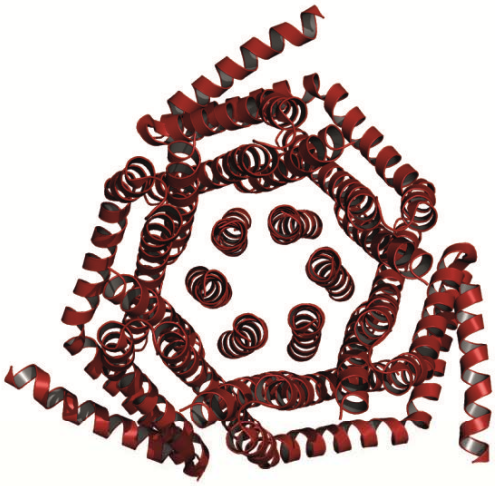

RECI VII 2019

VII CONGRESO RED ESPAÑOLA CANALES IÓNICOS

CÁCERES (SPAIN) 15-17 MAY

Welcome

Dear friends and colleagues:

In the past congress held in Santiago de Compostela in 2017, the Spanish Network of Ionic Channels (RECI) deposited in us the honor of organizing the VII meeting of the RECI in the city of Cáceres, a World Heritage Site. We are undertaking this task with enthusiasm and responsibility, with the difficult objective of maintaining the high scientific level achieved in previous meetings.

This is the first time that RECI meets in the city of Cáceres. The dates, from May 15 to 17, 2019, seem ideal to visit the city and its surroundings, and enjoy a cozy confluence between researchers from all areas of study of ion channels. Thus, next to the Monumental City is the congress venue, the San Francisco Cultural Complex, located in the old convent of San Francisco that was founded by the Franciscan Pedro Ferrer in 1472. There is a wide variety of hotels that are located in the environment of the congress venue and where we have reserved rooms for the attendees.

The main objective of this edition is to achieve a place of transmission, exchange and interaction between researchers of ion channels and provide a welcoming environment for the participation of young researchers in order to arouse their interest in this area.

The scientific program includes 3 plenary lectures, 6 symposia, including 13 conferences (25 + 5 min) and 19 oral communications (12 + 3 min), and sessions for the presentation and discussion of posters. The reception of the congress will take place in the García Matos cloister of the San Francisco Cultural Complex and the congress dinner will be held in Castillo de la Arguijuela, a walled enclosure built in the fifteenth century by Francisco de Obando "el Viejo" located at about 14 km from the city of Cáceres and declared an Asset of Cultural Interest.

We hope that you enjoy your stay in Cáceres, as well as the activities programmed for this RECI meeting.

Sincerely

Juan Antonio Rosado Dionisio (jarosado@unex.es)

President of the Organizing Committee

VII Meeting of the Spanish Network of Ionic Channels

Cáceres, May 15-17, 2019

University of Extremadura

The University of Extremadura was founded in 1973 (Decree 991/1973, 10th May 1973 BOE 18th May 1973). Its history is intimately linked to the region of Extremadura. Initially, the University was made up of the Faculty of Sciences in Badajoz, the University College in Caceres, the University Schools for primary teacher-training in Caceres and Badajoz and the School of Agricultural and Technical Engineering in Badajoz. Since its opening, UEx has seen a rise in student numbers, degrees and centres, expanding to a total of four separate centres, including the campuses in Badajoz and Caceres, the University Centre in Merida and the University Centre in Plasencia.

More than 24,000 under-graduate and post-graduate-level students study at UEx, along with a further 8,000 students who are completing doctoral studies or other courses. 1,500 teachers and more than 800 administrative, technical and service staff work at this University. The Rector is Dr. Píriz Duran and the Governing Body of the University is made up of different professional bodies, such as the Governing Senate and Council and university staff members such as the Vice-Rectors, Management and General Secretary.

Wednesday, May 15, 2019

Hour	Event
15:00	Registration office opens
16:00-16:30	Opening Ceremony
16:30-17:30	**Plenary Opening Lecture** Chair: Ana Gomis Speaker: **Thomas Voets** *(Laboratory of Ion Channel Research, KU Leuven)* Title: **TRP channels in acute and inflammatory pain**
Symposium 1: Mechanotransduction and piezo channels Chair: Ana Gomis	
17:30-18:00	Miguel A. Valverde (Universitat Pompeu Fabra) Title: **Mechano/osmosensitive ion channels in cell migration and invasion**
18:00-18:30	Jorge Fernández-Trillo (Inst. Neurociencias de Alicante) Title: **Piezo2 mediates corneal noxious mechanosensation**
18:30-19:00	Mark Hoon (National Institute of Dental and Craniofacial Research, Bethesda, MD, USA) Title: **Molecular and functional characterization of Piezo2 splicing**
19:00-19:15	Lucia Alonso-Carbajo (Lab Ion Channel Res., KU Leuven-VIB Center for Brain & Dis. Resea., Leuven, Belgium/IBGM-Universidad Valladolid/CSIC) Title: **Activation of the cation channel TRPM3 in sensory nerves induces vasodilation of resistance arteries**
19:15-19:30	Lola Rueda-Ruzafa (CINBIO-Universidad de Vigo) Title: **Increasing temperature activates TREK potassium channels in nodose ganglion neurons**
19:30-19:45	Maria Giustina Rotordam (Nanion Technologies GmbH, Munich, Germany) Title: **A high-throughput patch clamp method to investigate Piezo1 channels from healthy and anaemic red blood cells**
20:00-21:00	**Welcome Reception**

Thursday, May 16, 2019

Hour	Event
Symposium 2: Ion channels: from structure and fuction to drug discovery Chair: Pilar de la Peña	
9:00-9:30	Francisco Barros (Universidad de Oviedo) Title: **The cardiac hERG K^+ channel: a journey from cell biology to structure and function**
9:30-10:00	Donato del Camino Fernández-Miranda (Rheostat, Cambridge, MA, USA) Title: **Targeting TRP channels for drug development**
10:00-10:15	Aravind Kshatri (Universidad de La Laguna) Title: **Fragile X mental retardation protein differentially regulates the biophysical properties of BK channels**
10:15-10:30	Pablo Doñate-Macián (Universitat Pompeu Fabra) Title: **The TRPV4 channel links calcium influx to DDX3X activity and viral infectivity**
10:30-10:45	Fabiana Scornik (Universitat de Girona) Title: **Cardiac sodium current is severely impaired in induced pluripotent stem cell-derived cardiomyocytes from Brugada Syndrome patients**
10:45-11:00	Jesusa Capera (Universitat de Barcelona) Title: **Mitochondrial Kv1.3 channels in apoptosis**
11:00-12:00	**Coffee break and posters**
Symposium 3: Calcium signaling and cell function Chair: Tarik Smani	
12:00-12:30	Angel Nadal (Universidad Miguel Hernández) Title: **Bisphenol-A modulates ion channel expression and function via estrogen receptor β in mouse pancreatic beta cells**
12:30-13:00	María Fernández Velasco (Hospital Universitario La Paz) Title: **Immune system and Ca^{2+} signaling in cardiac cells: Role of NOD1**
13:00-13:15	Carmen Delgado (Biomed Res. Inst. Alberto Sols, CSIC) Title: **Beneficial effects of paricalcitol on cardiac dysfunction and deleterious remodeling after established heart failure**
13:15-13:30	Marta Martín-Bórnez (Inst. de Biomedicina de Sevilla) Title: **Essential role of Orai1 and SARAF in vascular remodeling**
13:30-13:45	Carles Solsona (Universitat de Barcelona) Title: **Calcium dependent Chloride Channels (CaCC) as a target of a bacterial toxin**
13:45-14:00	Ricardo Gómez (Universidad de La Laguna) Title: **NMDAR and BK channels form Ca^{2+} nanodomains in basal dendrites of barrel cortex layer 5 pyramidal neurons to control synaptic excitability and plasticity**
14:00-15:30	**Lunch break**

Symposium 4: Store-operated channels Chair: Carlos Villalobos	
15:30-16:00	Juan A. Rosado (Universidad de Extremadura) Title: **Role of TRPC6 in breast cancer cell Ca^{2+} homeostasis**
16:00-16:30	Carlos Villalobos (IBGM-Universidad de Valladolid/CSIC) Title: **Store-operated channels in pituitary physiology**
16:30-16:45	Isaac Jardín (Universidad de Extremadura) Title: **EFHB regulates store-operated calcium entry, migration and proliferation in breast cancer cells**
16:45-17:00	Lucia G. Gutiérrez (IBGM-Universidad de Valladolid/CSIC) Title: **Reversing Ca^{2+} channel remodeling in colon cancer cells by polyamine biosynthesis inhibition**
17:00-17:15	Isabel Mayoral-González (Inst. Biomedicina de Sevilla) Title: **Dysregulation of ion channels expression associated with the adverse cardiac remodeling due to heart revascularization**
17:15-17:30	José J. López (Universidad de Extremadura) Title: **A new role for adenylate cyclase 8 as modulator of store-operated calcium entry**
17:30-18:30	**Plenary Lecture** Chair: Juan A. Rosado Speaker: **Christoph Romanin** (Johannes Kepler University Linz, Linz, Austria) Title: **Regulation of STIM1 and Orai1 function**
18:30-20:30	Visit to the Monumental city of Cáceres
21:00-23:00	**CONGRESS DINNER**

Friday, May 17, 2019

Hour	Event
	Symposium 5: Cardiac ion channels are not alone Chair: Eva Delpón
9:00-9:30	José Jalife (University of Michigan, MI, USA) Title: **Dysfunction of the Cardiac Kir2.1-Na_V1.5 channelosome trafficking underlies arrhythmia susceptibility in the Andersen-Tawil Syndrome**
9:30-10:00	Eva Delpón (Univ. Complutense de Madrid) Title: **The odds couple: Na_v1.5 and Kir2.x channels**
10:00-10:15	Sendoa Tajada (University of California School of Medicine, CA, USA) Title: **BIN1 induces the formation of T-tubules, Cav1.2 channel clusters and adult-like Ca^{2+} release units in developing cardiomyocytes**
10:15-10:30	Clara Serrano-Novillo (Universitat de Barcelona) Title: **Endoplasmic reticulum-plasma membrane junctions hub the Iks complex**
11:30-11:30	**Coffee break and posters**
	Symposium 6: Drug discovery and ion channel pharmacology **Chair: Asia Fernández**
11:30-12:00	Francisco Nieto López (Universidad de Granada) Title: **Voltage-gated sodium channels as pharmacological targets for neuropathic pain relief**
12:00-12:30	Rosario Gonzalez Muñiz (Inst. de Química Médica, CSIC) **β–Lactam TRPM8 antagonists prevent chemotherapy-induced cold allodynia**
12:30-12:45	Mercè Izquierdo-Serra (Universitat Pompeu Fabra) Title: **Low-throughput evaluation of novel Cav2.1-modulators to treat Hemiplegic Migraine**
12:45-13:00	Diego A. Peraza (Instituto de Investigaciones Biomédicas Alberto Sols (IIBM), CSIC-UAM) Title: **Identification of IQM-266, a novel DREAM ligand that modulates KV4 currents**
13:00-14:00	**Closing Lecture** Chair: Juan A. Rosado Speaker: **Annarosa Arcangeli** (Università degli Studi di Firenze, Italia) Title: **Ion channel conformations regulate intracellular signaling and cell fate in tumors**
14:00-14:30	RECI Assembly
14:30-15:30	**Lunch and Farewell**

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Plenary Speakers

Thomas Voets
TRP CHANNELS IN ACUTE AND INFLAMMATORY PAIN
Laboratory of Ion Channel Research, VIB Center for Brain and Disease Research & KU Leuven Department of Cellular and Molecular Medicine, Leuven, Belgium.

Christoph Romanin
REGULATION OF STIM1 AND ORAI1 FUNCTION
Institute of Biophysics, Johannes Kepler University Linz, Austria

Annarosa Arcangeli
ION CHANNEL CONFORMATIONS REGULATE INTRACELLULAR SIGNALING AND CELL FATE IN TUMORS
Department of Experimental and Clinical Medicine, University of Florence, 50134 Firenze, Italy.

Plenaries

TRP CHANNELS IN ACUTE AND INFLAMMATORY PAIN

Thomas Voets

Laboratory of Ion Channel Research,
VIB Center for Brain and Disease Research & KU Leuven Department of Cellular and Molecular Medicine, Leuven, Belgium

Transient receptor potential (TRP) channels form a superfamily of cation channels involved in a wide variety of physiological and pathophysiological processes. In particular, several mammalian TRP channels are expressed in sensory neurons, where they act as molecular sensors of thermal and chemical cues. Using single and combined mouse knockout models, we have investigated the role of various somatosensory TRP channels in acute pain sensation, and assessed how their expression and function changes in inflammatory conditions associated with hyperalgesia and ongoing pain. We further explore how pharmacological targeting of specific TRP channels may be developed to treat a variety of chronic pain conditions.

REGULATION OF STIM1 AND ORAI1 FUNCTION

Christoph Romanin

Institute of Biophysics, Johannes Kepler University Linz, Austria

Ca^{2+} entry into the cell via store-operated Ca^{2+} release activated Ca^{2+} (CRAC) channels triggers diverse signaling cascades that affect cellular processes like cell growth, gene regulation, secretion and cell death. These store-operated Ca^{2+} channels open following depletion of intracellular Ca^{2+} stores and their main features are fully reconstituted by the two molecular key players: the stromal interaction molecule (STIM) and Orai. STIM represents an ER-located Ca^{2+} sensor, while Orai forms a highly Ca^{2+} selective ion channel in the plasma membrane. Functional as well as mutagenesis studies together with structural insights about STIM and Orai proteins provide a molecular picture of the interplay of these two key players in the CRAC signaling cascade. This talk highlights the main experimental advances in the understanding of the STIM1-Orai choreography thereby establishing a portray of key mechanistic steps in the CRAC channel signaling cascade. The focus will be laid on gain- and loss-of-function mutants of both STIM1 and Orai in providing a mechanistic understanding and biophysical characterization of their activation state.

ION CHANNEL CONFORMATIONS REGULATE INTRACELLULAR SIGNALING AND CELL FATE IN TUMORS

Annarosa Arcangeli

Department of Experimental and Clinical Medicine, University of Florence, 50134 Firenze, Italy.

Adhesive interactions among cells and between cells and the extracellular matrix (ECM) determines the choice between different cell fates, and is accompanied by substantial changes in ion transport. This is particularly relevant in tumors, where different constituents of the tumor microenvironment, including ECM proteins, are known to modulate malignancy of tumor cells. Moreover, recent evidence indicates that several ion transport mechanisms are dis-regulated in cancer cells. The widest evidence regards the bidirectional interplay occurring between integrin receptors and K^+ channels. These proteins can form signaling hubs that regulate cell proliferation, differentiation and migration. This occurs both in normal and neoplastic tissue, although with different underlying mechanisms. Recent results show that the physical interaction with integrins determines the balance of the open and closed K^+ channel states, and individual channel conformations regulate distinct downstream pathways. We propose a model of how these mechanisms regulate proliferation and metastasis in cancer cells. Such findings open a completely novel scenario, since neoplastic establishment and progression could be modulated by targeting specific ion channel conformations.

Symposium 1: Mechanotransduction and piezo channels

Chair: Ana Gomis

Invited

MECHANO/OSMOSENSITIVE ION CHANNELS IN CELL MIGRATION AND INVASION.

Miguel A. Valverde.

Universitat Pompeu Fabra, Barcelona, Spain.

In order to progress towards an invasive and/or metastatic phenotype cancer cells need to migrate through the extracellular matrix and often through narrow and tortuous extracellular routes. This process imposes important physical restrictions and generates mechanical stimuli on the migrating cells. Besides, during primary tumor growth cells also modify their environment at times increasing tumor stiffness, which, in turn, correlates with activation of the mechanosensitive transcription factors YAP and TAZ, and higher metastatic potential. Furthermore, epithelial cancer cells metastasizing in the brain protect themselves with the production of serpins. However, there are still several fundamental questions that remain to be answered: How do cells respond to confinement? How do they choose among the different pathways for migration? What are the mechanisms that trigger the secretion of serpins? We propose that all these questions share a common element, the participation of mechano/osmosensitive (MOS) channels.

Invited

PIEZO2 MEDIATES CORNEAL NOXIOUS MECHANOSENSATION

Jorge Fernández-Trillo

Instituto de Neurociencias, Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas, 03550 San Juan de Alicante, Spain.

Most of the principal physiological processes required for living organisms survival occur through detection and integration of mechanical stimuli. Activation of sensory terminals of low threshold mechanoreceptors evokes tactile and proprioceptive sensations, while sensations evoked by activation of nociceptors are painful. Although significant progress has been made in understanding the cellular and molecular transduction mechanisms in touch receptors and proprioceptors, the mechanisms underlying transduction of mechanical forces by nociceptors are poorly understood.

