avanzados en Zonas Aridas. 2 Department of Psychiatry and 3 Neurology, Washington University in St Louis Medical School.

The site of action potential (AP) initiation is located at the axon initial segment (AIS) where a high density of voltage dependent channels is present, determining both threshold and the shape and frequency of APs. In many principal cells GABAergic inputs to the AIS and soma come from separate types of interneurons. Here we studied the effect of axonal versus somatic GABA receptor activation by local application of the exogenous GABAA receptor agonist Muscimol to granule cells of the dentate gyrus, the gatekeepers of the hippocampal tri-synaptic circuit. Local application of 5 μM muscimol to the AIS robustly depolarized the voltage threshold for AP initiation (1.7 ± 0.2 mV, N=13) with relatively little change in input resistance (15%). Because presynaptic inputs to dentate granule

neurons produce small EPSPs (0.1-0.5 mV), this threshold change is significant to granule cell function. Somatic Muscimol application did not alter voltage threshold but strongly decreased input resistance (58 %), resulting in a large increase in rheobase. Application of synaptic-like stimulus that summate to fire an action potential, failed to reach threshold when Muscimol was applied to the AIS. Activation of AIS GABAA channels during spike trains diminished the number of AP and can change the precision in firing, having an impact in the transmission of information. In summary, our results show that AIS GABAA channels have a role not only setting up the voltage threshold for AP initiation, but also modulating the firing pattern in granule cells.

Acknowledgments: This work was supported by NIH grant NS54174 (SM), BioTecZA INNOVA-CORFO 06FC01IFC-71 and Proyecto de Inserción de Nuevos Investigadores de la Academia CONICYT 79090013 (PR).

OP 3

MODULATION OF TASK2 POTASSIUM CHANNEL ACTIVITY BY βγ SUBUNIT OF HETEROTRIMERIC G PROTEIN.

Añazco, C.C., Peña-Münzenmayer, G., Bórquez, M., Cid, L.P., Sepúlveda, F.V., Niemeyer, M.I.
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TASK2 is expressed in renal proximal tubule, where it participates in the regulation of cellular volume and bicarbonate reabsortion. Currently, as background knowledge, few is known about possible molecular mechanisms that govern these processes. Several studies have described direct effects of heterotrimeric G protein subunits (α and βγ) on ionic channels activity. Preliminary evidence suggests that TASK2 activity is being modulated by heterotrimeric G protein subunit. Therefore, the aim of the present study was identifying the mechanism responsible for the regulation of TASK2 by G protein subunits, including the recognition of the specific subtype of G protein subunit that regulates the process. For this purpose, the patch-clamp whole cell technique was used to test modulation of TASK2 activity in the presence of GTPγS, GDPβS and βγ purified protein, from HEK cells transfected with TASK2. GTPγS and purified βγ protein inhibit TASK2 current, unlike GDPβS. This effect of inhibition is not observed in some mutants in basic aminoacids in the carboxyl terminal portion. Protein interaction studies by means of co-inmuneprecipitation assays indicate that TASK2 interacts with β1 and β2 subunits but neither β3 nor β4. Our results demonstrate that the activity of TASK2 expressed in HEK cells, is modulated by βγ subunit of heterotrimeric G protein and that basic residues in the carboxyl terminal portion of the channel are important to this phenomenon. Modulation of TASK2 channels by signaling pathways associated to G protein coupled receptor might represent potential sites for hormonal modulation of K^+ transport in renal proximal tubule cells.

Funded by FONDECYT # 1090478 and FONDECYT # 3085021.

OP4

REGULATION OF POTASSIUM CHANNEL TASK-2 BY INTRACELLULAR PH/CO2 CHANGES

Gaspar Peña-Münzenmayer(1,2), Carolina Añazco(1), L. Pablo Cid(1), Francisco V. Sepúlveda(1,3), María Isabel Niemeyer(1). 1Centro de Estudios Científicos (CECS), Valdivia, Chile; 2Universidad Austral de Chile, Valdivia, Chile; 3Centro de Ingeniería de la Innovación del CECS, Valdivia, Chile.

TASK-2 potassium channel participates in the acid-base homeostasis by two mechanisms: regulation of the HCO_3^- reabsorption in the proximal tubule and modulation of the respiratory frequency in chemosensitive neurons at the retrotrapezoid nucleus (RTN) (Warth et al, 2004; Gestreau et al, 2010). Both functions have been proposed to require the extracellular pH sensitivity of TASK-2 as the principal regulatory mechanism. However, recently our laboratory has reported a new mechanism of regulation of TASK-2 by intracellular pH changes (Niemeyer et al, 2010). In this work we have explored whether the type of changes in intracellular pH brought about by HCO_3^- fluxes could regulate TASK-2. To this end we co-expressed TASK-2 and the renal Na^+/HCO_3^- cotransporter NBCe1-A in HEK-293 cells and recorded Na^+/HCO_3^- and K^+ fluxes in whole cell patch-clamp experiments. Cotransporter current was elicited by exposing the cell to a solution containing 5% CO_2 and 33 mM $NaHCO_3$. Appearance of cotransporter-mediated inward current was always accompanied by TASK-2 current activation. Reversing the flow of $NaHCO_3$ had the opposite effect. As bath solutions containing $CO_2/NaHCO_3$ inhibit the current in the absence of cotransporter, we interpret these results as implying a functional interaction between TASK-2 and NBCe1-A, presumably through an HCO_3^- influx-dependent intracellular alkalinization. Additionally, preliminary results indicate a CO_2-dependent regulation of TASK-2 that would be independent of intracellular pH. Intracellular CO_2/HCO_3^- variations could be important signals regulating TASK-2 in RTN and proximal tubule.