The Piezo2 channel is mainly responsible for touch sensation and proprioception. Interestingly, mechanosensitivity of skin nociceptors is unaffected in Piezo2-deficient mice. Newly, it has been suggested that Piezo2 is required for mechanical allodynia but has only a partial role in physiological painful mechanosensation.

Activation of corneal mechanoreceptors evokes painful sensations but the transducing channels involved in their mechanosensitivity have not been identified yet. We provide direct evidence for a role of Piezo2 ion channels in mechanotransduction at corneal sensory endings signaling mechanical pain.

Invited

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF PIEZO2 SPLICING

Mark Hoon

National Institute of Dental and Craniofacial Research, Bethesda, MD, USA.

Piezo2 is a mechanically gated ion-channel found in many tissues including touch sensory neurons. We uncovered that the Piezo2 gene is extensively spliced producing multiple Piezo2 isoforms with different properties. Interestingly, human and mouse sensory neurons express many different spliced forms of Piezo2 while non-sensory cells predominantly express a single isoform. We also found that different classes of sensory express different Piezo2 variants. Characterization of some of these Piezo2 variants demonstrates that splicing gives rise to ion-channels with different ion-permeability, sensitivity to calcium, and inactivation kinetics. These results suggest that splicing is a mechanism permitting a single gene to encode many receptors which are tuned to respond to multiple mechanical stimuli.

S1O1

Activation of the cation channel TRPM3 in sensory nerves induces vasodilation of resistance arteries.

Lucía Alonso-Carbajo[1,2], Yeranddy A. Alpizar[1], Justyna B. Startek[1], José Ramón López-López[2], María Teresa Pérez-García[2], Karel Talavera[1]

[1]Department of Cellular and Molecular Medicine, Laboratory of Ion Channel Research, KU Leuven; VIB Center for Brain & Disease Research, Leuven Belgium. [2]Departamento de Bioquímica y Biología Molecular y Fisiología, Instituto de Biología y Genética Molecular, Universidad de Valladolid y CSIC, Valladolid, Spain.

The Transient Receptor Potential Melastatin 3 (TRPM3) is a Ca^{2+}-permeable non-selective cation channel activated by the neurosteroid pregnenolone sulfate (PS). This compound was previously shown to contract mouse aorta by activating TRPM3 in vascular smooth muscle cells (VSMC), and proposed as therapeutic modulator of vascular functions. However, PS effects and the role of TRPM3 in resistance arteries remain unknown. Thus, we aimed at determining the localization and physiological role of TRPM3 in mouse mesenteric arteries. Real-time qPCR experiments, anatomical localization using immunofluorescence microscopy and patch-clamp recordings in isolated VSMC showed that TRPM3 expression in mesenteric arteries is restricted to perivascular nerves. Pressure myography experiments in wild type (WT) mouse arteries showed that PS vasodilates with a concentration-dependence that was best fit by two Hill components (effective concentrations, EC_{50}, of 14 and 100 μM). The low EC_{50} component was absent in preparations from *Trpm3* knockout (KO) mice and in WT arteries in the presence of the CGRP receptor antagonist BIBN 4096. TRPM3-dependent vasodilation was partially inhibited by a cocktail of K^+ channel blockers, and independent from β-adrenergic signaling. We conclude that, contrary to what was found in aorta, PS dilates mesenteric arteries, partly via an activation of TRPM3 that triggers CGRP release from perivascular nerve endings and a subsequent activation of K^+ channels in VSMC. We propose that TRPM3 is implicated in the regulation of the tone of resistance arteries and that its activation by yet unidentified endogenous damage-associated molecules lead to protective vasodilation responses in mesenteric arteries.

Keywords: TRPM3; sensory nerve; vasodilation; pregnenolone sulfate; CGRP.

Acknowledgements: this work was supported by grants from the Fund for Scientific Research Flanders (G.0C68.15), the Ministerio de Economía y Competitividad (MINECO, BFU2016-75360-R) and the Junta de Castilla y León (VA114P17).

S1O2

Increasing temperature activates TREK potassium channels in nodose ganglion neurons.

Lola Rueda-Ruzafa[1], Salvador Herrera[1], Ana Campos[1], Alba Rodríguez[1], Jose A. Lamas[1].

[1]Laboratory of Neuroscience, Biomedical Research Centre (CINBIO), University of Vigo, 36310, Vigo, Spain.

The nodose ganglion (NG) is composed of A, Ah and C-type neurons which have an important role in regulating visceral afferent function. Neurons of the NG express two-pore domain potassium (K2P) channels, mainly TREK-1 and TRESK [1]. The aim of this study was to investigate whether native TREK channels were activated by physiological temperature like reported for heterologously expressed channels.

To study TREK currents, perforated-patch and single channel recordings were carried out in mouse nodose neurons in culture. Initially, neurons were recorded at room temperature and afterwards the temperature of the bath solution was increased to physiological levels of ~ 37 ºC and/or to ~ 50 ºC.

The increase of temperature induced a hyperpolarization when reaching ~ 40 ºC, this effect was blocked by fluoxetine, known to block TREK channels. At higher temperatures a membrane depolarization was observed. The hyperpolarization was about -14, -16 and -12 mV for A-, Ah- and C-type cells respectively. In voltage-clamp, physiological temperature induced outward currents of about 94, 105 and 80 pA in A-, Ah- and C-type cells respectively. All these responses were abolished by fluoxetine. Additional single-channel experiments confirmed that TREK channels are essentially closed at room temperature, but strongly activated by physiological temperature.

Altogether, our results highlight an important role of TREK channels in setting neuronal excitability at physiological temperature levels. In this way, these K2P channels might compensate painful responses to temperature changes by acting as neuroprotective channels.

Keywords: K2P, TREK channels, physiological temperature, vagal afferent neurons

Acknowledgments: Funds from Spanish Government (MINECO, BFU2014-58999-P), Galician Government (GPC2015/022) and European Regional Development Fund (FP7-316265-BIOCAPS). Partially supported with FEDER Funds.

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S1O3

A high-throughput patch clamp method to investigate Piezo1 channels from healthy and anaemic red blood cells

Maria Giustina Rotordam[1,2], Elisa Fermo[3], Nadine Becker[1], Niels Fertig[1], Andrea Brüggemann[1], Stéphane Egée[4], Paola Bianchi[3], Lars Kaestner[2,5] and Markus Rapedius[1]

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Red Blood Cells (RBC) undergo large mechanical forces while passing through the tiny vessels in the blood circulation. The way they regulate the volume is strictly controlled by the interplay of ion channels. It has been postulated indeed that membrane deformation causes Ca^{2+} ions to enter via mechanosensitive channels like Piezo1 [1-3]. An increase in the intracellular free-Ca^{2+} leads to K^+ efflux via activation of Gardos channels, membrane hyperpolarization, and concomitant loss of anions through the conductive anion pathway. Although there is indirect evidence of Piezo1 activation (i.e. via Ca^{2+} imaging), an electrophysiological proof of Piezo1 activity in RBCs is still lacking. Since its discovery, the patch clamp technique has proven to be a powerful direct tool to investigate the ionic transport of RBCs in physiological and pathophysiological situations. With the advent of the automated patch clamp technology the empirical procedure has been simplified in favor of an unlimited experimental freedom and high-throughput [4,5].

In this work, planar patch clamp instruments have been used to perform a biophysical and pharmacological characterization of ion channels expressed in human RBCs, with particular emphasis on Piezo1. Piezo1 currents were successfully elicited by external application of Yoda1 [6] in cell culture (N2A) and RBCs, despite the lack of activation by pressure or shear stress. Yoda1 responder and non-responder cells were automatically identified following application of strict quality control filters. By upscaling the assay to high-throughput platforms it has been possible to measure a large number of cells at the same time, thus tackling the problems of heterogeneity and cell-to-cell variability typical of RBCs [7-8]. Moreover, RBCs from patient carrying a novel Piezo1 gain-of-function mutation were investigated in comparison to healthy RBCs [9], thus proving that high-throughput patch clamp technology provides assays for drug discovery and personalized treatment of anaemic disorders such as hereditary xerocytosis.

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Symposium 2: Ion channels: from structure and function to drug discovery

Chair: Pilar de la Peña

Invited

THE CARDIAC HERG K^+ CHANNEL: A JOURNEY FROM CELL BIOLOGY TO STRUCTURE AND FUNCTION

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Human *ether-á-go-go*-related gene (hERG, Kv11.1, KCNH2) K^+ channels are expressed in a variety of non-cardiac cells and play a key role in setting their electrical behaviour. Also, hERG channels mediate the cardiac IK_r current, an essential determinant of action potential repolarization in the human ventricle and of pacemaking activity in heart nodes. Thus, some mutations in the hERG gene or drug inhibition of hERG channels underlie inherited or acquired type-2 long-QT syndrome, that increases the risk of *torsade de pointes* arrhythmia, ventricular fibrillation and sudden cardiac death. The physiological roles of hERG derive from an unusual combination of gating kinetics, characterized by a slow rate of activation, very fast inactivation and inactivation recovery, and a particularly slow deactivation on repolarization. This makes hERG operate as an inward rectifier, although it has the six membrane-spanning topology with the voltage sensing and pore domain organization typical of the conventional depolarization-activated Kv channels. Based in functional data, partial structures of cytoplasmic regions, functional reconstitution of mutant channels with recombinant N-terminal fragments, and the demonstration of physical proximity between the hERG amino terminus and the N-terminal portion of the S4-S5 linker, we have proposed that a dynamic network of interactions between several cytoplasmic regions and/or between them and the transmembrane channel core constitutes either an essential component of the hERG gating machinery, or an important regulator of the gating process. Furthermore, in a pioneer study with both hERG and eag1 (Kv10.1) channels, we demonstrated recently that even when the covalent continuity between the voltage sensing and the pore domains of these channels is broken (i.e. in channels split at the S4-S5 linker), an almost unaffected voltage-dependent activation gating is observed. Therefore, at least these Kv channels cannot use for voltage-dependent activation the electromechanical lever system classically proposed for the conventional *Shaker*-like Kv channels, in which movements of the voltage sensing domain are transduced to channel opening by using a long alpha-helical S4-S5 linker that, acting as a rigid lever, pulls the gate located at the cytoplasmic end of helix S6 to open. The even more recent recognition of a non-domain-swapped architecture in these (and other) channels, in which the S4-S5 is very short (making the voltage sensor of one subunit of the channel tetramer contact the pore domain of the same subunit), further confirmed that, in these entities, the non-swapped voltage sensors should work to transmit force and operate the gate in a way different than through the S4-S5 lever mechanism. We will discuss the possibility that in this scenario, and also according to our last data with some hERG split channel variants, interactions between cytoplasmic domains and the channel core constitute an essential factor for a non-electromechanical but instead electro-allosteric voltage sensor-gate coupling mechanism.

Keywords: Voltage-dependent potassium channel; *Ether-á-go-go*-related gene; hERG; Kv11.1; gating; structure-function relationship; split channel

Acknowledgements: Supported by Grant BFU2015-66429-P (MINECO/FEDER UE) from the Spanish Ministerio de Economía y Competitividad, co-financed with European Fund for Economic and Regional Development (FEDER) funds.

Invited

TARGETING TRP CHANNELS FOR DRUG DEVELOPMENT

Donato del Camino Fernández-Miranda

(Rheostat, Cambridge, MA, USA)

The TRP (Transient Receptor Potential) family of channels comprises a diverse group of cation-selective ion channels that act as cellular sensors as well as signal integrators. These channels are involved in numerous physiological functions, and mutations in human genes encoding TRP channels are the cause of a number of hereditary diseases affecting the renal, skeletal and nervous systems, among others. Thus, there has been significant pharmaceutical interest in targeting TRP channels for therapeutic drug development, and indeed, compounds that modulate some members of this family have entered clinical trials. Here, we will discuss the results of such drug discovery efforts performed at Hydra Biosciences aimed at the development of novel small molecule TRP channel modulators.

S2O1

Fragile X mental retardation protein differentially regulates the biophysical properties of BK channels

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Fragile mental retardation protein (FMRP) is an RNA-binding protein prominently expressed in neurons. Missense mutations or complete loss of FMRP can potentially lead to fragile X syndrome (FXS), a common form of inherited intellectual disability [1, 2]. In addition to RNA regulation, FMRP was also proposed to modulate neuronal function by direct interaction with the large conductance Ca^{2+} activated potassium (BK) β_4 regulatory subunits (BKβ_4) [3]. However, the molecular mechanisms underlying FMRP regulation of BK channels were not studied in detail. We have used electrophysiology and super-resolution stochastic optical reconstruction microscopy (STORM) to characterize the effects of FMRP on pore-forming BKα subunits, as well as their interactions with regulatory subunits BKβ_4 and BKγ_1. STORM experiments revealed clustered multi-protein complexes containing BKα or BKβ_4 and FMRP, consistent with FMRP binding not only to BK$\alpha\beta_4$ (in agreement with previously published data [3]), but also to BKα. Interestingly, our data indicate that FMRP alters the steady state properties of BKα channels by increasing their activation and deactivation rates. Analysis using the Horrigan-Aldrich model revealed alterations in the parameters associated with voltage sensor function (J_0) and channel opening (L_0). However, no significant effects of FMRP were observed on the biophysical properties of BK$\alpha\beta_4$ channels. Conversely, STORM experiments showed that, in the presence of FMRP, interactions between BKα and BKγ_1 subunits are significantly diminished. Consistently, electrophysiological recordings of cells coexpressing FMRP, BKα and BKγ_1 subunits, did not show BK$\alpha\beta_1$ currents. In summary, our data show that FMRP regulates BKα channels, does not affect BK $\alpha\beta_4$ channel function, and inhibits modulation by BKγ_1 subunits.

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S2O2

The TRPV4 channel links calcium influx to DDX3X activity and viral infectivity.

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Ion channels are well placed to transduce environmental cues into signals used by cells to generate a wide range of responses, but little is known about their role in the regulation of RNA metabolism. Here we show that the TRPV4 cation channel binds the DEAD-box RNA helicase DDX3X and regulates its function. TRPV4-mediated Ca^{2+} influx releases DDX3X from the channel and drives DDX3X nuclear translocation, a process that involves calmodulin (CaM) and the CaM-dependent kinase II. Genetic depletion or pharmacological inhibition of TRPV4 diminishes DDX3X-dependent functions, including nuclear viral export and translation. Furthermore, TRPV4 mediates Ca^{2+} influx and nuclear accumulation of DDX3X in cells exposed to the Zika virus or the purified viral envelope protein. Consequently, targeting of TRPV4 reduces infectivity of dengue, hepatitis C and Zika viruses. Together, our results highlight the role of TRPV4 in the regulation of DDX3X-dependent control of RNA metabolism and viral infectivity.

Keywords: TRPV4, DDX3X, Viral infectivity

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S2O3

Cardiac sodium current is severely impaired in induced pluripotent stem cell-derived cardiomyocytes from Brugada Syndrome patients

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Brugada Syndrome (BrS) is a cardiac arrhythmogenic disease that predisposes to sudden cardiac death. It is associated with mutations in the SCN5A gene, encoding the cardiac sodium channel alpha subunit (NaV1.5). We investigated a single nucleotide variation (SNV) in the SCN5A gene (c. 4573 G>A; NaV1.5_p.V1525M), associated to BrS. This SNV is located in a highly conserved region of the cytoplasmic loop connecting domains 3 and 4 of the channel. Our objective was to determine the sodium current (INa) properties of induced pluripotent stem cell-derived cardiomyocytes (iPS-CM) obtained from two mutation carriers from the same family. We also studied the SNV in heterologous expression in tsA201 cells (NaV1.5V1525M).

Dermal fibroblasts from patients and a non-related healthy control were reprogrammed to iPS cells. Clones were characterized and differentiated to form iPS-CM beating monolayers. tsA201 cells were transfected to express NaV1.5V1525M and compared with the wild type NaV1.5 (NaV1.5WT). Sodium currents were studied using the whole-cell patch clamp technique.

Our results showed a strong effect of the SNV on iPS-CM INa density. A total loss of INa was observed in a high percentage of the cells recorded from both patients (79%, n=71 and 67%, n=34 respectively). In the remaining cells for which we could record INa, we observed a 75.6% (n=19) and an 80.7% (n=13) reduction in INa density, compared with the control (n=40). Interestingly, the recovery from inactivation time constant was increased only in one of the two patients.

This was in sharp contrast to what we observed using recombinant channels expressed in tsA201 cells. Only a small fraction of cells transfected with NaV1.5V1525M did not express any current (7%). Moreover, heterozygous cotransfection of NaV1.5V1525M and NaV1.5WT, produced a proportional reduction in INa density (53% reduction). Unlike iPS-CM results, we also observed a positive shift of the voltage dependence of activation, and a negative shift of steady-state inactivation. However, we did not observe any increase in the recovery from inactivation time constant.

Overall, our results show a strong loss-of-function of NaV1.5 derived from patients' iPS-CM, which could not be observed using heterologous expression systems.

This suggests that, in native sodium channels, NaV1.5V1525M may exert a dominant negative effect over NaV1.5WT.

Keywords: Brugada Syndrome; Pluripotent stem cells; Cardiomyocytes; tsA201 cells; Sodium current.

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S2O4

Mitochondrial Kv1.3 channels in apoptosis

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The voltage-gated potassium channel Kv1.3 is expressed at the inner mitochondrial membrane of different cell lines. Mitochondrial Kv1.3 regulates apoptosis by direct interaction with Bax and has been suggested as a therapeutic target against cancer transformation and proliferation. However, molecular mechanisms regulating the mitochondrial targeting of the channel remain elusive. We previously identified a Caveolin Binding Domain (CBD) at the N-terminus of Kv1.3. This domain is highly hydrophobic (FQRQVWLLF) and mediates the channel interaction with caveolin-1. Such interaction is essential to regulate Kv1.3 affinity to lipid raft microdomains. Although Kv1.3-CBD deficient (Kv1.3CBD) channel is misslocalized from the plasma membrane, it is properly folded and forms functional homotetramers. Yet, Kv1.3-CBD expression seriously impairs cell homeostasis. We report that mutating the CBD of Kv1.3 enhances the channel pro-apoptotic properties by directly affecting the mitochondrial membrane potential and ROS production. Moreover, Kv1.3-CBD expression dramatically affects mitochondrial morphology, leading to cristae withdrawal. Our results shed light on the mechanisms regulating Kv1.3 mitochondrial activity and targeting.

Symposium 3: Calcium signaling and Cell Function

Chair: Tarik Smani

Invited

BISPHENOL-A MODULATES ION CHANNEL EXPRESSION AND FUNCTION VIA ESTROGEN RECEPTOR B IN MOUSE PANCREATIC BETA CELLS.

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According to the American Diabetes Association, “Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both” [1]. The most common form of diabetes, namely type 2 diabetes (T2D), results from a deficiency in insulin action on peripheral tissues together with a lack of compensatory insulin production and release by pancreatic beta cells. T2D is considered a multifactorial disease and the present model for explaining its etiology suggests that both genetic predisposition and environmental factors play key roles in the disease development. Bisphenol-A (BPA) is a widespread endocrine disrupting chemical that affects different physiological systems [2]. BPA has been associated with T2D development in epidemiological studies. Moreover, low doses of BPA modify pancreatic beta cell function and induce insulin resistance, being some of these effects mediated by estrogen receptors α (ERα) and β (ERβ). In the present study we investigated whether low doses of BPA regulate the expression and function of ion channels involved in beta cell function through activation of estrogen receptors.

Microarray analysis of islets from vehicle- and BPA-treated (100 µg/kg/day for four days) mice showed that BPA modulated the expression of 1440 probe sets (1192 upregulated and 248 downregulated genes), from which more than 50 genes encoded important sodium and potassium channel subunits, such as *Scn9a*, *Kcnb2*, *Kcnma1*, and *Kcnip1*. These findings were confirmed by quantitative RT-PCR in islets from BPA-treated mice or whole islets treated *ex vivo*.

Electrophysiological measurements showed a decrease in sodium, calcium and total potassium currents in BPA-treated islets. The pharmacological profile indicated that BPA reduced Kv2.1/2.2, KCa1.1, and Cav2.3-mediated currents, which is in line with BPA effects on gene expression. BPA-induced changes were abrogated in beta cells derived from ERβ-/- mice, suggesting that ERβ mediates BPA effects in pancreatic beta cells. Moreover, BPA increased burst duration, reduced the amplitude of the action potential, and enlarged the action potential half-width, leading to alterations in the beta cell electrical activity.

Our study suggests that BPA modulates the expression and function of sodium and potassium channels via ERβ in mice pancreatic islets. Furthermore, BPA alters beta cell electrical activity. Altogether, these BPA-induced changes in beta cells might play a role in the diabetogenic action of BPA described in animal models.

Keywords: Potassium channels, sodium channels, beta cell, bisphenol-A, diabetes, endocrine disrupting chemicals, estrogen receptor β.

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Invited

IMMUNE SYSTEM AND CA^{2+} SIGNALING IN CARDIAC CELLS: ROLE OF NOD1

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Heart failure (HF) is a complex syndrome that harbors functional, structural and molecular alterations. Recent studies point to some mediators of the innate immune system as new partners in the HF progression. NOD1 (nucleotide-binding oligomerization domain containing 1) is a family member of the innate immune system involved in certain cardiovascular diseases. Our group has previously demonstrated that the selective activation of this receptor induces cardiac dysfunction and Ca^{2+} mishandling, common hallmarks of HF. So the aim of the present study was to determine the role of NOD1 in HF progression.

NOD1 expression was examined in human failing myocardium and in a mice model of HF induced by myocardial infarction, *wt*-PMI. The NOD1 pathway was up-regulated in human and murine failing myocardium. Next, we determined whether the absence of NOD1 impairs HF progression. Compared to *wt*-PMI, hearts from $Nod1^{-/-}$-PMI mice showed less cardiac dysfunction and attenuated structural remodelling. We performed cardiomyocyte isolation of the different experimental groups to study the intracellular Ca^{2+} handling. Improved cardiac function in $NOD1^{-/-}$-PMI mice was associated with a prevention of Ca^{2+} dynamics impairment associated to HF, including lower and longer $[Ca^{2+}]_i$ transients and a small SR Ca^{2+} load induced by a down-regulation of the SR Ca^{2+}-ATPase expression, and by increased protein levels of the Na^+/Ca^{2+} exchanger. Augmented diastolic Ca^{2+} release in *wt*-PMI cells was related to over-phosphorylation of ryanodine receptor (RyR), which was prevented in $NOD1^{-/-}$-PMI cardiomyocytes. Pharmacological blockade of NOD1 also blunted Ca^{2+} mishandling in *wt*-PMI mice. Under isoproterenol administration, $NOD1^{-/-}$-PMI mice showed significantly lower ventricular arrhythmias and mortality rates. These beneficial effects were associated with minor abnormal systolic Ca^{2+} release and with normalization in the phosphorylation state of RyR under isoproterenol administration in $NOD1^{-/-}$-PMI mice. Our results support that the absence of NOD1 prevents cardiac dysfunction linked to HF though modulating intracellular Ca^{2+} handling.

Keywords: innate immune system; NOD1; Ca^{2+} handling; heart failure; cardiomyocytes

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S3O1

Beneficial effects of paricalcitol on cardiac dysfunction and deleterious remodeling after established heart failure

Carmen Delgado[1], María Tamayo[1], Laura Martín[1], Almudena Val[2], María José García-Piedras[3], Jose A. Navarro[4], Eduardo Lage[5], Patricia Prieto[1], Gema Ruiz[4], María Fernández[2]

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Chronic heart failure (HF) is a major health concern in aging societies. It is commonly accompanied by progression to maladaptive hypertrophy with cardiac dilation and diminished left ventricular (LV) ejection fraction (EF). Adverse cardiac remodeling is an important determinant of HF clinical outcomes and is linked to disease progression and poor prognosis. Ventricular remodeling involves cardiomyocyte hypertrophy, pro-fibrotic responses and down-regulation of K^+ currents, which lead to altered electrotonic coupling between cells and prolonged QT intervals, increasing the risk of ventricular arrhythmias and sudden cardiac death. Furthermore, depressed cardiac function in HF is commonly associated with impairment of intracellular Ca^{2+} homeostasis. Paricalcitol (PC) is a synthetic vitamin D3 analog that acts as a selective activator of vitamin D receptor (VDR). There is clear evidence demonstrating cardioprotective properties associated with the VDR pathway. However, less information is available on the structural and functional cardiac effects of PC on established HF, and especially regarding its effects on electrophysiological or Ca^{2+}-handling remodeling associated with HF. In the present study, we used a murine model of HF induced by pressure overload (transverse aortic constriction; TAC). Mice were divided into two experimental groups: sham and TAC-operated. Cardiac magnetic resonance image (CMRI) was performed 4 weeks after surgery and only TAC-operated animals with EF <60% were included in the study. Treatment with 300 ng/kg PC or vehicle was initiated 4 weeks after surgery over 5 consecutive weeks and CMRI was repeated 9 weeks after surgery. Animals were sacrificed and hearts were used for biochemical and histological studies. In some cases, hearts were retrograde perfused to isolate ventricular myocytes for electrophysiological and intracellular calcium imaging studies. CMRI analysis showed that LV end-diastolic and end-systolic volumes were increased 4 weeks after TAC relative to sham animals, indicating dilation of the LV, which was significantly greater 9 weeks after surgery. PC treatment for 5 weeks prevented the progression of both parameters and similar results were observed for EF. The progressive decline in EF from 4 to 9 weeks after TAC was prevented by PC treatment. This beneficial effect on cardiac dysfunction was related to prevention of intracellular Ca^{2+}-mishandling remodeling by improving the amplitude of the intracellular calcium transients and preventing their slower time decay. Histological examination of hearts and the heart ratio weight/tibia length confirmed the presence of cardiac LV hypertrophy in the TAC group. Treatment with PC had antihypertrophic effects by attenuating calcineurin/NFAT signaling. Additionally, PC had antifibrotic effects linked to prevention of the expression of the profibrotic genes *Serpine-1*, *Col1a1* and *Col3a1*. Electrocardiographic recordings on mice 9 weeks after TAC showed long QT intervals when compared with sham groups, and this was mitigated by PC treatment. Finally, electrophysiological study of K^+ currents (IK^+) showed that the IK^+ density was reduced in TAC mice with established HF after 9 weeks, and PC treatment prevented this reduction. Overall, these data suggest that PC treatment in established HF attenuates disease progression by preventing adverse cardiac remodeling at the cellular and molecular level.

Keywords: Chronic heart failure, cardiac hypertrophy, QT interval, paricalcitol, vitamin D receptor, Calcineurin/NFAT pathway, myocardial fibrosis, cardiac cellular electrophysiology, ventricular cardiomyocytes, K^+ currents.

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S3O2

Essential role of Orai1 and SARAF in vascular remodeling

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Background: Key molecular components of Store-Operated Calcium Entry (SOCE) Orai1 and STIM1 have been associated with vascular smooth muscle cells (VSMCs) proliferation and vascular remodelling [1]. However, the role of SARAF (SOCE Associated Regulatory Factor), a new regulatory protein involved in STIM1 inhibition, hasn't been explored. The aim of this study was to examine the role of SARAF and Orai1 in VSMC proliferation and neointima formation after balloon injury of rat carotid arteries.

Methods and Results: Experiments were conducted in an animal model of rat carotid angioplasty, to evaluate neointima formation. We also used VSMC isolated from rat coronary artery to examine cell proliferation. First, we confirmed the formation of neointima after balloon injury of rat carotid arteries by haematoxylin and eosin staining of tissue sections up to 3 weeks after surgery. Next, we found significant higher expression of SARAF, STIM1 and Orai1 in injured arteries compared to control tissues, corroborating the presence of these regulatory proteins in the neointima layer. Using immunostaining and proximity ligation assay, we demonstrated that SARAF markedly colocalize with Orai1 in the neointima. Furthermore, we found that selective silencing of SARAF and Orai1 by small interfering RNA (siRNA) inhibited IGF-1-induced VSMC proliferation.

Conclusions: Our data suggest that SARAF interact with Orai1 to modulate SOCE and VSMC proliferation after vascular injury.

Keywords: SARAF; Orai1; carotid lesion; Smooth muscle proliferation; store-operated calcium entry

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S3O3

Calcium dependent Chloride Channels (CaCC) as a target of a bacterial toxin

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CaCCs are a subtype of chloride channels that are present in a wide range of excitable and non-excitable cells. Because *Xenopus laevis* oocytes endogenously express these channels, they have been used for their biophysical characterization. CaCCs are activated by voltage. In an I/V representation, CaCCs rectify at positive potentials. CaCCs are activated by both voltage and intracellular Ca^{2+}, showing at +60 mV an EC_{50} of 1 μM. The channels preferentially permeate large anions, the ionic selectivity being $NO_3^- > I^- > Br^- > Cl^- > F^-$. Pharmacological blockers of other chloride channels such as niflumic and flufenamic acids, NPBB and DIDS also block CaCCs; however, MONNA is more selective with an IC_{50} of 80 nM. The CaCCs of *Xenopus* oocytes correspond to TMEM16A and play a fast block of polyspermy.

TMEM16 proteins are a family of ten members (from TMEM16A to J) which have ten transmembrane segments. These proteins are also described as Anoctamins. Some of the members of the family are considered so far as pure CaCCs, for example TMEM16A and TMEM16B, which are present in the plasma membrane. The functional conformation of channels is a dimer. Other members of the family are scramblases, a membrane protein that transfer, with no ATP hydrolysis, phospholipids from the inner leaflet to the external leaflet of the cellular membranes. Some TMEM16 members are considered to have both chloride channel and scramblase activity. Either the chloride channel activation or the scramblase activity is dependent of the cytoplasmic Ca^{2+} concentration. Two Ca^{2+} ions bind to each monomer. The structure of the calcium sensor has been elucidated. Five amino acids have been shown to be critical for calcium sensing, glutamates 650, 698, 701, 730 as well as aspartate 734 have been proposed to be the Ca^{2+} binding sites in TMEM16A. These amino acids are located in close proximity to a cavity of each monomer, where the putative ion conduction pore is proposed to be located [1].

The scramblase activity of TMEM16 proteins is activated by the increase in cytoplasmic Ca^{2+} in the same loci which activates chloride conductance. A scrambling domain has been described in TMEM16E and TMEM16F. The scrambling domain of TMEM16F has been transferred to TMEM16A, conferring this protein a scramblase activity. Through molecular simulations, the phospholipids have been proposed to cross the molecule through a hydrophilic intramolecular groove which is the same pathway which Cl^- crosses the plasma membrane [2-3].

TMEM16A is mainly located at lipid rafts and its N-terminal domain is in a direct molecular contact with the IP_3 receptor of the endoplasmic reticulum. TMEM16A can be activated by the entry of Ca^{2+} from the extracellular milieu or be active by a direct contact with the IP_3 receptor even in the absence of extracellular Ca^{2+}.

Studying the effect of Epsilon toxin (Etx), produced by *Clostridium perfringens*, we found that, in *Xenopus* oocytes, the toxin caused an internal calcium mobilization through the TMEM16A, activated CaCCs and the release of ATP. On the other hand, in MOLT4 human lymphocytic cell line, Etx activated the scramblase activity. We suggest that these effects are dependent on the expression of an accessory subunit, as the human the myelin and lymphocyte protein (MAL) is mandatory to be expressed in order to obtain the effect of Etx. Additional details of the action Etx on TMEM16A are presented in a poster defended by Mercè Cases.

Keywords: TMEM16 proteins, Anoctamin, internal calcium mobilization, Epsilon toxin, *Clostridium perfringens*

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S3O4

NMDAR and BK channels form Ca^{2+} nanodomains in basal dendrites of barrel cortex layer 5 pyramidal neurons to control synaptic excitability and plasticity

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Large conductance Ca^{2+}- and voltage-gated K^+ channels (BK) form complexes with Ca^{2+}-permeant ion channels, coupling neuronal Ca^{2+} to membrane voltage signals. This association has been largely described for BK and voltage-gated Ca^{2+} channels (Cav) presynaptically. Postsynaptically, functional association of BK with N-methyl-D-aspartate receptors (NMDAR) has been shown in olfactory bulb [1] and dentate gyrus granule cells [2]. The existence of NMDAR-BK complexes in other brain regions and their physiological relevance in neuronal function remain unknown.

Here we studied the role of NMDAR-BK complexes in somatosensory "barrel" cortex layer 5 pyramidal neurons (BC-L5PN), where two neuronal populations (A- or B-type neurons) were characterized according to the presence or absence of NMDAR-mediated Ca^{2+} spikes following the action potential [3]. We hypothesized that this difference relays on the presence of NMDAR-BK complexes in B-type BC-L5PN. Additionally, we predict that they would make B-type BC-L5PN less sensitive to afferent synaptic inputs and induction of synaptic plasticity. We combined electrophysiological, immunochemistry, and imaging experiments to test these hypotheses.

Local application of NMDA at basal dendrites induced a biphasic current response in 35% of BC-L5PN at depolarizing voltages (from -20 mV), consisting on an fast-activating inward current immediately followed by a long-lasting outward current. NMDAR antagonist AP5 abolished both currents, whereas BK blocker paxilline abolished just the outward current. The application of selective NMDAR-NR2A or NMDAR-NR2B antagonists partially abolished the outward current, demonstrating that functional NMDAR-BK complexes can be formed both by NR2A- and NR2B-containing NMDAR. Intracellular application of Ca^{2+} chelators allowed us to estimate a range for the NMDAR-BK interaction. Finally, BC-L5PN expressing NMDAR-BK complexes (B-type neurons) are less sensitive to the induction of long-term potentiation (LTP) at basal dendrites when compared to A-type neurons after the application of a spike-timing-dependent plasticity protocol (STDP-LTP).