Supported by Fondecyt Grant 1090478, Conicyt Doctoral Grant 24081049, F Fondecyt Postdoctoral Grant 3085021.

S5

L-TYPE CALCIUM CHANNEL β SUBUNITS MODULATE ANGIOTENSIN II RESPONSES IN CARDIOMYOCYTES

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The Angiotensin II regulation of L-type calcium currents in cardiac muscle is controversial. Moreover, the possible role of auxiliary subunit composition of the channels in the Angiotensin II modulation of L-type calcium channels and the molecular mechanism responsible for this modulation has not been explored. For this reason, we used electrophysiology, confocal microscopy and calcium imaging to explore the L-type Ca^{2+}-current response to Angiotensin II and to establish the role of calcium channel β subunits in L-type calcium current modulation by Angiotensin II. In cardiomyocytes, Angiotensin II exposure induces rapid inhibition of L-type Ca^{2+}-current with a magnitude that correlates with the rate of inactivation of the current. Likewise, over-expression of individual β subunit in heterologous systems reveals that the magnitude of Angiotensin II inhibition and $Ca_V1.2$ internalization is dependent on the $Ca_Vβ$ subunit isoform, with $Ca_Vβ1b$ containing channels being more strongly regulated. $Ca_Vβ2a$ containing channels were insensitive to modulation and this effect was partially due to the N-terminal palmitoylation sites of this subunit. Imaging data suggest that the mechanism of Angiotensin II action involves channel internalization. Finally, we show that in intact cardiomyocytes, the magnitude of calcium transients on spontaneous beating cells is modulated by Angiotensin II in a $Ca_Vβ$ subunit manner. These data demonstrate that $Ca_Vβ$ subunits alter the magnitude of inhibition of L-type Ca^{2+}-current by Angiotensin II.

S6

THE $Ca_Vβ$ SUBUNIT PREVENTS RFP2-MEDIATED UBIQUITINATION AND PROTEASOMAL DEGRADATION OF L-TYPE CHANNELS.

Christophe Altier, Agustin Garcia-Caballero, Brett Simms, Haitao You, Lina Chen, Jan Walcher, H William Tedford, Tamara Hermosilla & Gerald W Zamponi.

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It is well established that the auxiliary Cavβ subunit regulates calcium channel density in the plasma membrane, but the cellular mechanism by which this occurs has remained unclear. We found that the Cavβ subunit increased membrane expression of Cav1.2 channels by preventing the entry of the channels into the endoplasmic reticulum–associated protein degradation (ERAD) complex. Without Cavβ, Cav1.2 channels underwent robust ubiquitination by the RFP2 ubiquitin ligase and interacted with the ERAD complex proteins derlin-1 and p97, culminating in targeting of the channels to the proteasome for degradation. On treatment with the proteasomal inhibitor MG132, Cavβ-free channels were rescued from degradation and trafficked to the plasma membrane. The coexpression of Cavβ interfered with ubiquitination and targeting of the channel to the ERAD complex, thereby facilitating export from the endoplasmic reticulum and promoting expression on the cell surface. Thus, Cavβ regulates the ubiquitination and stability of the calcium channel complex.

OP5

WNT-5A AFFECTS SYNAPTIC ACTIVITY THROUGH MEMBRANE DEPOLARIZATION INDUCED BY NITRIC OXIDE IN MAMMALIAN HIPPOCAMPUS.

Jorge Parodi and Nibaldo C. Inestrosa.

Centro de Envejecimiento y Regeneración (CARE), Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.

Introduction. Wnt signaling plays an important role in synaptic activity of the central nervous system. We report here that Wnt-5a increases the amplitude of miniature postsynaptic current (minis) on hippocampal cells, secondary to nitric oxide production. Material and Methods. We obtain electrophysiological recordings from hippocampal neurons obtained from 18 day pregnant rat embryos. Currents were measured with the whole-cell patch-clamp. Spontaneous minis, were isolated by the addition of TTX (0.1μM, Sigma) and MgCl2 (2mM) to the external solution, Wnt-5a was obtained from conditioned medium of expressing cells.
Results. We found that acute application of Wnt-5a, reduces a Kv potassium current and generated a change in the membrane resistance (control: 493±33MΩ to Wnt-5a: 685±60MΩ). These effects are blocked by the nitric oxide inhibitor 7-NI (1 μM) or by the addition of sFRP (a soluble blocker of Wnt). The effect of Wnt-5a is concentration depended (dilution 0.001 to 0.1). The alterations in the membrane resistance induced synaptic activity changes. Wnt-5a increases the frequency of minis (control: 1.9±0.15 Hz and Wnt-5: 4.1±0.5 Hz) and the amplitude (control: 53±7 pA and Wnt-5a: 89±6.7

pA) all these effects were prevented by 7-NI and sFRP.
Discussion. These results suggest that Wnt-5a modulates different aspects of synaptic activity through nitric oxide production in the mammalian central nervous system.

Supported by BASAL project (CONICYT-PEFB12/2007)

Structure II

S7

STOCHASTIC BEHAVIOR OF ION CHANNELS: NUMERICAL SIMULATION AND CONSEQUENCES FOR NEURONAL EXCITABILITY.