In summary, our results show that NMDAR-BK channels are functionally coupled forming Ca^{2+} nanodomains at basal dendrites of BC-L5PN. These complexes constitute a newly described mechanism to control synaptic excitability and plasticity in somatosensory layer 5 pyramidal neurons.

Keywords: Ca^{2+} nanodomains; NMDAR; BK; synaptic plasticity; barrel cortex

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Symposium 4: Store-operated channels

Chair: Carlos Villalobos

Invited

ROLE OF TRPC6 IN BREAST CANCER CELL CA^{2+} HOMEOSTASIS.

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Transient receptor potential channels mediate Ca^{2+} influx as well as voltage changes across the plasma membrane in response to a number of stimuli. Different members of the TRP familiy have been reported to play a role in the progression of certain types of cancer. In luminal and triple negative breast cancer cell lines TRPC6 is overexpressed as compared to non-tumoral breast epithelial cells. In breast tumoral cells, TRPC6 plays a relevant role in the activation of store-operated Ca^{2+} entry (SOCE), a major Ca^{2+} influx mechanism in non-excitable cells that plays a relevant role in a wide range of cancer hallmarks, from cell proliferation and migration to apoptosis resistance. SOCE in luminal and triple negative breast cancer cells is strongly dependent on the Ca^{2+} channels Orai3 and Orai1, respectively [1], and we have recently reported that TRPC6 interacts with both Orai1, in triple negative breast cancer cells, and Orai3, in luminal tumoral cells. By silencing TRPC6 protein expression using shRNA and expressing a pore-dead dominant-negative TRPC6 (TRPC6dn) mutant we have found that TRPC6 is required for specific Orai1 and Orai3 trafficking to and recycling at the plasma membrane in triple negative and luminal breast cancer cells, respectively [2]. *In vitro* TRPC6 knockdown and expression of TRPC6dn impaired luminal and triple negative cell proliferation, migration and invasion detected by BrdU incorporation, wound healing and Boyden chamber assays, respectively. These effects are likely explained by its role in the modulation of Orai channel recycling and thus the activation of SOCE. Oleocanthal (OLCT), a phenolic compound isolated from olive oil with anti-tumoral properties, has been found to attenuate luminal and triple negative breast cancer cell viability, as well as their ability to migrate and proliferate, without having any effect on non-tumoral epithelial breast cells. In breast cancer cells, treatment with OLCT results in transient Ca^{2+} influx that was found to be dependent on TRPC6 expression. Furthermore, treatment with OLCT for 24-72 h downregulates TRPC6 expression, which, in turn, is likely to result in the attenuation of SOCE in these cells, and thus, the impariment of the ability of breast cancer cells to proliferate and migrate [3]. These findings introduce TRPC6 as a relevant modulator of Ca^{2+} influx and the development of different cancer hallmarks in luminal and triple negative breast cancer cells

Keywords: MCF7; MDA-MB-231; Orai1; Orai3; TRPC6; store-operated calcium entry

Acknowledgements: supported by MINECO grant BFU2016-74932-C02-01 and Junta de Extremadura-FEDER grants IB16046 and GR18061.

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Invited

STORE-OPERATED CA^{2+} CHANNELS IN PITUITARY PHYSIOLOGY

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The Anterior Pituitary (AP) gland contains five different cell types that release six major AP hormones controlling most of the entire endocrine system. AP hormone release is modulated by Ca^{2+} signals induced by different hypothalamic releasing hormones (HRHs) acting on specific receptors in AP cells. TRH and LHRH both induce Ca^{2+} release and Ca^{2+} entry in responsive cells while GHRH and CRH only induce Ca^{2+} entry. Ca^{2+} involves both voltage-operated Ca^{2+} channels related to electric activity and store operated Ca^{2+} entry (SOCE). SOCE is the most important Ca^{2+} entry pathway in non-excitable cells but also may play a pivotal role in excitable cells as AP cells. SOCE has been proposed to contribute to Ca^{2+} responses induced by TRH and LHRH but the channels involved remain unknown. In other cells, SOCE is mediated by the interaction between stromal interaction protein 1 (STIM1) and Orai1 channels at the plasma membrane. However, whether canonical TRP (TRPC) channels contribute to SOCE is a long-term debate that remains controversial. Accordingly, we used AP cells isolated from mice devoid of Orai1 channels (noted as Orai1-/- or Orai1 KO mice) and mice lacking expression of all seven canonical TRP channels (TRPC) from TRPC1 to TRPC7 (noted as heptaTRPC KO mice) to investigate contribution of these putative channel proteins to SOCE and intracellular Ca^{2+} responses induced by HRHs in AP cells. We found that thapsigargin-evoked SOCE is lost in AP cells from Orai1$^{-/-}$ mice but totally unaffected in cells from heptaTRPC KO mice. Conversely, while spontaneous intracellular Ca^{2+}-oscillations related to electrical activity were not affected in the Orai1$^{-/-}$ mice, they were dampened in AP cells from heptaTRPC KO mice. We also found that Ca^{2+} entry induced by TRH and LHRH is decreased in AP cells isolated from Orai1$^{-/-}$. In addition, Ca^{2+} responses to several HRHs, particularly TRH and GHRH, are decreased in the heptaTRPC KO mice. These results indicate that expression of Orai1, and not TRPC channel proteins, is necessary for thapsigargin-evoked SOCE and is required to support Ca^{2+} entry induced by TRH and LHRH in mouse AP cells. In contrast, TRPC channels contribute to spontaneous Ca^{2+}-oscillations and Ca^{2+} responses induced by TRH and GHRH. We conclude that Orai1 and TRPC contribute to calcium signals and AP physiology [1].

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S4O1

EFHB regulates store-operated calcium entry, migration and proliferation in breast cancer cells.

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Ca^{2+} homeostasis is finely tuned by a plethora of processes in eukaryotes. Store-operated calcium entry (SOCE) is the main Ca^{2+} entry pathway in non-excitable cells, appearing in electrically excitable cells as well [1]. SOCE and its key mediators STIM, Orai and TRPC proteins are activated upon intracellular Ca^{2+} stores depletion. There are a number or regulators that orchestrate and coordinate the whole mechanism. Modification of SOCE, its main components or its regulators has been associated to a number of pathological conditions, including cancer [2]. STIM, Orai and TRPC proteins, as well as their regulators, are expressed in breast cancer cells with different levels of expression and function. By using molecular biology, biochemistry and fluorescence imaging microscopy techniques we have found that the EF-Hand domain family member B (EFHB), a novel regulator of SOCE that controls the dynamic association between SARAF and STIM1, required to activate Orai1 and its subsequent modulation via slow Ca^{2+}-dependent inactivation, is over-expressed in the ER^{+} MCF7 and triple negative MDA-MB-231 breast cancer cell lines, when compared with the non-tumoral MCF10A breast cell line. Knockdown of EFHB in both tumoral cell lines impairs significantly SOCE, while it has no effect in SOCE in non-tumoral MCF10A cells. Furthermore, we have demonstrated that inhibition in SOCE leads to an impairment in proliferation, migration and invasion, which are relevant breast cancer features. In sum, we present an important and novel role of EFHB by subtly modulating SOCE, and subsequently, proliferation, migration and invasion in breast cancer cells.

Keywords: SOCE, Orai1, STIM1, SARAF, EFHB, Breast Cancer,

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S4O2

Reversing Ca^{2+} channel remodeling in colon cancer cells by polyamine biosynthesis inhibition

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Store-operated Ca^{2+} entry (SOCE) is the most important Ca^{2+} entry pathway in non-excitable cells. Colorectal cancer (CRC) show decreased Ca^{2+} store content and enhanced SOCE that correlate with cancer hallmarks and are associated to remodeling of store-operated channels (SOCs). Normal colonic cells display small, Ca^{2+}-selective currents driven by Orai1 channels [1]. In contrast, CRC cells display larger, non-selective currents driven by Orai1 and TRPC1 channels. Difluoromethylornithine (DFMO), a suicide inhibitor of ornithine decarboxylase (ODC), the limiting step in polyamine biosynthesis, strongly prevents CRC, particularly when combined with sulindac [2]. We asked whether DFMO may reverse SOC remodeling in CRC. We found that CRC cells overexpress ODC and treatment with DFMO decreases cancer hallmarks including enhanced cell proliferation and apoptosis resistance. Consistently, DFMO enhances Ca^{2+} store content and decreases SOCE in CRC cells. Moreover, DFMO abolish selectively the TRPC1-dependent component of SOCs characteristic of CRC cells and this effect is reversed by the polyamine putrescine. Combination of DFMO and sulindac inhibit both SOC components and abolish SOCE in CRC cells. Finally, DFMO treatment inhibits expression of TRPC1 and STIM1 in CRC cells. These results suggest that polyamines contribute to Ca^{2+} channel remodeling in CRC and DFMO may prevent CRC by reversing channel remodeling.

Keywords**:** Colorectal cancer; DFMO; Store-operated Ca^{2+} entry; Store-operated currents; Polyamines; TRPC1; Sulindac.

Acknowledgements: We greatly appreciate expert technical assistance by Mr. David del Bosque. We thank Dr. Mary Pat Moyer, INCELL Corporation, San Antonio, TX, USA, for providing NCM460 cells.

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S4O3

Dysregulation of ion channels expression associated with the adverse cardiac remodeling due to heart revascularization.

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Objectives: Despite of prompt and successful revascularization the adverse cardiac remodeling still occurs both at the site myocardial infarction, known as risk zone, and in remote zone leading to heart failure (HF). Cardiac remodeling is characterized by abnormalities in the handling of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), because of ion channels dysregulation. This study sought to evaluate the role of different proteins related to Ca^{2+} homeostasis in ischemic heart after revascularization.

Methods and Results: The effect of Ischemia and Reperfusion (I/R) was evaluated *in vivo* in Wistar rat undergoing transient ligation of the left coronary artery, and *in vitro* in isolated Neonatal Rat Ventricular Myocytes (NRVM).

We observed that I/R produced significant decrease in cytosolic $[Ca^{2+}]$ transients in adult cardiomyocytes isolated from risk and remote zones. I/R also reduced sarcoplasmic reticulum Ca^{2+} content in both zones. To determine the molecular participants involved in I/R-induced alteration of $[Ca^{2+}]_i$, we performed a PCR-based micro-array to evaluate the expression of 45 genes associated with Ca^{2+} homeostasis. We found significant upregulation of 14 genes, most of them belonging to TRPC family (TRPC1/3/5/6), and to the store operated Ca^{2+} entry (SOCE) signaling pathway, namely Orai1/2 and STIM1/2. Using qRT-PCR and western blot we confirmed that under I/R the expression of STIM1, Orai1, TRPC5 was upregulated either in risk or remote zones. Using NRVM, we first determined that I/R exacerbated the SOCE induced by thapsigargin. Secondly, we demonstrated that silencing of Orai1 and TRPC5 inhibited I/R-induced SOCE. Finally, using proximity ligation assay we demonstrated that Orai1 interacts with TRPC5 under I/R [1].

Conclusions: Altogether, we demonstrated that ion channels genes are differentially expressed in risk and remote zone under I/R. We suggest that store operated calcium channels play a critical role in the adverse cardiac remodeling.

Keywords: ischemia and reperfusion / adverse remodeling / Ca^{2+} dysregulation / store operated Ca^{2+} channels / TRPC

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S4O4

A new role for adenylate cyclase 8 as modulator of store-operated calcium entry

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Calcium ion (Ca^{2+}) and cyclic adenosine monophosphate ($_c$AMP) signalling are two of the most important second messengers that are involved in the activation of many cellular functions and also, for the development of cancer hallmarks in tumor cells [1, 2]. Store-operated Ca^2 entry (SOCE) is the major pathway of Ca^{2+} influx across the plasma membrane in non-excitable cells. This mechanism, initiated by the depletion of the intracellular Ca^{2+} stores, requires the activation of Orai1, the channel pore-forming located on the plasma membrane [1]. Recent studies have demonstrated the direct interaction between the amino termini of Ca^{2+}-stimulated adenylyl cyclase-8 (AC8) and Orai1, suggesting that Orai1 plays a pivotal role in the coordination of Ca^{2+} and $_c$AMP signalling pathways [3]. Using RNAi-mediated AC8 silencing and two different approaches based on measurement of cytosolic free-Ca^{2+} concentration ($[Ca^{2+}]_c$) in single cells, we demonstrate that AC8 silencing significantly reduced Orai1-mediated SOCE. Western blotting and immunoprecipitation assays revealed that the AC8-Orai1 interaction is dependent on SOCE-mediated extracellular Ca^{2+} influx, but it does not dependent on Ca^{2+} released from intracellular stores. Finally, we demonstrate that AC8 is required for, cell migration estimated by wound healing assay, in MDA-MB-231 cells. Summarizing, our findings suggest a new role for AC8 as a modulator of SOCE by its interaction with Orai1, reinforcing the interplay between these two ubiquitous signalling pathways.

Keywords**:** SOCE; Orai1; Adenylate Cyclase-8; cell migration; MDA-MB-231 cells

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Symposium 5: Cardiac ion channels are not alone

Chair: Eva Delpón

Invited

DYSFUNCTION OF THE CARDIAC KIR2.1-NAV1.5 CHANNELOSOME TRAFFICKING UNDERLIES ARRHYTHMIA SUSCEPTIBILITY IN THE ANDERSEN-TAWIL SYNDROME

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From the University of Michigan, Ann Arbor (D.P.-B., G.G.-S., C.R.V., E.N.J.-V., R.J.R., A.M.d.R., T.J.H., K.F.C., B.C.W., M.Z., K.K., H.H.V.F.J.A., H.H.V., J.J.): Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain (J.J.); CIBERV, Madrid, Spain (J.J.); Universidad Complutense, Madrid, Spain (R.C., M.P.-H., M.M., E.D.); Centro de Biología Molecular Severo Ochoa (UAM-CSIC), Universidad Autónoma de Madrid, Spain (F.J.D.-G.).

Traditionally, ion channel diseases have been called “monogenic” and studied solely on the improbable assumption that there is a direct relationship between the ion channel mutation and the disease phenotype [1]. However, ion channel proteins do not function in isolation [2]. They are part of large, multi-protein complexes comprising not only the ion channels and their auxiliary subunits, but also components of the cytoskeleton, regulatory kinases and phosphatases, trafficking proteins, extracellular matrix proteins, and even other ion channels. We have recently discovered that the main cardiac voltage-gated Na^+ channel ($Na_V1.5$) and a strong inward rectifier K^+ channel (Kir2.1) form at least two different “channelosomes” at the cardiomyocyte membrane, and function within macromolecular complexes to control cardiac excitability [3]. We have also demonstrated that NaV1.5 and Kir2.1 form a complex that pre-assembles early in its forward trafficking pathway [4]. Furthermore, we found that similar to Kir2.1, NaV1.5 channels may be selected as cargo into Golgi export carriers in a signal-dependent manner through an adaptor protein complex 1-clathrin adaptor interaction. Moreover, trafficking deficiency and retention of Kir2.1 channel at the Golgi apparatus affects trafficking of $Na_V1.5$ channels, and vice versa [4,5]. Our data highlight the importance of considering effects of trafficking defective mutations on functional expression of other ion channels or proteins that may be a part of the macromolecular complex within which the proteins interact. The results offer a novel paradigm about the molecular mechanism of arrhythmia susceptibility in monogenic ion channels diseases, such as the Andersen–Tawil Syndrome, and potentially other diseases, including Brugada syndrome.

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Invited

THE ODDS COUPLE: NAV1.5 AND KIR2.X CHANNELS.