Patricio Orio, Andrés Canales & Erick Olivares
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Stochastic opening and closing of ion channels (channel noise) is a major source of variability in the behavior of neurons and their integration properties. The effect of channel noise on action potential timing and threshold variability has been extensively studied and its biological relevance is undisputed. In the case of more complex phenomena, such as sub-threshold oscillations and bursting, it has been shown that noise interacts with non-linear dynamics shaping oscillatory regimes. We study the effect of channel noise in a generalized model of bursting driven by a temperature-modulated sub-threshold oscillation, comparing its effects with that of additive white noise introduced into the current equation (current noise). In the vicinity of a bifurcation between chaotic regimes, channel noise induces regular bursting firing patterns more efficiently than current noise. Thus, channel noise can shape dramatically the sub-threshold dynamics of neurons and their integration properties, in a way that cannot be explained by current noise.
We are also studying the implementation of a diffusion approximation (DA) algorithm for stochastic channel simulation. The DA algorithm permits a much faster simulation than explicit simulation of Markov chains, but it is only applied for two-state channels because a scheme with more states implies obtaining the square root of a matrix. Here I show that this matrix can obtained analytically, thus allowing for faster simulation. The performance of the DA is compared with Markov chain modeling in two different models for action potential generation. The DA presented may be suitable for its implementation in dynamic clamp experiments.

Supported by Fondecyt 11090308. CINV is a Millennium Institute.

S8

CONKUNITZINS – Kv CHANNEL BLOCKERS WITH SERINE PROTEASE INHIBITOR ACTIVITY FROM THE VENOM OF CONUS STRIATUS.

Rocio K. Finol-Urdaneta(1), Stefan Becker(2), Heinrich Terlau(3), Robert J. French(1).
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The so-called Kunitz-domain proteins contain three highly conserved disulfide bridges and include well-characterized serine protease inhibitors like the bovine pancreatic trypsin inhibitor. These protease inhibitors show striking structural similarities with certain venom peptides that block voltage-gated K channels (Lancelin et al. 1994, Nat. Struct. Biol. 1:246-250). Conkunitzin-S1 (Conk-S1) and Conk-S2 from the venom of Conus striatus show a backbone fold similar to that of the canonical Kunitz inhibitors, but with only 2 disulfide bonds to stabilize their structure. Conk-S1 inhibits Shaker potassium currents and Kv1.7 mammalian channels. In rodents, Conk-S1 enhances insulin secretion, in a glucose-dependent fashion, likely via block of Kv1.7 mediated currents (Finol-Urdaneta et al.). Here, we demonstrate that Conk-S1 (at pH 7.6 and 4.2) and Conk-S2 (at pH 4.2) inhibit trypsin activity. Conkunitzins are larger than most pore-blocking conopeptides (Conk-S1: 60, and Conk-S2: 65 amino acids), with several basic and aromatic residues which could potentially interact with cation-binding surfaces. The substitution Y51A, in Conk-S1, both abolishes protease inhibition and decreases affinity for hKv1.7 channels. Voltage-clamp studies confirm Conk-S1's selective targeting of Kv1.7 channels and suggest that its inhibitory mechanism may rely on a combination of direct pore block and gating modulation. Alanine replacement of either of two basic residues from Conk-S1 results in increased IC50 (K21A: wt and pore mutant H341D; or R34A: H341D only). Additionally, a gating shift, likely associated with protonation of H341, is inhibited by Conk-S1. Together, these results suggest that inhibition of trypsin and Kv1.7 block are mediated by overlapping parts of Conk-S1.

Acknowledgements: We are grateful to Nina Strüver and Catherine Diao for technical assistance. Support was provided by the Canadian Institutes of Health Research and a BioFuture Prize of the German Ministry of Education and Research.

S9

ROLE OF KCNN4 CHANNELS IN CYSTIC FIBROSIS.

Carlos A. Flores, Viviana Bustos, Texia Riquelme, Pablo Cid & Francisco V. Sepúlveda.
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Cystic fibrosis (CF) is characterized by a deficience in chloride transport due to mutations in the gene coding for the CFTR channel. The impaired transport produce a deficient hidration of mucosa, diminished secretion of macromolecules and electrolyte sweat loss. Some of these tisular defects can develop into obstructive episodes specially in airways, intestine and pancreas, that often are lethal. CF disease is normally accompained with inflammatory symptoms, that could be expressed subclinically. The KCNN4 calcium activated potassium channel can regulate chloride secretion in the intestine and also participates in the activation of several cells of the inmunne system. To evaluate the role of KCNN4 in CF we generated a double mutant animal with CF (ΔF508 Cftr mutation) and null for KCNN4. We observed that the double mutant animals have a reduced lethality compared with CF mice.

Ussing chamber experiments of CF and double mutant intestines determined that there was no parallel chloride conductance compensating for the absence of CFTR that can explain the dimished lethality in the double mutant animals. Moreover weight rates, water fecal content and mucous accumulation, were similar in CF and their double mutant littermates. We observed that CF small intestine had a 4 fold increase in the number of mast cells when compared to controls (0.35 ± 0.28 v/s 1.46 ± 0.4 mast cells/field, respectively). The mast cell count was reversed near control numbers in double mutant animals (0.4 ± 0.19 mast cells/field). Pharmacological inhibition of KCNN4 in CF mice by injection of the specific blocker TRAM-34, reduced small intestine mast cells (0.17 ± .0.11 mast cells/field).