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Cardiac excitability and refractoriness are largely determined by the functional interplay between the Na (I_{Na}) and the inward rectifier K (I_{K1}) currents. The I_{Na} is generated by Nav1.5 channels (encoded by the *SCN5A* gene) in the atria and the ventricles, while the I_{K1} is generated by Kir2.1, 2.2 and 2.3 channels, which are differently expressed in both chambers. The number and function of each channel type expressed in the cardiomyocytes determine the properties of the currents. In fact, loss-of-function mutations in *SCN5A* underlie several inherited arrhythmogenic syndromes, including the Brugada Syndrome (BrS) which increases the risk of ventricular arrhythmias and sudden cardiac death. We have analyzed the molecular determinants that govern Nav1.5/Kir2.x reciprocal modulation in the atria and the ventricle. We compared some of the biological properties of Nav1.5 and Kir2.1 channels when they are expressed together or separately. Finally, we also investigated whether the presence of BrS-associated mutations concomitantly alters the I_{K1} density. The results demonstrated the critical role of the N-terminal domain of Nav1.5 channels (determined by Ser at position 20) in Nav1.5-Kir2.1 and Nav1.5-Kir2.2 reciprocal interactions and suggested that the molecular mechanisms controlling atrial and ventricular excitability may be different since Nav1.5 channels do not interact with Kir2.3 channels which are expressed in human atria [1]. Moreover, results demonstrate that binding to α1-syntrophin is necessary for the Nav1.5-Kir2.x positive reciprocal modulation. Furthermore, there is a pool of Nav1.5 and Kir2.1 channels that forms complexes that are built up at early stages of channel processing and with anterograde and retrograde trafficking routes similar to those of Nav1.5 channels alone [2]. In fact, Nav1.5-Kir2.1 complexes interact with 14-3-3 proteins, are modulated by CaMKII, are ubiquitinated by Nedd4-2 ubiquitin-protein ligase and degraded by the proteasome. Finally, using mouse models of *SCN5A* haploinsufficiency, and the overexpression of native and mutated Nav1.5 channels in expression systems, rat ventricular cardiomyocytes, and human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), we demonstrated that the endoplasmic reticulum (ER) trafficking-defective Nav1.5 channels significantly decreased the I_{K1} since they did not positively modulate Kir2.1/2.2 channels. Moreover, Golgi trafficking-defective Nav1.5 mutants produced a dominant negative effect on Kir2.1/2.2 and, thus, an extra I_{K1} reduction. Otherwise, ER trafficking-defective Nav1.5 channels can be partially "rescued" by Kir2.1/2.2 channels through an unconventional secretory route which involves the Golgi reassembly stacking proteins (GRASPs)[3]. Thus, cardiac excitability would be greatly affected in BrS patients harbouring Nav1.5 mutations with Golgi trafficking-defects since these mutants can concomitantly trap Kir2.1/2.2 channels, thus unexpectedly decreasing the I_{K1} besides the I_{Na}.

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S5O1

BIN1 induces the formation of T-tubules, Cav1.2 channel clusters and adult-like Ca^{2+} release units in developing cardiomyocytes.

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Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) are at the center of new cell-based therapies for cardiac disease, but may also serve as a useful *in vitro* model for cardiac cell development. An intriguing feature of hESC-CMs is that although they express contractile proteins and have sarcomeres, they do not develop transverse-tubules (T-tubules) with adult-like Ca^{2+} release units (CRUs). We tested the hypothesis that expression of the protein BIN1 in hESC-CMs promotes T-tubules formation, facilitates Cav1.2 channel clustering along the tubules, and results in the development of stable CRUs. Using electrophysiology, $[Ca^{2+}]_i$ imaging, and super resolution microscopy, we found that BIN1 expression induced T-tubule development in hESC-CMs, while increasing differentiation towards a more ventricular-like phenotype. Voltage-gated Cav1.2 channels clustered along the surface sarcolemma and T-tubules of hESC-CM. The length and width of the T-tubules as well as the expression and size of Cav1.2 clusters grew, as BIN1 expression increased and cells matured. BIN1 expression increased Cav1.2 channel activity and the probability of coupled gating within channel clusters. Interestingly, BIN1 clusters also served as sites for sarcoplasmic reticulum (SR) anchoring and stabilization. Accordingly, BIN1-expressing cells had more Cav1.2-ryanodine receptor junctions than control cells. This was associated with larger $[Ca^{2+}]_i$ transients during excitation-contraction coupling. Our data support the view that BIN1 is a key regulator of T-tubule formation and Cav1.2 channel delivery. By studying the role of BIN1 during the differentiation of hESC-CMs, we show that BIN1 is also important for Cav1.2 channel clustering, junctional SR organization, and the establishment of EC coupling.

Keywords: hESC, cardiac myocytes, BIN1, T-tubules, Cav1.2 clusters, calcium release units.

Acknowledgements: This work was supported by grants from the US National Institutes of Health (NIH): R01-HL085686 and 1K99AG056595-01; the American Heart Association: 15SDG25560035.

S5O2

Endoplasmic reticulum-plasma membrane junctions hub the Iks complex

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The voltage-gated potassium channel Kv7.1, when associated to the KCNE1 β-subunit, generates the IKs current, an important slowly activating delayed rectifying potassium current of the cardiac action potential. KCNE1 profoundly shapes the function of the channel. The functional Kv7.1/KCNE1 complex is widely studied for more than 20 years. Thus, it is known that relevant cardiac channelopathies, such as long QT syndrome, are caused by mutations in either subunit. However, the subcellular compartment hosting the assemblage of the complex and routes driving the functional complex to the plasma membrane raise an intense debate. Our results based on FRET experiments, cell unroofing preparations (CUPs) and TEM do not support an interaction early in their biogenesis. Endoplasmic reticulum to Golgi transport experiments revealed that Kv7.1 and KCNE1 used different pathways to reach the cell surface. However, upon association, Kv7.1 redirects KCNE1 to a non-conventional secretory pathway that bypasses Golgi. Proteins that organize and stabilize membrane specific subcellular domains improved Kv7.1 surface targeting. dSTORM superresolution imaging studies in cardiomyocytes revealed a relation between Kv7.1 and KCNE1 in specific membrane regions of the cell. Our results indicate that t-tubules are important compartments for the Kv7.1/KCNE1 association and localization in cardiomyocytes. Our data on the cardiac Iks complex reveals an emerging and hidden anterograde trafficking mechanism for oligomeric ion channels in mammalian cells.

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Symposium 6: Drug Discovery and ion channel Pharmacology

Chair: Asia Fernández

Invited

VOLTAGE-GATED SODIUM CHANNELS AS PHARMACOLOGICAL TARGETS FOR NEUROPATHIC PAIN RELIEF.

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Voltage-gated sodium channels (VGSCs) play an essential role in neuronal and non-neuronal function, being responsible for the initiation and propagation of action potentials in excitable cells by allowing the influx of sodium ions. The VGSCs are large integral membrane proteins composed of a 240-260 kDa pore-forming α-subunit and one or more auxiliary β-subunits of smaller size (30–40 kDa). Ten mammalian α-subunit isoforms have been identified, 9 of which ($Na_v1.1$– $Na_v1.9$) are voltage-gated and one non-voltage-gated member, Nax, which is involved in salt sensing. The different VGSC subtypes have distinct kinetics and voltage-dependent properties and differ in their tissue localization and sensitivity to tetrodotoxin (TTX). In adulthood, the VGSCs α-subunits $Na_v1.1$, $Na_v1.6$, $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ are all normally expressed in primary sensory neurons in the PNS, whereas $Na_v1.3$ is normally expressed in the CNS. This tissue localization makes these α-subunits have a critical role in neuronal function under both physiological and pathological conditions. Genetic studies in animals and humans have led to significant advances in our understanding of the role of VGSCs in pain pathways. In fact, a growing number of pain syndromes have been attributed to mutations in genes encoding VGSCs. In addition, alterations in the expression and/or function of some specific VGSCs have been implicated in a number of chronic pain conditions, including neuropathic pain, which remains a huge unmet health need. Some of these VGSCs have been proposed as targets for analgesic drug development. In particular, the finding of new molecules or known compounds that selectively can block one or several VGSCs subunits expressed in the PNS, to avoid CNS and cardiac side effects, can be of great interest for neuropathic pain relief.

Keywords: Voltage-Gated Sodium Channels; VGSCs; $Na_v1.7$; $Na_v1.8$; $Na_v1.9$; Chronic pain; Neuropathic pain.

Invited

B–LACTAM TRPM8 ANTAGONISTS PREVENT CHEMOTHERAPY-INDUCED COLD ALLODYNIA

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Transient receptor potential melastatin type 8 (TRPM8) is considered an attractive therapeutic target in the search for new analgesics and antitumor agents [1]. Cumulative experimental evidences identify increased TRPM8 expression in sensory neurons after nerve injury or inflammation, resulting in enhanced sensitivity to cold allodynia and hyperalgesia, while activation of TRPM8 appears also important in attenuating pain in certain acute and inflammatory pain states [2]. TRPM8 expression is also up-regulated in different tumor cells (i.e. prostate and skin melanoma cancers, among others) [1,2].

Following an HTS screening campaign of our in house library of compounds, we discovered some β,γ-diaminoesters having TRPV1, TRPM8 and TRPA1 antagonist properties [3]. To restrict the conformational flexibility, the hydrophobic substituents important for TRPM8 blockade were attached on a rigid β-lactam scaffold. This lead to a new family of compounds that selectively blocked all modalities of TRPM8 activation (menthol, voltage, and temperature) [4]. Further optimization of the first series of β-lactam TRPM8 antagonists afforded compounds with improved properties. In this presentation I will talk about different aspects of this research, from the *in vitro* pharmacological characterization to the *in vivo* activity in a model of chemotherapy-induced allodynia. Molecular modeling studies that point to possible modes of interaction with the channel will also be commented.

Key words: TRPM8; antagonists; chemotherapy-induced allodynia; β–lactams

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S6O1

Low-throughput evaluation of novel $Ca_v2.1$-modulators to treat Hemiplegic Migraine

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Human mutations in the *CACNA1A* gene, encoding the pore-forming α_{1A} subunit of the voltage-gated $Ca_V2.1$ calcium channel, cause most of the familial and sporadic hemiplegic migraine (FHM/SHM) cases. Hemiplegic Migraine (HM) mutations induce a gain of $Ca_V2.1$ channel activation that specifically enhances cortical excitatory transmission to favor initiation and propagation of cortical spreading depression, a key process in migraine pathophysiology [1]. Accordingly, there are pharmacological evidences suggesting that reduction of $Ca_V2.1$ activity can provide a new therapeutic approach for the treatment of HM and the relief of common migraine [2, 3]. Currently the only truly $Ca_V2.1$-selective inhibitors are peptide toxins of the agatoxin family, which are not suitable therapeutic tools: their mode of inhibition can give rise to undesirable side effects and peptides have limited utility for *in vivo* studies.

Here we have employed two strategies to identify novel selective CaV2.1-inhibitors: a) chemical modifications from a start-point-molecule (state-dependent inhibitor of $Ca_V2.1$ and $Ca_V2.2$); and b) a structure-based design approach to identify ligands that selectively bind to the calmodulin binding (IQ-like) domain of the $Ca_V2.1$ channel, whose amino acid sequence differ from the IQ-site of other Ca_V family members. Indeed, Ca_V channel blockers with tunable selectivity, kinetics and potency have been previously characterized via targeting of the IQ motif [4]. From an initial set of 80 compounds recognized as potential $Ca_V2.1$ blockers *in silico*, our results highlighted six novel small organic molecules with higher selectivity for $Ca_V2.1$ inhibition (over $Ca_V2.2$ and $Ca_V1.2$ blockade) as prospective elements to develop HM therapeutic tools. In a low-throughput evaluation of the compounds, we studied their effect on the wild-type "healthy" and gain-of-function FHM mutant $Ca_v2.1$ channels. We identified novel potent and selective inhibitors of $Ca_V2.1$ capable of preventing the excessive activity of $Ca_V2.1$ channels produced by human mutations leading to HM, with minor effects on the wild-type channel (in order to reduce/prevent potential side effects). In addition, to validate the potential therapeutic use of the best candidates, we tested if the reduction of excessive $Ca_V2.1$ activity was sufficient to prevent hyper-excitability on cortical networks observed in HM. Interestingly, our study featured two selective $Ca_v2.1$ inhibitors that diminished excitability of the neuronal network, alone or in combination with other drugs. These novel molecules aimed at modulating the $Ca_v2.1$ channel activity and revert the pathological gain-of-function effect might contribute to the development of a new, effective and safe treatment for both hemiplegic and common migraine

Keywords: $Ca_V2.1$, Ca^{2+} influx, inhibitors, gain-of-function, hemiplegic migraine, cortical neurons, cortical neural networks, glutamate release.

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S6O2

Identification of IQM-266, a novel DREAM ligand that modulates K_V4 currents.

Diego A. Peraza[1,2], Pilar Cercós[3], Pablo Miaja[1], Yaiza G. Merinero[1], Laura Lagartera[3], Paula G. Socuéllamos[1,4], Carolina Izquierdo García[3], Sara A. Sánchez[1], Alejandro López-Hurtado[5,6], Mercedes Martín-Martínez[3], Luis A. Olivos-Oré[4], José R. Naranjo[5,6], Antonio R. Artalejo[4], Marta Gutiérrez-Rodríguez[3], Carmen Valenzuela[1,2].

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Downstream Regulatory Element Antagonist Modulator (DREAM)/KChIP3/calsenilin is a neuronal calcium sensor (NCS) with multiple functions, including the regulation of A-type outward potassium currents (I_A). This effect is mediated by the interaction between DREAM and K_V4 potassium channels and it has been shown that small molecules that bind to DREAM modify channel function. A-type outward potassium current (I_A) is responsible of the fast repolarization of neuron action potentials and frequency of firing. Using surface plasmon resonance (SPR) assays and electrophysiological recordings of $K_V4.3$/DREAM channels, we have identified IQM-266 as a DREAM ligand. IQM-266 inhibited the $K_V4.3$/DREAM current in a concentration-, voltage-, and time-dependent-manner. By decreasing the peak current and slowing the inactivation kinetics, IQM-266 led to an increase in the transmembrane charge ($QK_V4.3$=DREAM) at a certain range of concentrations. The slowing of the recovery process and the increase of the inactivation from the closed-state inactivation degree are consistent with a preferential binding of IQM-266 to a pre-activated closed state of $K_V4.3$/DREAM channels. Finally, in rat dorsal root ganglion neurons, IQM-266 inhibited the peak amplitude and slowed the inactivation of I_A. Overall, the results presented here identify IQM-266 as a new chemical tool that might allow a better understanding of DREAM physiological role as well as modulation of neuronal I_A in pathological processes.

Keywords: $K_V4.3$ channels; DREAM; DREAM ligands; KChIP; A-type current; Alzheimer.

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Posters

P001

Study chronic effect of 17-b-estradiol over trp channels in nociceptor terminals in microfluidic chambers.

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Pain is a major problem for current healthcare representing a source of great human suffering and monetary costs. Standard analgesic treatments to date have side effects. Thus, targeting peripheral pain transduction offers a complementary way to manage pain. Transient receptor potential (TRP) family play a key role in pain transduction in nociceptive terminals which makes these ion channels potential peripheral antinociceptive targets. In this project we study the possible sensitizing effect of 17-β-estradiol over TRP activity that could explain sex differences in a nitroglicerine-induced migraine model. In order to study TRPV1/A1/M8 in neural projections we perform long lasting trigeminal cultures in microfluidic devices, the use of this innovative technique allows to apply stimuli in the nociceptor's terminal in contrast to the classical culture. Taking profit of the advantages of this new type of culture, we study the effect of chronic application of sexual hormone 17ß-estradiol over nociceptor sensitivity, which is applied at different physiologically relevant concentrations. Then we perform calcium imaging assays and qRT-PCR to describe TRP activity and expression.

Keywords: Microfluidic chambers; Trigeminal culture; Transient Receptor Potential (TRP); 17-β-estradiol; Calcium imaging.

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P002

***In vivo* role of TRP channels in an experimental model of chronic migraine.**

González-Rodríguez S, Alarcón-Alarcón D, Fernández-Carvajal A, Ferrer-Montiel A.

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The current available treatments to manage chronic pain are often insufficient or their use is limited by side effects such as addiction, respiratory depression or tolerance in the case of opioids or gastrointestinal bleeding, ulcers and cardiovascular complications induced by non-steroideal anti-inflammatory drugs. Thus, new drugs are urgently needed. Among the mediators already identified as potential targets to develop new drugs, TRP channels superfamily has been identified as one of the most relevant channels. Several pharmacological strategies have been used to target these ion channels, however, their proper role in some pathologies such as migraine is unknown especially due to the limited number of existing experimental models. The use of animal pain models and more specifically knock out mice (TRPV1$^{-/-}$, TRPA1$^{-/-}$ or TRPM8$^{-/-}$) might constitute a useful tool to study the role of the channels in different diseases and allows us to understand the neurochemical changes during the establishment and maintenance of pain.

Keywords: migraine, TRP channels, pain.

P003

Expression of the cold thermoreceptor TRPM8 in peripheral sensory neurons: evidence for distinct subpopulations

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The ion channel TRPM8 is mainly expressed in a fraction of peripheral sensory neurons and is the main sensor of environmental cold in mammals. The function of this ion channel in sensory neurons of the dorsal root ganglia (DRG) and trigeminal ganglia (TG) has been extensively studied using calcium imaging and electrophysiological techniques. However, the lack of reliable antibodies against TRPM8 has limited the use of antibody-based detection methods so far.

In this work, we screened several antibodies against TRPM8 on two transgenic TRPM8 reporter mouse models: TRPM8-green fluorescent protein (GFP) knock-in mice, *Trpm8*^{EGFPf} [1] and TRPM8-yellow fluorescent protein (YFP) transgenic mice, *Trpm8*^{BAC}*-EYFP*$^{+}$ [2].