KCNN4 inhibition reduced CF mice lethality by a mechanism that might involve the immune response and not transport properties of the affected epithelial tissues. The use of specific KCNN4 blockers can be of use for future therapy in human patients.

Funded by FONDECYT 11100408. CECS is a Milenio Institute.

OP 6

TRANSIENT RECEPTOR POTENTIAL MELASTATIN CATION CHANNELS INVOLVED IN CELL DEATH.

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Endothelial dysfunction is decisive in cardiovascular diseases progression. Moreover, neuronal death is linked to several neurodegerative diseases. Lipopolysaccharide-induced ROS-mediated endothelial and neuronal cell death are a main feature observed in inflammation secondary to endotoxemia, becoming the leading causes of death among hospitalized critically ill patients in intensive care units. However, the molecular mechanism underlying LPS-induced endothelial and neuronal cell death is not well understood.

Transient receptor protein melastatin-4 (TRPM4) is a non-selective cationic channel associated to cell death which is expressed in endothelium and modulated by ROS. Our results showed that suppression of TRPM4 expression by siRNA or activity suppression with a dominant negative, were effective in to confer endothelial cell resistance to LPS challenge.

On the other hand, TRPM7 is a calcium channel highly expressed in neurons. We observed that LPS or H2O2 exposure were both able to strongly increase TRPM7 expression. Knockdown of TRPM7 expression using siRNA technology was effective in to protect neurons from the LPS exposure.

We conclude that TRPM4 is critically involved in LPS-induced endothelial cell death. In addition, LPS promotes an abnormal ROS-dependent TRPM7 over-expression, which plays a crucial role in pathological events leading to neuronal death. These findings are useful in sepsis drug design and develop of new strategies for sepsis therapy.

This work was supported by: UNAB DI-33-11/R (FS), FONDECYT: 11080119 (FS), 11080019 (DV), 3090030 (OP).

OP 7

SYNAPTOTOXICITY OF ALZHEIMER BETA AMYLOID CAN BE EXPLAINED BY IT'S PORE-FORMING PROPERTY.

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The working hypothesis of Alzheimer´s Disease (AD) is that excess Aβ either i) binds to membrane receptors affecting their functions, ii) interferes with signaling cascades or iii) directly disrupts neuronal membranes causing pore formation leading to alterations in ionic homeostasis. Although the latter is an attractive hypothesis because it could explain several

effects of Aβ on brain neurons, it has not been demonstrated to occur in brain neuronal membranes. In the present study, we found that synthetic Aβ aggregates were able to localize in synaptic regions and increase intracellular Ca^{2+} by a pore-forming mechanism. Patch clamp experiments in cell-attached mode using Aβ (500-1000 nM) inside the patch pipette showed that Aβ has a rapid, concentration-dependent and potent perforating property in neuronal membranes. Analysis of the charge transferred during the capacitative response indicates that the effect of Aβ was similar to those of gramicidin and amphotericin, two other peptides commonly used to form perforates in neurons (Aβ1-40 = 219±22, Aβ1-42 = 250±24, gramicidin = 235±23, amphotericin = 210±15 fC, n=15). Cell imaging experiments using 500 nM Aβ and ethidium bromide (EtBr, 5 µM) in the patch pipette showed the presence of EtBr inside the neurons after ~ 20 min, thus providing direct evidence that small molecules can pass through these Aβ perforations.

In conclusion, the present results indicate that Aβ perforations were critical to induce an increase in intracellular calcium in hippocampal neurons. Furthermore, these results strongly suggest that amyloid pores could be involved in synaptic dysfunction mediated by Aβ aggregates.

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Channels and Disease II

S10

INITIAL MOLECULAR MECHANISMS IN THE PATHOLOGY OF THE ADULT NEURO-DEGENERATIVE DISEASE ALS.

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Amyotrophic lateral sclerosis (ALS) is an incurable disease caused by adult degeneration of cranial and spinal motoneurons (MNs). Mutations in superoxide dismutase (SOD1) are responsible for a proportion of familial ALS by a gain of unknown toxic function(s). The only drug that is available to treat ALS is riluzole, which only prolongs patient survival by a few months when started at symptomatic stages. We recently showed that months before MN degeneration and clinical symptoms appear, MNs and interneurons (INs) in acute slice preparations from neonatal hSOD1G93A mice (P4-10) displayed morphological and functional changes, including increases in persistent sodium current (PCNa), excitability, and synaptic neurotransmission (van Zundert et al., 2008 Journal Neuroscience). These functional changes in neuronal activity are the earliest yet reported for the hSOD1G93A mouse, and are present 2-3 months before MN degeneration and clinical symptoms appear in these mice. During my talk I will also present new data obtained with a novel in vitro ALS model system and discuss the importance of distinguishing primary target(s) of mutant SOD1 from secondary effects and compensatory mechanisms to allow the development of therapeutic drugs in ALS and other neurodegerative diseases such as Alzheimer disease and Parkinson´s disease

S11

DISEASE MECHANISMS IN EAST SYNDROME WITH MUTATIONS IN KCNJ10

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INTRODUCTION

Mutations in KCNJ10 lead to epilepsia, ataxia, sensorineural hearing loss and a tubulopathy, with hypomagnesemia and mild salt loss reminiscent of Gitelman's syndrome. All mutations studied so far affect channel activity and/or trafficking.

OBJECTIVES

KCNJ10 mutations were expressed in mammalian cells and oocytes to investigate the properties of mutant channels in more detail. Previous data had suggested that some mutations are partially functional and we hypothesized – based on structural considerations, that these mutations impinge on channel regulation. Animal models were studied to elucidate physiological aspects of KCNJ10 function.