We performed "*in situ*" hybridization (ISH) against TRPM8, combined with GFP immunofluorescence, in TG sections from hemizygous *Trpm8*$^{EGFPf/+}$ mice and found that nearly all GFP(+) TG neurons expressed TRPM8 mRNA. The GFP expression pattern in cryosections of adult TG and DRG ganglia revealed the presence of two populations of labeled neurons: small, intensely fluorescent neurons, and medium-size, lightly fluorescent cells. In DRG sections, they represented about 7 and 15% out of all the neurons respectively. A similar finding was obtained in short term cultures from adult DRG and TG, with two clearly differentiated labeled populations. Then, we performed immunohistochemical experiments in cryosections and cultured neurons, testing a battery of antibodies against TRPM8, optimizing the protocol conditions for specific labeling. Finally, we used the iDISCO clarification technique [3] in both transgenic models, to examine the presence and distribution of TRPM8 in mouse embryonic and adult tissues.

Our results suggest the presence of distinct subpopulations of TRPM8-expressing neurons in mouse peripheral ganglia, possibly subserving different functions.

Keywords: thermoreceptor; nociceptor; somatosensory; TRP channel; dorsal root ganglia.

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P004

Expression of the cold thermoreceptor TRPM8 in mouse brain circuits involved in thermal homeostasis

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The ion channel TRPM8 is the principal sensor of environmental cold in mammalian sensory nerve endings. Although it is mainly expressed in a subpopulation of peripheral sensory neurons, it has also been identified in certain non-neuronal tissues. Little is known about the expression of this thermosensitive ion channel in the central nervous system. The objective of this work was to study the expression and anatomical distribution of TRPM8 channels in mouse brain.

We used RT-PCR and "in situ" hybridization (ISH). Furthermore, GFP immunohistochemistry was carried out in two transgenic TRPM8 reporter mouse models: TRPM8-green fluorescent protein (GFP) knock-in mice, $Trpm8^{EGFPf}$ [1] and TRPM8-yellow fluorescent protein (YFP) transgenic mice, Trpm8BAC-EYFP+ [2]. Finally, we performed patch-clamp recordings in $Trpm8^{BAC}$-$EYFP^{+}$ septal neurons.

We found that TRPM8 is expressed in mouse central nervous system, although with much lower levels of expression than in peripheral sensory ganglia. Positive cells were mainly identified in the preoptic hypothalamus, septal area, reticular thalamic nucleus and limbic regions with projections widely distributed within the brain and brainstem. Electrophysiological recordings in brain slices revealed the functionality of these ion channels.

Our results showing expression of TRPM8 in the central nervous system open a new window in TRPM8 physiology. Further experiments are required to fully understand the potential roles of this molecular sensor within the brain.

Keywords: TRP channels; hypothalamus; septum; reticular thalamus; patch-clamp; thermoregulation.

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P005

Comprehensive biophysical assays: From single channel electrophysiology to overall cell behavior

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Integral membrane proteins, predominantly ion channels and transporters, have been the focus of basic biophysical research, as well as drug discovery and safety projects for decades. Electrophysiological experiments on fully functional artificial lipid bilayers enable the investigation of basically any membrane-affecting agent. In combination with automated patch clamp and impedance/electrical field potential (EFP)-like recordings of relevant targets expressed in heterologous systems, as well as of human iPSC-derived cardiomyocytes and neurons, we demonstrate broad biophysical application assays, connecting single channel electrophysiology with overall single cell and cell population behavior.

Here, we present the temperature dependent activation or deactivation of different Transient Receptor Potential (TRP) channels by means of planar patch clamping on our medium and high throughput screening (HTS) platforms Patchliner and SyncroPatch 384PE, as well as with highest resolution on a single channel level on our recently introduced Orbit mini setup. Additionally, the effect of drugs on action potentials as recorded in iPSC-cardiomyocytes is important for assessing the interaction of the cardiac ion channel ensemble. We present our advances in development of iPSC-cardiomyocytes “ready-to-use” assays for automated patch clamp. We also show, short and long-term impedance/EFP-like recordings of diverse cell-types, such as drug safety experiments on iPS cardiomyocytes and cancer tox-assays. In summary, medium and high throughput screening (HTS) assays such as automated electrophysiological patch clamp and impedance-based assays allow for the determination of drug effects on a whole cell level whereas artificial bilayers provide a robust environment for the assessment of single ion channel molecules.

P006

Role of GirK channels in dorsal hippocampus functionality

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The G-protein-gated inwardly rectifying potassium (Kir3/GirK) channel is the effector of many G-protein-coupled receptors. Its dysfunction has been linked to the pathophysiology of Alzheimer disease, Down syndrome, psychiatric diseases, epilepsy or alcoholism. GirK channels are constitutively active in the dorsal hippocampus contributing to both, resting membrane potential, and compensate any excesses of excitation. Despite the critical involvement controlling neuronal excitability, it remains to be stablished their role in dorsal hippocampus physiology. Here, we have examined the role of GirK-dependent signalling in the mouse dorsal hippocampus at different levels of complexity, and its relevance in the maintenance of hippocampal-dependent cognitive functions. For that purpose, GirK-dependent signal was pharmacologically modulated by specific drugs. Our data shows that the disruption of GirK-dependent signalling in the dorsal hippocampus, whether gain or loss of function, induces learning and memory deficits by impairments in LTP induction. These results support the contention that an accurate control of GirK activity must take place in the hippocampus to sustain cognitive faculties.

Key words: Kir3; GirK; hippocampus; excitability; LTP; Learning and Memory; mouse

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P007

Ion transport in confined geometries below the nanoscale: interfacial effects and scaling behavior in membrane channels.

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Many protein channels have in common the importance of electrostatic interactions between the permeating ions and the nanochannel. Since ion transport occurs under confinement conditions, interfacial effects such as access resistance (AR) may play a significant role. We measure AR in a large ion channel, the bacterial porin OmpF, by means of single channel conductance measurements in electrolyte solutions containing varying concentrations of high molecular weight PEG, sterically excluded from the pore. We found that AR might reach up to 80% of the total channel conductance in diluted solutions, where electrophysiological recordings register essentially the AR of the system and depend marginally on the pore characteristics [1]. These results show that a sound interpretation of electrophysiological measurements in terms of channel ion selective properties requires the consideration of interfacial effects. On the other hand, charged polar groups of the lipid may have a strong influence on the electric potential and the ionic concentration near the membrane-solution interface. Charged residues within the protein located near the pore mouth can also play a role, although to a lesser extent than AR and membrane surface charges. These three factors are obviously coupled and are strongly dependent on the channel aperture size, 3D structure and channel-lipid assembling. Comparison of experiments performed in charged and neutral planar membranes shows that lipid surface charges modify the ion distribution and determine the value of AR, indicating that lipid molecules are more than passive scaffolds even in the case of large transmembrane proteins. These findings are relevant to the fact that ionic conductance in membrane channels exhibits a power-law dependence on electrolyte concentration ($G \sim c^{\alpha}$). The many scaling exponents, α, reported in the literature usually require detailed interpretations concerning each particular system under study. We critically evaluate the predictive power of scaling exponents by analyzing conductance measurements in four biological channels with contrasting architectures [2]. We show that scaling behavior depends on several interconnected effects whose contributions change with concentration so that the use of oversimplified models missing critical factors could be misleading. In fact, the presence of interfacial effects could give rise to an apparent universal scaling that hides the channel distinctive features. We complement our study with 3D structure-based Poisson−Nernst−Planck (PNP) calculations, giving results in line with experiments and validating scaling arguments. Our findings not only provide a unified framework for the study of ion transport in confined geometries but also highlight that scaling arguments are powerful and simple tools with which to offer a comprehensive perspective of complex systems, especially those in which the actual structure is unknown.

Keywords: access resistance; scaling behavior; PEG; ion electrodiffusion; single-molecule electrophysiology

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P008

Deciphering the PKA-dependent Kv1.3 endocytosis

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The voltage-gated potassium channel $K_V1.3$ is expressed in many tissues but playing a special role during the immune response, being crucial for the activation and proliferation of leukocytes. An altered expression of the channel is at the onset of several autoimmune diseases. Therefore, Kv1.3 is considered a potential therapeutic target against pathologies such as multiple sclerosis, psoriasis or rheumatoid arthritis.

In this context, the study of the mechanisms involved in the modulation of $K_V1.3$ deserves considerable attention. The down-stream signal produced by the channel is a balance between the positive and negative inputs targeting the channel to the cell surface. Therefore, the $K_V1.3$ turnover, caused by channel internalization and degradation, influences the inflammatory response. Thus, understanding endocytosis is crucial for the knowledge of the $K_V1.3$ physiology. Adenosine (Ado), a potent endogenous immunomodulator, induces $K_V1.3$ endocytosis in leukocytes by stimulating PKC. In addition, Ado also activates PKA-signaling pathways. In this study we have investigated the role of PKA in $K_V1.3$ turnover. To that end, we examined the channel internalization, membrane abundance, ubiquitination and endocytic mechanisms. Our results show that PKA activation increased the number of channels endocytosed . Moreover, we found that the $K_V1.3$ internalization is ubiquitin independent and seems to target the channel to both lysosomal and proteasomal degradation. Therefore, Ado, exerting an effective anti-inflammatory mechanism, mediates two alternative and redundant PKC and PKA-mediated molecular mechanisms modulating the abundance of $K_V1.3$ at the cell surface. Our results bring light to the effective Ado-dependent immunosuppression in leukocytes.

Keywords: Protein turnover; Endocytosis; Ubiquitination; Protein Kinase A; Voltage-gated potassium channels.

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P009

S-Acylation of the regulatory Kvβ2.1 subunit

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Kvβ proteins are cytoplasmic peptides mainly known as voltage dependent K^+ channel (Kv channels) regulatory subunits. Three members belong to this family, Kvβ1, 2 and 3, presenting a four-fold symmetric arrangement. In addition, Kvβs exhibit an oxidoreductase activity in permanent association with the NADPH cofactor. Kvβ subunits alter electrophysiological properties of some Kv1 and Kv4 channels. Moreover, they function as chaperone-like proteins promoting the channel surface expression. In this study, we focus on the Kvβ2.1 subunit. This peptide associates with the Kv1.3 channel leading to multiple physiological effects. Due to their wide expression in both immune and nervous systems, we aimed to elucidate the relationship between these two proteins within the Kv1.3 functional oligomeric complex. Kv1.3 and Kvβ2.1 may be located in specific membrane microdomains, named lipid rafts. S-Acylation is a post-translational modification which mainly occurs on cysteine residues. Some soluble proteins can target to the plasma membrane and lipid rafts by being S-Acylated. Therefore, we have explored this mechanism in Kvβ2.1. By mutating the entire Kvβ2.1 cysteine repertoire, we identify a basal cysteine-dependent S-Acylation of the peptide. Our results indicate that the largest number of mutated cysteines correlates with a markedly reduced surface expression of the regulatory subunit. In addition, the S-Acylation of both proteins affects their interaction.

Keywords: Kv channels; Regulatory Subunits; Lipid Raft Microdomains; Palmitoylation; Cysteine Modification.

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P010

The calmodulin-binding domain of KCNE4 is responsible for the association with Kv1.3

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The voltage-dependent potassium channel Kv1.3 plays a crucial role in leukocyte activation, proliferation and apoptosis. Aberrant expression levels of this channels corelate with autoimmune diseases, and the modulation of this channel has been studied as a therapeutic target for such disorders. In leukocytes, Kv1.3 is coexpressed with the ancillary protein KCNE4. This peptide acts as a dominant negative that both retains Kv1.3 intracellularly and accelerates inactivation kinetics. Therefore, the Kv1.3/KCNE4 complex is crucial for the immune physiology. However, the molecular determinants involved in this association remain unknown. In the present work, we show that a tetraleucine motif in the carboxy-terminal domain of KCNE4 is responsible for Kv1.3 binding and intracellular retention. Furthermore, we propose a structural model of the Kv1.3/KCNE4 complex. In agreement with our experimental data, the *in silico* model suggests that KCNE4 interaction hides a forward-trafficking motif within Kv1.3. Furthermore, KCNE4 transfers a strong endoplasmic reticulum retention signalling to the complex. Our data indicates that the association of KCNE4 to the Kv1.3 channelosome regulates the balance between anterograde and intracellular retention elements that control the levels of Kv1.3 at the plasma membrane. The cell surface expression of the channel is essential for its function in regulating the immune system.

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P011

Distance constraints on activation of TRPV4 channels by AKAP150-bound PKCα in arterial myocytes.

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TRPV4 (transient receptor potential vanilloid 4) channels are Ca^{2+}-permeable channels that play a key role in regulating vascular tone. In arterial myocytes, TRPV4 activity can be enhanced by the action of the vasoconstrictor angiotensin II. Opening of TRPV4 channels creates local increases in Ca^{2+} influx, detectable optically as "TRPV4 sparklets". Modulation of TRPV4 sparklets depends on subcellular signaling domains that involve the activation of protein kinase C bound to the anchoring protein AKAP150. Here, we used super-resolution nanoscopy, patchclamp electrophysiology, and Ca^{2+} imaging approaches to test the hypothesis that AKAP150-dependent modulation of TRPV4 channels is critically dependent on the distance between these two proteins in the sarcolemma of arterial myocytes. Our data show that the distance between AKAP150 and TRPV4 channel clusters varies with sex and arterial bed. Consistent with our hypothesis, we further found that basal and angiotensin II-induced TRPV4 channel activity decays exponentially as the distance between TRPV4 and AKAP150 increases. Indeed, our data suggest a maximum radius of action of ~200 nm for local modulation of TRPV4 channels by AKAP150- associated proteins.

Keywords: sexual dimorphism; vascular physiology; super resolution; calcium; sparklets.

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P012

Mechanisms linking Kv1.3 to proliferation

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Potassium channels, by modulating membrane potential (E_M), cell volume and/or Ca^{2+} influx, are important elements in cell cycle regulation. However, growing evidence shows that non-canonical functions can influence cell proliferation in the absence of (or in addition to) K^+ efflux. The voltage-dependent potassium channel Kv1.3, has been implicated in the control of cell cycle in different cells and in different ways. The specific requirement of this channel in proliferation in several cell types, suggests the involvement of molecule-specific interactions via mechanisms that are just starting to be identified.

In a heterologous expression system, we have previously shown that the expression of Kv1.3 channels increased proliferation, while the expression of Kv1.5 (another member of the Kv1 family) decreased it. In this system, we demonstrated that Kv1.3-induced proliferation does not require K^+ fluxes, but needs an intact voltage-sensing mechanism. We identified the molecular determinants of Kv1.3-induced proliferation at the cytosolic-C-terminal domain. In this region, two individual point mutations at putative phosphorylation sites (Y447A and S459A) abolished proliferation.

Here, we further explore the mechanisms involved in Kv1.3 proliferation by analyzing: 1) the effect of changes on membrane potential (E_M) on Kv1.3-induced proliferation, 2) the effect of Kv1.3 mutants with altered gating properties on proliferation and 3) the interactions of Kv1.3 with proteins that activate signaling pathways.

Kv1.3 or Kv1.3-WF (pore mutant channel) channels were co-transfected with WT-KATP channels (composed of SUR1+Kir6.2) or gain of function (GOF-KATP) ATP–insensitive channels (SUR1+Kir6.2G334D). Resting E_M was significantly hyperpolarized and Kv1.3-induced proliferation was abolished in cells co-expressing GOF-KATP, and was restored by increased [K+]e. To confirm the voltage-dependence of Kv1.3 induced proliferation, we designed Kv1.3-mutants with a shift of the activation towards more depolarized potentials, and we tested their effect on proliferation. Finally, we found that Kv1.3 interacts with IQGAP3, a scaffold protein involved in proliferation which facilitates MEK/ERK signaling. Kv1.3-induced proliferation and Kv1.3 phosphorylation was impaired by MEK/ERK signal inhibition. Moreover, Kv1.3 phosphorylation and Kv1.3 interaction with IQGAP3 were facilitated by membrane depolarizations. Altogether, these data indicate that voltage-dependent conformational changes of Kv1.3 are an essential element in Kv1.3-induced proliferation.

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P013

Activation of Calcium dependent Chloride Channels (CaCCs) by Epsilon toxin from *Clostridium perfringens* through the required accessory MAL protein.