METHODS

KCNJ10 WT and R65P, G77R and R175Q were expressed in CHO cells and oocytes and assessed using whole cell patch-clamp, single channel recordings and macropatch recordings.

RESULTS

All mutations investigated strongly reduced the open probability of KCNJ10 and KCNJ10/KCNJ16 heteromeric channels. R65P led to a reduction in mean channel open time, and all mutations shifted the pH-dependence of activation to more alkaline pH values. R175Q showed a strong reduction in PIP2 affinity.

Animal models for KCNJ10 deficiency highlight physiological functions of KCNJ10 in the kidney, inner ear and brain.

DISCUSSION

These results provide a framework to understand, and possibly modify, loss of function in patients with mutations in KCNJ10. In the kidney, KCNJ10 is critically involved in salt reabsorption via NCC, both by providing pump-leak coupling for the Na+/K+-ATPase as well as by providing the driving force for chloride exit through ClC-K/barttin channels. KCNJ10 is critical for stabilizing the neuronal membrane voltage by allowing glial cells to remove K+ from

sites of high neuronal activity, and it is critical for the generation of the endocochlear potential.

CONCLUSION

Several mutations in KCNJ10 found in EAST syndrome patients affect channel regulation by intracellular pH. Pharmacological intervention could therefore restore channel function in these patients.

OP 8

TRPM4 ENHANCES CELL PROLIFERATION THROUGH UP-REGULATION OF THE β-CATENIN SIGNALING PATHWAY. Marcelain K, Armisén R, Simon F, Tapia JC, Toro J, Quest AF, Stutzin A.

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Altered expression of some members of the TRP ion channel superfamily has been associated with the development of pathologies like cancer. In particular, TRPM4 levels are reportedly elevated in diffuse large B-cell non-Hodgkin lymphoma, prostate, and cervical cancer. However, whether such changes in TRPM4 expression may be relevant to genesis or progression of cancer remains unknown. Here we show that reducing TRPM4 expression decreases proliferation of HeLa cells, a cervical cancer-derived cell line. In this cell line, constitutive TRPM4 silencing promoted GSK-3β-dependent degradation of β-catenin and reduced β-catenin/Tcf/Lef-dependent transcription. Conversely, overexpression of TRPM4 in T-REx 293 cells (a HEK293-derived cell line) increased cell proliferation and β-catenin levels. Our results identify TRPM4 as an important, unanticipated regulator of the β-catenin pathway, where aberrant signaling is frequently associated with cancer.

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Physiology and Regulation III

S12

THE ROLE OF TRPM8-DEPENDENT COLD THERMORECEPTORS OF THE CORNEA AS REGULATORS OF THE SURFACE WETNESS OF THE EYE.

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The neural mechanisms involved in detecting dryness of the ocular surface are unclear. We hypothesized that thermal receptors activated by evaporation-induced cooling serve as 'humidity detectors' to regulate basal tearing rate. By using extracellular recording of nerve terminal impulse activity of intact cold thermoreceptors of the mouse cornea in vitro for the first time, we show that cold-sensitive nerve endings display a background impulse activity that changes significantly with the small oscillations in corneal temperature occurring during regular interblink periods. The high sensitivity of corneal cold thermoreceptors depends critically on the expression of the cold-transducing channel TRPM8. In $TRPM8^{-/-}$ mice, corneal cold thermoreceptors are silent and insensitive to cooling and menthol. In parallel, the basal tearing rate is markedly reduced in $TRPM8^{-/-}$ mice, while they conserve intact the irritative tearing response mediated by TRPV1- and TRPA1-expressing corneal nociceptors. In $TRPA1^{-/-}$ mice, cold sensitivity and tearing rate are very similar to wild type animals. Likewise, in human subjects basal tear flow decreased by warming of the cornea and raising its temperature from 33ºC to 36ºC, a temperature that silences cold receptor impulse activity and reduces tearing in mice. These findings indicate that TRPM8-dependent ongoing activity in corneal cold receptors provides an essential tonic sensory input to maintain basal tear fluid secretion, suggesting that cold receptor activity plays an important role in the regulation of ocular surface dryness (Parra et al., 2010).

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Parra A, Madrid R, Echevarria D, Del OS, Morenilla-Palao C, Acosta MC, Gallar J, Dhaka A, Viana F, Belmonte C (2010) Ocular surface wetness is regulated by TRPM8-dependent cold thermoreceptors of the cornea. Nat Med 16:1396-1399.

S13

TITLE: HOP-DIFFUSION ON COLD SENSING CHANNELS, A KISS THAT'S SURE TO LINGER.