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Epsilon toxin (Etx) is a pore forming toxin produced by *Clostridium perfringens* toxino-types B and D. The oligomerization of the toxin on the plasma membrane would alter the cell's integrity and will cause an ionic imbalance. Conversely, Etx may act on a receptor, triggering a downstream signaling and rising eventually the Ca^{2+} concentration within host cells. Etx has been described as pore forming protein due to its structural similarities to the aerolysin-like β-barrel pore forming toxins. However, a cellular receptor has been claimed and recently it has been demonstrated that the Myelin and Lymphocyte protein (MAL) is required and necessary for the action of the toxin [1]. Even though the hypothesis of pore formation and/or receptor signalling are still in debate, our results do not indicate a pore formation activity on the plasma membranes of *Xenopus laevis* oocytes expressing human MAL. In any case, the toxin causes a release of ATP only in cell lines expressing MAL and we have therefore investigated the intracellular cascades triggered by the interaction between Etx and MAL protein. We observed that ATP release was dependent on the intracellular Ca^{2+} mobilization. Moreover, this calcium mobilization activated the endogenous CaCCs of *Xenopus* oocytes which are also described as TMEM16A or Anocatmin1. TMEM16A belongs to a family of proteins with functions varying from Cl^- channels to scramblase [2]. The switch from a channel function to a scramblase activity is restricted to one or two amino acids. These channels despite their recent molecular and structural characterization have shown to have a vast relevance in different pathologies: the Scott syndrome affecting the coagulation cascade, in some epithelial tumours and craniocervical dystonia as examples. TMEM16A/Anoctamin 1 has been proved to have a direct contact with the IP_3 receptor of the endoplasmic reticulum and consequently has the ability to modify the balance of ER-stored or cytoplasmic Ca^{2+}, allowing the trigger of different internal cascades. When MAL is present in the plasma membrane, our results indicated that Etx activated this particular pathway, because inhibitors of PLC or STIM1/Orai complexes as well as Ryanodine receptor and IP_3 inhibitors did not inhibit the release of ATP. Correspondingly, the internal calcium mobilization caused by Etx, stimulated the activation of the TMEM16A currents, abolished through TMEM16A antisense nucleotides. In addition, MOLT4 cell line expressing MAL released ATP and developed a scramblase activity, which was recorded by labelled AnnexinV, when treated with Etx. Conversely, cell lines devoid of MAL did not have a scramblase activity. Hence, TMEM16A is behaving as a scramblase when MAL is present and exposed to the toxin. We hypothesize that MAL protein acts as an accessory protein for TMEM16A in the lipid raft, where the interaction with the toxin causes a change in conformation of TMEM16A-MAL complex and by this, activates the IP_3 receptor transporting Ca^{2+} into the cytoplasm, activating the TMEM16A channel which also acquires the scramblase configuration.

Keywords: Epsilon toxin; Myelin and Lymphocyte protein; TMEM16A; Anoctamin; internal calcium mobilization; *Clostridium perfringens.*

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P014

K_V1.3 channel inhibition by indolic compounds in chronic lymphocytic leukemia cells

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Introduction: Chronic lymphocytic leukemia (CLL) is the most common leukemia in western countries and it's based on B cell clonal expansion. This disease has no cure and the appearance of pharmacological resistance is very common. We have identified indole-3-carbinol (I3C) and its main metabolite, 3,3'-diindolylmethane (DIM), as active compounds with pharmacological activity against CLL. K_V1.3 potassium channels are involved in B cells and T cells function controlling plasmatic membrane potential, Ca^{2+} entry and cellular proliferation. Therefore, these channels could be a new therapeutic target against CLL. Here, we have analysed if these indolic compounds can act, in part, by modulating the K_V1.3 function.

Material and Methods: we assessed the expression, activity and effect of the indolic compounds on K_V1.3 channels in CLL cells that are either sensitive or resistant to I3C cytotoxicity. Currents were registered by whole-cell patch-clamp. Statistical significance was determined by t-Student test or by nonparametric Mann-Whitney test.

Results: the K_V1.3 current magnitude on CLL cells correlates with their sensitivity to the cytotoxic effect of I3C, resistant- exhibiting CLL cells ≈2.5-fold higher K_V1.3 current amplitude and K_V1.3 expression than sensitive-CLL cells. Both I3C and DIM inhibited K_V1.3 current in CLL cells, being DIM ≈4-fold more potent. However, indole-3-carboxylic acid (I3CA), a non-cytotoxic compound, did not inhibit the K_V1.3 current.

Conclusions: I3C sensitive-CLL cells exhibit greater K_V1.3 current magnitude than I3C resistant-CLL cells, which can be due to the existence of different canalosomes in these cells. The K_V1.3 inhibitory effect of the indolic compounds tested, correlates with their cytotoxic effect on CLL cells. Our results suggest that K_V1.3 channel could be involved in CLL cells pharmacological resistance mechanism and its inhibition could be part of the cytotoxic mechanism of I3C and DIM, which open new venues to the treatment of this disease.

Key words: Chronic lymphocytic leukemia (CLL), K_V1.3, indole-3-carbinol (I3C), 3,3'-diindolylmethane (DIM), B cell

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

P015

Biophysical interaction of NMDAR and BK channels in heterologous systems.

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Large conductance Ca^{2+}- and voltage-gated K^+ channels (BK) are regulated by changes in the membrane potential and intracellular Ca^{2+} concentration. Different ion channels have been described as Ca^{2+} sources for BK channel physiological activation, including voltage-gated Ca^{2+} channels (VGCC) [1], TRPV channels [2] and ryanodine receptors [3]. In addition, it has been shown that N-methyl-D-aspartate receptors (NMDAR) and BK channels are functionally coupled in the central nervous system [4,5]. Recent results from our group demonstrate the relevance of this interaction for synaptic plasticity in basal dendrites of barrel cortex layer 5 pyramidal neurons (BC-L5PN) (unpublished data). However, the biophysical properties of NMDAR-BK complexes function remain elusive.

In this work, we used a combination of electrophysiological and fluorescence techniques to characterize NMDAR-BK function. Inside-out patch-clamp recordings from transfected HEK293T cells showed similar BK activation levels when they are associated with NR2A- or NR2B-containing NMDAR. This result is consistent with the experiments performed in acute slices, where BK channel outward currents are both partially abolished by selective antagonists of either NR2A- or NR2B-containing NMDAR channels. Proximity ligation assay (PLA) experiments to quantify NMDAR-BK association levels showed similar interaction events between BK and NR2A- or NR2B-containing NMDAR. These interactions most likely occur at distances below 50 nm, as further confirmed by acceptor photobleaching FRET experiments.

In summary, we demonstrate that BK is functionally coupled with both NR2A- and NR2B-containing NMDAR, forming nanodomains (20-50 nm) at the plasma membrane. Integration of NMDAR and BK channels in complexes may alter the biophysical properties of individual channels, e.g. inducing synergistic effects in activation and deactivation kinetics. To address this question, studies of NMDAR (including NR2A or NR2B)-BK complexes using whole cell patch-clamp fluorometry are currently underway.

Keywords: BK channel; NMDAR; electrophysiology; nanodomain; protein-protein interactions.

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P016

Development and optimization of BK-cpGFP Ca^{2+} sensors.

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Calcium ions trigger intracellular signals that are related to multiple cellular functions. In the nervous system, they regulate the neurotransmitter release, which happens at the microsecond scale. Genetically encoded Ca^{2+} indicators (GECIs) entail a powerful tool for visualizing and quantifying intracellular calcium signals, with high temporal resolution. In addition, some Ca^{2+} signaling events occurring at localized neuronal regions require high spatial resolution. At the synapse, large-conductance Ca^{2+} and voltage-activated potassium (BK) channels form Ca^{2+} nanodomains with voltage-dependent Ca^{2+} channels (VDCC). The action potential at these nanodomains triggers simultaneous membrane depolarization and intracellular Ca^{2+} increase. As a consequence, BK channels are activated, leading to repolarization. Function of these molecular "brakes" for neuronal function and neurotransmission has been elusive due to the lack of appropriate sensors. In this context, we hypothesized that BK channels, which are the physiological intracellular Ca^{2+} sensors located in neuronal Ca^{2+} nanodomains, may constitute an excellent molecular basis to generate local reporters of Ca^{2+} concentration. This approach would produce unprecedented tools to study this physiological process in vivo.

Based on FRETable BK channels previously developed in our laboratory [1], we have generated a potential Ca^{2+} sensor (BK-860cpGFP) by inserting a circularly permutated green fluorescent protein (cpGFP) into the BK amino acid position 860. Here we show that BK function is not altered by the cpGFP insertion. Addition of ionomycin to BK-860cpGFP-transfected HEK293 cells induced an increase in cpGFP fluorescence, which was associated to Ca^{2+} binding to specific sites in the BK channel. Simultaneous imaging and electrophysiology experiments in excised membrane patches allowed us to characterize the Ca^{2+} and voltage-sensitivity of the fluorescent signal. Although our initial results show robust fluorescent signals, the signal-to-noise ratio from BK-860cpGFP signals may not be high enough to record Ca^{2+} in vivo. A modification that has proved to improve the dynamic range of sensors signals consists on the alteration of the amino acid linkers between the host protein and the cpGFP [2]. In BK-860cpGFP constructs, the proteic linkers between the cpGFP and BK consist on 9 and 12 aminoacids at each end of the cpGFP. These amino acid sequence and length is determined by the mosaic ends of the transposon Tn5 and the restriction sites designed during the in vitro transposition process used to generate the original BK-FRET library of constructs [1]. In this work, we have shortened the existing linkers to a total of three aminoacids. By employing degenerated oligonucleotides, 5'-VVCVVCVVC-3', where V can correspond to an adenine, cytosine or guanine, around 700 possible combinations are obtained. The resultant constructs were transfected in HEK293T cells a screened using a semi high-throughput fluorescence assay with a Victor X5 plate reader. Fluorescence readings were obtained in basal conditions and after Ca^{2+} addition. We obtained at least four constructs showing a higher dynamic range of the cpGFP than the original BK-860cpGFP. Biophysical characterization of the optimized BK-860cpGFP constructs is currently underway by using Patchclamp fluorometry (PCF).

Keywords: BK channels, GECIs, cpGFP, Ca^{2+} nanodomains, genetic engineering optimization.

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P017

Role of the Neuronal BKβ4 Subunit in the Formation of Calcium Nanodomains Constituted by BK and Voltage-Gated Calcium Channels.

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Voltage and calcium dependent-potassium channels (BK, Slo1 or maxi-K channels) are present in a number of tissues in association with voltage-gated calcium (Ca_v) channels, being this interaction essential to accomplish a variety of biological roles such as regulation of the contractile tone in smooth muscle, mediation of flow-stimulated kaliuresis in the kidney and control of neurotransmitter release in the brain [1]. These functional properties rely on the unique gating of BK channels, since they are allosterically activated by two separate stimuli: elevations of intracellular Ca^{2+} and membrane depolarization, leading higher intracellular Ca^{2+} concentrations to pore opening into the physiological voltage range. Apart from α pore-forming subunits, the gating properties that BK channels display in a number of cells and tissues are dramatically modified by the presence of different specific regulatory subunits (β and γ subunits). In brain tissue, the interaction with the $BK\beta_4$ subunit is key to understand the role of BK channels in terms of neuronal excitability [2]. $BK\beta_4$ containingchannels display slow gating kinetics, resistance to iberiotoxin and charybdotoxin blockade and ethanol sensitivity, among other electrophysiological properties.

In this work, we have explored the potential influence of $BK\beta_4$ on the reconstitution of nanodomains formed by a subset of Ca_v channels with distinct functional properties and BK channels in HEK293T cells. On the one hand, the formation of complexes between Ca_v and BK channels has been assessed by Proximity-ligation assay (PLA) allowing in situ detection and quantitation of protein-protein interactions with high sensitivity. Additionally, using superresolution stochastic optical reconstruction microscopy (STORM) we have directly visualized with sub-diffraction resolution the intimate association of individual molecules revealing nanometer-sized BK-Ca_v clusters with precise detail and spatial accuracy. Complementary, functional coupling of these channels was evaluated by electrophysiological studies.

Our results demonstrate that $Ca_v2.1$, $Ca_v2.2$ and $Ca_v1.2$ (but not $Ca_v2.3$) are located in close proximity forming nanodomains with BK channels when co-expressed in heterologous systems, in consistency with prior studies [3]. Interestingly, the $BK\beta_4$ subunit exhibits an enhancing effect on the formation of complexes, since its presence increases the proportion of heteromeric clusters of BK-Ca_v channels.

Our findings offer an unprecedented view into the clustering of BK and Ca_v channels at the plasma membrane providing new structural information to understand their spatial relationship.

Keywords: BK channels; Ca_v channels; nanodomains; superresolution; electrophysiology.

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P018

Functional implications of L-type calcium channels association with neuronal lipid rafts.

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Neuronal lipid rafts can be seen as plasma membrane nanodomains particularly relevant for rapid and efficient cross-signaling between calcium and reactive oxygen and nitrogen species [1,2]. In previous works, we have shown that neuronal L-type calcium channels (LTCCs) and N-methyl-D-aspartate receptors (NMDAR) are major calcium entry systems associated with lipid rafts nanodomains, and this allows for focalized production of nitric oxide in neurons upon their activation [2-3]. The tight association of these calcium entry systems with lipid rafts leads to high calcium concentration submicrodomains in their vicinity upon activation of either LTCCs or NMDAR. In addition, the integrity of neuronal lipid rafts plays a major role in the activation of LTCCs by partial plasma membrane depolarization, because its disruption with methyl-β-cyclodextrin impairs the phosphorylation of LTCCs by CaMK and PKA and leads to its inactivation [4]. In addition, cytochrome b_5 reductase is also associated with these neuronal lipid rafts [5], and this is a redox system that upon deregulation by cytochrome *c* in the early stages of neuronal apoptosis releases superoxide anion [6]. Therefore, neurotoxic pro-oxidant stimuli can activate the focalized production by lipid rafts of peroxynitrite, and LTCCs activity is highly sensitive to oxidative modification by peroxynitrite [1]. Thus, impairment of LTCCs activity should also be expected for pro-oxidant neurotoxic agents that bind to neuronal lipid rafts. Indeed, this point has been confirmed with amyloid β peptide 1-42 in primary cultures of rat cerebellar granule neurons.

Keywords: L-type calcium channels; lipid rafts; neurons; calcium microdomains; amyloid β

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P019

Calcium dependent control of mucin secretion

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Calcium-induced mucin secretion from goblet cells by exogenous agonist-dependent (stimulated) and -independent (baseline) manner is essential to create the mucus layer that protects the epithelial lining from external particles, such as pathogens and allergens. Here we present a new model for mucin secretion in which extracellular calcium entry is necessary for stimulated secretion, whereas intracellular calcium oscillations are required for baseline secretion. Our data reveal that cooperation between the sodium channels TRPM4-TRPM5 and sodium-calcium exchangers (NCXs) promote the entry of extracellular Ca^{++} to release mucin from goblet cells [1]. Baseline secretion, on the other hand, is triggered by intracellular calcium oscillations mostly by the function of ER-located ryanodine receptors (RYRs). These calcium oscillations act via a small Ca^{++} binding protein, KChIP3 to control the fusion propensity of mucin granules [2]. Our data prompt us to propose that KChIP3 is a high affinity Ca^{++} sensor specific for baseline mucin secretion. Upon binding to calcium, KChIP3 dissociates from mature granules, which are then processed for fusion to plasma membranes. In other words, KChIP3 bound to granules, acts as a break- a negative regulator- of fusion of mucin granules to plasma membrane. Altogether, we suggest the existence of chemically distinct pools of mucin granules for baseline and stimulated mucin secretion and the ability to control the release propensity of these granules provides novel means to control dysregulated mucin secretion dependent human pathologies of airway and the colon.

Keywords: TRPM4, NCX, KChIP3, Mucin secretion, Calcium oscillations

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P020

Proteomics-based gene disease associations and drug discovery approaches to understand elusive roles for TRPV2 in central nervous system.

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TRP channels are important pharmacological targets in physiopathology. The elusive, but widely expressed TRPV2 channel is the closest homologue to TRPV1, by far the best studied TRP channel. TRPV2 plays distinct roles in cardiac and neuromuscular function, immunity, and metabolism, and is associated with pathologies like muscular dystrophy and cancer. However, the lack of specific pharmacological profiles for TRPV2 hinders the study of the physiopathology relationships of this channel and specific syndromes. Here, we report two parallel strategies to gain insight on physiopathology and pharmacology of this ion channel. On one hand, interactomics allowed us to describe a TRPV2-interactome signature, which strongly associates with glioblastoma multiforme prognosis, progression, recurrence, and chemotherapy resistance [1]. Both approaches have opened the possibility to study novel TRPV2 gene-disease associations in the central nervous system, such as demyelination and hypomyelination syndromes.

Keywords: TRPV2; interatomics; gene-disease association; glioblastoma multiforme; drug discovery.

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P021

Effects of aging on K^+ and Ca^{2+} currents and glucose-regulated Ca^{2+} signals in mouse pancreatic alpha and beta-cells.