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TRPM channels have been described not exclusively at the plasma membrane (PM), but also at intracellular membranes where its role is not understood. Knowledge regarding channel intracellular localization, trafficking, and recruitment to the PM represent an important

piece of information needed to integrate channel activity with more complex cellular responses.
TRPM8 is a non-selective cation channel expressed on a subset of peripheral neurones, and is the molecular machine that allow us to detect cold signals from our surroundings. There is growing evidence linking TRPM8 to the development and maintenance of cold allodynia and hyperalgesia in the somatosensory system. Additionally, TRPM8 has been also identified in different tissues and associated with an important variety of tumors, where its role is not entirely understood.
Some members of the TRP channel family changes their cellular distribution in response to stimulation. Here, we will describe membrane/near-membrane dynamics of TRPM8-GFP containing particles in both, HEK-293T and F-11 transfected cells. 2D and 3D trajectories together with the velocity of individual protein containing vesicles were obtained by Total Internal Reflection of Fluorescence Microscopy (TIRFM) and single particle tracking (SPT). Trajectories were analyzed by plotting the mean-square displacement against time. Four characteristic types of motion were observed: (a) stationary; (b) simple Brownian diffusion; (c) directed diffusion; and (d) confined diffusion, in which particles undergoing Brownian diffusion are confined within a limited area. Our data suggests that TRPM8, when inserted into the plasma membrane, is confined into small domains of about 3 um in diameter, in which receptor molecules resides in the time scale of 2-8 s. In the absence of stimuli TRPM8 vesicles constantly move along a network that cover the plasma membrane, periodically stopping, most often just briefly. Stimulation halted this hop-diffusion probably by stabilizing TRPM8 channels, as a result, release from plasma membrane became significantly slower. This slower release of TRPM8 determined the overall increase of available receptors.

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S14

HYDROGEN PEROXIDE REMOVES TRPM4 CURRENT DESENSITIZATION CONFERRING INCREASED VULNERABILITY TO NECROTIC CELL DEATH.

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Necrosis is associated with 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 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 Cys(1093) 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 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 re-established 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 knockdown.

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OP 9

CONTRIBUTION OF N-GLYCOSYLATION TO THERMAL AND CHEMICAL SENSITIVITY OF TRPM8 ION CHANNEL IN RECOMBINANT AND NATIVE SYSTEMS.

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TRPM8, an ion channel activated by cold, cooling compounds and voltage, is the main molecular entity responsible for cold transduction in somatosensory neurons. Activation of TRPM8 by cold and menthol takes place through shifts in voltage-activation curve, allowing channel opening at physiological membrane potentials. Here we studied the N-glycosylation occurring at the pore loop of TRPM8, by using electrophysiological and calcium imaging tools. This post-translational modification has been described in several ion channels, and could play an important role in their gating and transport. We found that the unglycosylated TRPM8 mutant (N934Q) expressed in HEK293 cells displays marked functional differences compared with the wild-type channel, including a shift in the threshold of temperature activation and a reduced response to menthol and cold stimuli. Electrophysiological experiments confirmed that these effects are due to a shift in the voltage-dependence of TRPM8 activation towards more positive potentials. Treatment with tunicamycin, induced a similar reduction in the responses of TRPM8 to cold and menthol, mimicking the behaviour of the unglycosylated mutant N934Q. By using tunicamycin we evaluated the effect of the N-

glycosylation on the responses of trigeminal sensory neurons expressing TRPM8. Suppression of glycosylation also affects the function of native TRPM8, causing an important shift of the temperature threshold of cold-sensitive thermoreceptor neurons. Our results suggest that this post-translational modification of TRPM8 is an important mechanism modulating cold-thermoreceptor function, that must be considered in order to understand the diferences between mean temperature threshold of recombinant and native TRPM8 ion channels.

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OP10

NEAR-MEMBRANE DYNAMICS OF TRPM8 CHANNELS AND THE EFFECT OF AGONIST STIMULATION

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Introduction. Modulation of TRP channel distribution and availability by vesicular translocation has been reported as a regulatory mechanism for TRP channel function. Plasma membrane (PM) vesicle insertion regulates the number of functional thereby dictating total ionic flux and the cellular consequences of TRP channel function. These consequences will depend on both, the amplitude of current and its ionic composition. Na^+ current drives membrane depolarization and may be a trigger for opening other voltage-dependent ion channels, whereas Ca^{2+} influx drives fundamental cellular signaling cascades that control secretion, protein translocation, and vesicular fusion. Therefore, a tight control of TRP channel trafficking and delivery to various membrane compartments is thus a potentially critical step in regulating the flow of sensory information. Here we show whether agonist treatments affect the membrane dynamics of the cold and menthol receptor TRPM8. Methods. TRPM8-GFP fusion channels were transfected on F11 cells. Through-the-objective TIRF microscopy was used to perform single particle tracking and to record changes in mobility. As the tracking and subsequent analysis of the MSD was slow and tedious, we build our own automatized software for image analysis. Results. We present here a detailed diffusion analysis based on single particle measurements. Our data clearly show that TRPM8 agonists stimulate an increase in receptor density at the PM, and the effect on single vesicle dynamics. These changes are calcium-dependent. Importantly, by the use of the selective blocker BCTC we show that this process is dependent on TRPM8 channel activity. Conclusion. We picture that stimulation of vesicle exocytosis by TRPM8-mediated Ca2+ flux may increase cell-surface expression in a positive feedback loop. Such amplification power may represent a central theme in their function.

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OP11

FUNCTIONAL EXPRESSION OF THE A7 AND A4-CONTAINING NICOTINIC ACETYL-CHOLINE RECEPTORS ON THE NEONATAL RAT CAROTID BODY.

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The carotid bodies (CBs) are chemosensory organs that respond to hypoxemia with transmitter neurosecretion, leading to respiratory reflex response. It has been proposed that acetylcholine (ACh) is a key regulator of transmitter release through activation of presynaptic nicotinic ACh receptors (nAChRs). Since the identity of such nAChRs is currently unknown we studied the functional presence of the nAChR subtypes present in this system.

Rats CBs were placed in a recording chamber for electrochemical recordings or enzymatically disassociated for patch-clamp studies on isolated cells. Fast nicotine (1mM) superfusion increases catecholamine release (CAeff) from intact CBs. This response was diminished reversibly by the non-selective nAChR blocker hexamethonium, by the selective α7 blocker α-bungarotoxin and by the α4-containing nAChR blocker erysodine.

In isolated CB cells the nAChR agonists nicotine, ACh and cytisine all evoke inward currents with similar potencies. The nicotine-evoked current was fully blocked by mecamylamine and partially inhibited by α-bungarotoxin or erysodine. However, the combination of both α-bungarotoxin an erysodine failed to suppress this response. Immunodetection studies confirm the presence of α7 and α4 subunit in isolated dopaminergic CB cells.

Our results show that activation of α7 and α4-containing nAChR subtypes regulate catecholamine release from intact CB due to activation of fast inward currents expressed in chemoreceptor cells. Therefore, our results support the role of ACh as a presynaptic regulator of the carotid body system.

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Data Blitz Session

DB1

A NOVEL MECHANISM FOR VOLTAGE-DEPENDENCE IN THERMO-VOLTAGE SENSITIVE CHANNEL (TRPV1).

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TRPV1 is a thermoTRP channel that presents a fully temperature activation beyond of 42 °C at positive membrane voltage. Experimental evidences show that TRPV1 posses a structural organization similar to the K+ voltage-dependent ion channels (Kv). Nevertheless TRPV1 is weakly voltage dependence (0.7 e0) and it have just one arginine located in the fourth transmembrane segment (S4), in contrast with Kv channel (12 e0 gating charges). We hypothesize that the arginine 557 (located in the S4 segment) is the charge responsible of voltage-dependence channel activation. Using site-directed mutagenesis by PCR we neutralize this positive charge: R557A. The mutated channel was expressed in oocytes and the currents were elicited by voltage-clamp techniques (macro-patch and cut open vaseline-clamp). TRPV1-R557A channel in response to a square voltage protocol (-160 to 200 mV) shows a classical outward rectifying macroscopic current as a wild-type channel. The slope value obtained from the Boltzmann equation fit (corresponding to total gating charge) was similar to Wt-channel (~ 0.7 e0). However, a single channel analysis showed that the mutation alters the permeations properties of the channel, presenting a weakly inward rectification on the instantaneous current-voltage curve, in contrast to the wild-type that present a linearly relationship. These results suggest that the arginine 557 is not involved with gating voltage activation of these channels, furthermore seems that the R557 is more relative with the permeation pathway, mediated by a possible electrostatic interaction with the aspartate E570 in the fifth segment. These findings could be a new way that a six-segment ion channel senses the voltage changes across the membrane.

DB2

ISOLATION, CLONING AND CARACTERIZATION OF XCAT-1: A NEW TRP CHANNEL FROM XENOPUS LAEVIS OOCYTES.

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TRP channels superfamily is involved in various processes ranging from sensory activity to epithelial transport. There are TRP channels in eukaryotes, from yeast to mammals, however some physiological functions of all of them remain unclear. Xenopus oocytes have been widely used as expression systems for the study of transmembrane proteins (particularly ionic channels). However, the identification and characterization of endogenous channels has not been fully studied. Using Gene Bank database with a sequence of xCaT-1 a putative epithelial calcium transporter from Xenopus laevis we had cloning a new TRP channel from Xenopus leavis oocytes (Acc. Nº: AB085630). The sequence predicts a six transmembrane protein homologous to TRP family memmbers. The heterologous expression in HEK-293 cells of XCAT-1 identified a 75 KDa protein. Imaging studies determine a poor localization of the protein in plasmatic membrane and it found mainly associated with endoplasmic reticulum. However we detected the voltage activation activity of this channel by the technique of reconstitution in artificial planar lipid bilayers. Through this study attempts to contribute to a deeper understanding of these real tools such as cell Xenopus laevis oocytes and deepen the knowledge on protein-type TRP channels.

Financing: Fondecyt Proyect # 1070049.

DB3

TRP ION CHANNELS ON THE UNICELLULAR ALGAE *Chlamydomonas reinhardtii*.

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Introduction. Transient Receptor Potential ion channels are important in sensory physiology; TRP channels enable moving animals to sense diverse stimuli such as light, pressure, smell, taste, sound, and temperature. Sensory modalities are fundamental for navigation through an ever changing environment. Members of the TRP channel superfamily can be found in insects and mammals, just one has been reported to be active in a unicellular organism (TRPY, in yeast vacuolae), and none in higher plant cells. At present, TRP channels are mostly studied on mammalian cells, however, they were originally cloned from Drosophila melanogaster, where they form part of the phototransduction machinery. As we are interested on the evolution of TRP channel proteins as part of sensory systems we choose the unicellular aglae *Chlamydomonas reinhardtii* as a model. *Chlamydomonas* is a motile single celled green algae of about 10 micrometers in diameter, that swims helped by its two flagella. These algae are commonly found in soil and fresh water. They have a large chloroplast making them able to use photosynthesis as energy source, and an "eyespot" that senses

light and is composed by a few Channel-rodhopsin (CHR1 and CHR2) molecules. The electrical signals from *Chlamydomonas* have been studied for a long time, focused primarily on Channel-rodopsin activity, cellular phototaxis, and flagelar movement. After the release of *Chlamydomonas* genome sequence in late 2007, more than 60 putative ion channels has been reported as probable gene products. Methods. *Chlamydomonas* cell lines cc-124 and cc-503 were grown under 12h light/dark periods and constant agitation. Current recordings of light response were performed on a population of 1 million chlamy cells using a custom made current amplifier. Calcium imaging of temperature response was performed using a StepOne real-time pcr thermocycler (Applied Biosystems). Results. Our analysis in silico suggested that Chlamydomonas reinhardtii has genomic sequences encoding for TRP ion channels similar to the mammalian TRP channels from the Clasical and Melastatin families. We present here current recordings performed on chlamy cells suggesting the presence of TRP conductances and temperature responses that are affected by known TRP channel blockers.

Financiamiento: FONDECYT 11070190-1110906, PEW Program in Biomedical Sciences, and SeedingLabs.

DB4

$K_{Ca}3.1$ ENHANCES INTRACELLULAR CALCIUM INCREASE INDUCED BY FMLF IN U937 CELLS.

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Upon exposure to chemotactic agents competent immune cells respond by activating chemotaxis, cytokine release and production of reactive oxygen species (ROS). The chemotactic agent fMLF acts on plasma membrane receptors triggering different signaling pathways that induce an increase in $[Ca^{2+}]i$. This increase in $[Ca^{2+}]i$ is due to Ca^{2+} release from intracellular reservoirs as well as influx of extracellular Ca^{2+}. In this work we explored the electrophysiological response of U937 cells (a monocyte-derived cell line) to the fMLF and its impact on the intracellular calcium increase.
The results indicate that upon fMLF stimulation, differentiated U937 monocytes responded by increasing $[Ca^{2+}]i$ paralleled with a significant plasma membrane hyperpolarization. The increase in $[Ca^{2+}]i$ relied on Ca^{2+} release from intracellular reservoirs as well as influx of Ca^{2+}. The observed hyperpolarization enhanced the increase in $[Ca^{2+}]i$ and was found to be due to the activation of a calcium-activated K^+ channel. This current and the calcium increase induced by fMLF is specifically inhibited by clotrimazole and TRAM-34 and enhanced by DC-EBIO, a pharmacological profile compatible with $K_{Ca}3.1$ channels.
In conclusion, we propose that the response of monocytes to fMLF is mediated by $K_{Ca}3.1$ enhancing the inflow of Ca^{2+} by modifying the electrochemical gradient for Ca^{2+} through changes in the membrane potential.

DB5

A METHODOLOGICAL APPROACH TO MONITOR FLUCTUATIONS ON VESICULAR MEMBRANE POTENTIAL.

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Introduction. Synaptic vesicles are intra-cellular vessels carrying neurotransmitters and constitute a fundamental component for inter-cellular coupling during synaptic transmission. Widely studied since early '50s, what really capture our attention was the small information available regarding the contribution of different ion channel conductances to the resting potential of the vesicle. Several ion channel are likely present on vesicular membranes, these include Na^+, K^+, and Cl^- ion channels together with Zn^{+2} transporters. Additionally, there is an important H^+ flow coming from vesicular pumps and neurotransmitter transporters. Moreover, we showed that the conductance of TRPM7 vesicular channels ultimately affect the number of fused vesicles. An obvious question was whether the vesicular TRPM7 channel was active and its possible contribution to the resting potential of the vesicle. Methods. A direct consequence of ion permeation through a membrane separating media with different ionic conditions is a change in the membrane potential. As the structures we want to record are extremely small for the size of a patch pipette, we adapt an optical method to record changes in membrane potential. Our methodological approach uses GFP quenching by the hydrophobic anion dipicrylamine (DpA). DpA molecules are distributed inside the dielectric according to the membrane potential, and whenever DpA and GFP are close enough, GFP fluorescence is quenched. DpA transitions are fast enough to follow action potential. Conveniently, the GFP molecule can be fused to any transfectable marker, making this technique very specific for the organelle we want to study. We set up the preparation of membrane sheets. This technique allow us to have full access to the intact docked vesicle. Briefly, the cells are being prepared on polylysine treated coverslips, after 24hr transfection the preparation is sonicated in the presence of calcium free extracellular ringer solution. All what is left after sonication is a sheet of membrane glued to the coverslip with all the intact machinery associated. Imaging was performed using a laser TIRF microscope coupled to a Hamamatsu Orca 12ER CCD camera. Results. After proper DpA loading, we perfused different solutions and

we were able to record fluorescence fluctuations at the level of single vesicle.

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DB6

THE ROLE OF TRPM4 IN PROSTATE CANCER.

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TRPM4, a member of the melastatin subfamily of TRP channels operates as a Ca2+ activated and voltage-dependent monovalent cation channel that depolarizes the plasma membrane thereby modulating Ca2+ influx through Ca2+-permeable pathways. TRPM4 expression has been found to be elevated in a number of cancers, such as cervical uterine cancer and prostate cancer. It has been suggested that TRPM4 overexpression constitutes a proliferative advantage for these cells. A variety of microarrays analysis studies with prostate cancer patients have shown overexpression of the TRPM4 gene in cancer samples. Therefore, we propose to study whether TRPM4 is relevant in the development and progression of prostate cancer by using the TRAMP model (Transgenic Adenocarcinoma of the Mouse Prostate), and two prostate transgenic mouse models that overexpress the TRPM4 channel or a TRPM4 negative dominant, respectively. Preliminary immunohistochemistry results indicate that overexpression of the TRPM4 channel is related with an increase of the abnormal morphology in mouse transgenic prostate reflected by appearance of hyperplasia, suggesting a potential role of this gene in the progression and development of this type of cancer.

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