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The prevalence of diabetes and deterioration of glucose homeostasis increases with age. Peripheral insulin resistance (IR) is frequently found in elderly individuals. Under IR states, the endocrine pancreas undergoes different functional and structural adaptations in order to increase insulin secretion and maintain normoglycemia. However, if this adaptation is not sufficient, hyperglycemia and diabetes can emerge. Glucose homeostasis mainly depends on the coordinated secretion of insulin and glucagon from pancreatic beta and alpha-cells, respectively. In both cases, secretion relies on glucose-regulated metabolic, electrical and Ca^{2+} signaling events. In the present study, we aim to study the functional adaptations of the pancreatic alpha and beta-cells in a mouse model of aging: 3 and 20 month old animals were compared. Aged mice were also distributed in two groups according their IR (aged-IR) or non-IR state (aged-NIR). While aged animals exhibited hyperglucagonemia, only aged-IR animals displayed hyperinsulinemia. K^+ and Ca^{2+} currents were evoked by depolarizing voltage pulses in alpha and beta-cells identified by their cell capacitance and sodium inactivation current. Both current types showed a high tendency to be increased in aged-IR beta-cells. Glucose-regulated Ca^{2+} signals in alpha and beta-cells were analyzed by confocal microscopy within intact islets. In all groups, the characteristic alpha-cell Ca^{2+} signals at low glucose levels (0.5 mM) were inhibited when glucose was increased to 11 mM. However, this effect was more pronounced in the aged groups. The percentage of alpha-cells that responded to glutamate was also different during aging. Finally, we detected that the trigger of glucose-induced Ca^{2+} signals in the beta-cells was delayed in the aged-NIR. Thus, these preliminary results indicate that the electrophysiological and Ca^{2+} signaling patterns are altered in pancreatic islets during aging.

Keywords: pancreatic alpha-cell, pancreatic beta-cell, aging, islet of Langerhans, Ca^{2+} signals, hormonal secretion, K^+ and Ca^{2+} currents.

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P022

Role of LTCCs and alterations in Ca^{2+} dynamics in a rat model of subarachnoid haemorrhage

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Background: Subarachnoid haemorrhage (SAH) is a neurovascular disease where the rupture of brain blood vessels causes blood release into the perivascular space. Frequently, sustained vasospasm develops a few days after the haemorrhagic episode, producing severe injury with a high morbidity-mortality. L-type Ca^{2+} Channels (LTCCs) are involved in sustained arterial contraction and may play a role in the mechanism underlying arterial vasospasm, where vascular smooth muscle cells (VSMC) are depolarized [1].

Objectives: To explore whether LTCCs and Ca^{2+} dynamics could be altered in a SAH rat model.

Methodology: All experiments were conducted in accordance with Spanish R.D. 53/2013 and European Union 2010/63/EU legislation on protection of experimental animals. Using a SAH rat model we explored whether pathways involved in regulation of arterial contraction could be affected. Animals were divided in 3 experimental groups: control, sham and SAH. Electrophysiological recordings were performed on days 5, 7 and 9 after surgery, using Circle of Willis VSMCs. Resting potential was measured using the patch-clamp technique in current-clamp configuration. Cytoplasmic Ca^{2+} dynamics was evaluated on day 7 after surgery using Circle of Willis VSMCs incubated with 1 µM Fura 2-AM. Different extracellular high K^+ solutions were used to evaluate basal Ca^{2+} levels and Ca^{2+} response in the experimental groups. Nifedipine was used to evaluate the role of LTCCs in the basal conditions and under 30 mM K^+ (30K) depolarization.

Results: Electrophysiological analysis showed that the resting membrane potential of VSMCs isolated from cerebral vessels was depolarised in SAH animals *versus* sham on days 5 and 7 after surgery. Microfluorimetric recordings showed that basal Ca^{2+} levels were increased in SAH VSMCs. Application of nifedipine did not produce any significant effect on basal Ca^{2+} levels, indicating that LTCCs are not activated in resting conditions. Application of nifedipine on 30K evoked depolarization produces higher reduction in Ca^{2+} level in SAH versus sham and control.

Conclusions: SAH can induce alterations in ions homeostasis causing the depolarization of VSMCs from the cerebral arteries affected by the haemorrhage.

Keywords: Subarachnoid haemorrhage; vasospasm; LTCCs; Ca^{2+} dynamics; arterial depolarization.

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P023

Aging enhances expression of IP_3 receptors and ER-mitochondria colocalization leading to Ca^{2+} remodelling in rat hippocampal neurons.

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Aging is associated to cognitive decline and susceptibility to neuron death, two processes related recently to subcellular Ca^{2+} homeostasis. One of the most common pathologies associated to aging is Alzheimer's disease (AD) that has been related to excess of neurotoxic oligomers of amyloid β peptide (Aβo), loss of intracellular Ca^{2+} homeostasis and mitochondrial damage. However, the intimate mechanisms underlying this aging-related disease remain obscure. We have reported recently that long-term cultures of rat hippocampal neurons resembling aging neurons are prone to damage and display critical changes in intracellular Ca^{2+} homeostasis including increased resting cytosolic Ca^{2+} concentration, enhanced Ca^{2+} store content and increased Ca^{2+} transfer from the endoplasmic reticulum (ER) to mitochondria. This may favor energy production in aging at the expense of increased susceptibility of neuron cell death. In addition, we showed that store-operated Ca^{2+} entry (SOCE), a Ca^{2+} entry pathway involved in memory storage is decreased in aging. To disentangle the basis for the above changes we have used immunofluorescence to investigate expression of all IP_3 receptors in aged neurons, fluorescence imaging to monitor ROS generation and mitochondrial potential and confocal microscopy to estimate ER-mitochondrial colocalization in aged neurons. In addition, the effect of treating cultured neurons with Aβo was tested as well. We found that expression of all three IP_3 receptor isoforms increases with in vitro aging in rat hippocampal neurons. In addition, ROS generation was enhanced in aging neurons and this effect is further enhanced by treatment with Aβo. Mitochondrial potential, which is the driving force for mitochondrial Ca^{2+} uptake and ATP synthesis in mitochondria, is decreased in aging neurons and this effect is also exacerbated by Aβo. Finally, ER-mitochondria colocalization is also enhanced in aging neurons and this process is further exacerbated by Aβo. These results suggest that aging favors Ca^{2+} transfer from ER to mitochondria probably to increase efficiency of energy production. However, these changes may contribute to enhance susceptibility to cell death and decrease SOCE and memory storage in aging and AD.

Keywords: Aging; hippocampal neurons; store-operated calcium entry; endoplasmic reticulum; mitochondria.

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P024

Illuminating transcriptomic analysis of calcium remodeling in colorectal cancer cells.

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Colorectal cancer (CRC) cells undergo a remodeling of intracellular Ca^{2+} homeostasis that contributes to cancer hallmarks such as enhanced proliferation, invasion and survival. Important changes at the functional level include upregulation of store-operated Ca^{2+} entry (SOCE) and mitochondrial Ca^{2+} uptake together with depletion of intracellular Ca^{2+} stores (1)(2). Some of these changes have been investigated at the molecular level. However, since nearly 250 genes are involved in intracellular Ca^{2+} transport we have assessed possible differences in expression of these genes using Next Generation Sequencing (NGS) technologies. For this end, we used normal human colonic NCM460 cells and human colon adenocarcinoma HT29 cells. We had previously used IonTorrent® technology for analysis of differential expression of 77 genes (3). Now, we have extended our analysis to all calcium related genes and the whole transcriptome of NCM460 and HT29 cells using Illumina® technology. Data analysis has been carried out using R and bioconductor packages. We found that the outcomes from the 77 genes reported previously were very similar using both IonTorrent® and Illumina® technologies. Specifically, we observed similar differences in expression of selected voltage-operated Ca^{2+} channels and molecular players involved in store-operated Ca^{2+} entry (SOCE). Moreover, changes in expression of TRP channels, Ca^{2+} release channels, Ca^{2+} pumps and Na^{+}/Ca^{2+} exchangers were similar in both cases. Finally, genes involved in activation of mitochondria Ca^{2+} transport were similarly upregulated in CRC while genes involved in preventing mitochondrial Ca^{2+} uptake were downregulated. These results may provide a comprehensive view of calcium remodeling in CRC and its contribution to cancer hallmarks.

Keywords: Next Generation Sequencing; Calcium Homeostasis remodeling; Colon Cancer; store-operated Ca^{2+} entry; Mitochondrial Ca^{2+} transport.

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P025

ORAI1 translocation to the leading edge triggered by RAC1 potentiates lamellipodia formation and cell migration

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The plasma membrane Ca^{2+} channel ORAI1 and its activator, the endoplasmic reticulum protein STIM1, regulate plasma membrane ruffling in migrating cells [1]. Although STIM1 is a modulator of other Ca^{2+} channels, the pool of STIM1 phosphorylated at ERK1/2 sites (Ser575, Ser608, and Ser621) is known to activate ORAI1 [2]. It is also known that in migrating cells there is an enrichment of phospho-STIM1 at the leading edge, where phospho-STIM1 colocalized with ORAI1 [1]. However, it remains unclear the molecular mechanism for this specific localization of ORAI1 at the front of migrating cells, where the cytosolic free Ca^{2+} level is lower than in the rear part of the cell (trailing edge). In this work, we have found that epidermal growth factor (EGF) potentiated the co-precipitation and co-localization of ORAI1 with cortactin, a specific cytoskeletal protein enriched at the leading edge in the osteosarcoma cell line U2OS. RAC1, a small GTPase that regulates the localization of cortactin, co-precipitated with ORAI1 in cells stimulated with EGF. Because these co-precipitations were sensitive to the RAC1 inhibitor NSC23766, our results suggested a role for RAC1 in the relocalization of ORAI1 to the leading edge. To confirm this hypothesis, we used U2OS cells stably expressing Flag-RAC1 (wild-type), Flag-RAC1 G12V (constitutively active mutant), or Flag-RAC1 T17N (dominant negative mutant) to study ORAI1-cortactin localization. We found that RAC1 G12V triggered a robust translocation of ORAI1-cortactin to the cell periphery, whereas RAC1 T17N inhibited the translocation of ORAI1 to the leading edge. Because NSC23766 inhibited the externalization of ORAI1 (measured by a biotinylation assay) and also the extension of the lamellipodia at the leading edge, our results demonstrate that ORAI1 regulates lamellipodia formation, which is fully required for efficient cell migration.

Keywords: Calcium, lamellipodia, migration, ORAI1, RAC1.

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P026

STIM1 regulates ITPR3 levels and controls ER-mitochondria Ca^{2+} shuttling.

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STIM1 protein is the main regulator of store-operated Ca^{2+} entry (SOCE), a Ca^{2+} influx pathway regulated by the Ca^{2+} concentration within the endoplasmic reticulum (ER). As an ER-resident protein, STIM1 acts as a Ca^{2+} sensor within the ER lumen, and binds and activates plasma membrane Ca^{2+} channels (SOC channels) upon partial Ca^{2+} depletion at the ER. STIM1 is ubiquitous and plays an essential role in Ca^{2+} homeostasis, which is required for cell survival, since the dysregulation of Ca^{2+} mobilization has been linked to several pathologies, including autoimmune and neurodegenerative diseases. On the other hand, inositol 1,4,5-trisphosphate receptors (IP_3Rs) are involved in modulating store-operated calcium entry (SOCE). IP3R activation enhances STIM1-IP_3Rs association leading to an increased Ca^{2+} influx through SOC channels [1]. In this regard, STIM1 and IP_3Rs play an important role in Ca^{2+} shuttling between ER and mitochondria, an essential event to maintain an adequate Ca^{2+} homeostasis and mitochondrial function. We have recently reported that SH-SY5Y cells engineered by CRISPR to knockout STIM1 gene showed a significant mitochondrial dysfunction and loss of cell viability [2]. With the aim to study further the role of STIM1 in mitochondria, in this report we studied the correlation between STIM1 expression and IP_3Rs mRNAs levels. We found that STIM1-KO cells showed a strong decrease of ITPR3 (also known as IP_3R3) mRNA and protein expression, compared to wild-type cells. These results, together with a significant decrease of the mitochondrial Ca^{2+} uniporter (MCU) led to a decrease in the free Ca^{2+} concentration in mitochondrial ($[Ca^{2+}]_{mito}$) in STIM1-KO cells, measured with the genetically-encoded Ca^{2+} sensor 4mtD3cpv. On the contrary, wild-type SH-SY5Y cells overexpressing STIM1 showed an increase of ITPR3 expression levels. Finally, the overexpression of the ITPR3 receptor in STIM1-KO cells contributed to the normalization of $[Ca^{2+}]_{mito}$, a result that demonstrates that STIM1 controls ITPR3 expression and consequently the steady-state levels of $[Ca^{2+}]_{mito}$.

Keywords: Calcium; CRISPR; ITPR3; mitocondria; STIM1.

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P027

SOCE is essential for leukemia and breast cancer cell migration

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Eukaryotic organisms possess extraordinary and highly sophisticated machinery that controls and modulates Ca^{2+} homeostasis. Among them, store-operated calcium entry (SOCE) and its main components, STIM, Orai and TRPC, fine-tune the Ca^{2+} influx from the extracellular medium, triggered by intracellular Ca^{2+} stores exhaustion, and needed for the activation of several physiological events, including migration [1]. Therefore, up- or down-regulation in the expression of STIM, Orai or TRPC might modify the migration rate of breast cancer cells. Wound healing assay is an expeditious approach that provides valuable information about the role of a specific protein in migration and, therefore, in the pathophysiology of breast cancer cells. By creating a "scratch" or wound in a cell monolayer and taking pictures at regular intervals until the wound is completely closed, we can quantify the migration rate of a cell population and compare it with another [2]. Moreover, for suspension cell cultures, such as the human promyelocytic leukemia cell line (HL-60), we have adopted the transwell migration assay that allows us to study the ability of cell motility and invasiveness toward a chemo-attractant gradient [3]. By using molecular biology, biochemistry and imaging microscopy techniques we have demonstrated that knockdown of Orai1 and Orai2 in the human promyelocytic leukemia cell line (HL-60), and TRPC6 in the luminal ER^{+} (MCF7) and the triple negative (MDA-MB-231) breast cancer cells remarkably impaired SOCE and, subsequently, cell migration. Silencing Orai1 and Orai2 in HL-60 led to impairment of FAK tyrosine phosphorylation, while knockdown of TRPC6 in MCF7 and MDA-MB-231 resulted in significant reduction of the translocation of Orai3 and Orai1 to the plasma membrane, respectively, thus inhibiting SOCE and migration. These data support a role for SOCE in the high migration rate exhibited by leukemia and breast cancer cells, which could lead, eventually, to the establishment of biomarkers and therapies targeting those proteins and improving the diagnosis and prognosis for breast cancer.

Keywords: SOCE; Breast Cancer; Migration; Wound healing assay; Transwell migration assay.

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P028

Progesterone induces Ca^{2+} entry in triple negative MDA-MB-231 breast cancer cells by a mechanism involving STIM2, Orai1 and TRPC1.

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MDA-MB-231 cells lack the classical nuclear progesterone (P_4) receptor [1], thus other proteins would act as surrogates to accommodate this hormone; hence, it is probable that different cell signalling pathways could be driven by progesterone in these cells, which was aimed in the present investigation. Incubation for 72 h with P_4 (1 μM) reduced MDA-MB-231 cell proliferation. P_4 (1 μM) evoked Ca^{2+} mobilization in MDA-MB-231 cells, which resulted enhanced by knocking down the progesterone receptor membrane component 1 (PGRMC1) that has been reported as a P_4 target receptor. STIM2 silencing almost fully impaired P_4-evoked Ca^{2+} mobilization, and furthermore, Orai1 and TRPC1 silencing also blocked P_4-driven Ca^{2+} entry. In contrast, STIM1 silencing did not alter P_4-dependent Ca^{2+} entry; therefore, P_4-evoked Ca^{2+} entry is strongly dependent on STIM2, Orai1 and TRPC1 in MDA-MB-231 cells. Interestingly, PGRMC1 silencing impaired the inhibitory effect of P_4 on proliferation. Finally, we evaluated the intracellular localization of p53 and NFAT1 in order to ascertain possible changes in their function evoked by P_4. Incubation of MDA-MB-231 cells for 72 h with P_4 significantly increased p53 translocation to the cell nuclei at 24h and 72 h as compared with control untreated cells, which has been already reported [2]. Contrary, in cells lacking PGRMC1, p53 values in the nucleus resulted similar to those found in mock non-stimulated cells. Regarding NFAT1, as previously reported by others, Orai1 activation by the P_4.STIM2 pathway was able to enhance NFAT1 migration to the cell nuclei after 48 h. In addition, in cells lacking PGRMC1, NFAT1 migrated to the nuclei even at 24 h, finding a significant greater NFAT1 accumulation in the nuclei at 48 h as compared to mock cells treated with P_4. Altogether these findings suggest that despite P_4 favours a proproliferative Ca^{2+} dependent mechanism, driven by STIM2, but it is overcome by activation of p53 through PGRMC1.

Keywords**:** P_4, PGMRC1, STIM2, MDA-MB-231, NFAT1.

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P029

Store operated Ca^{2+} entry and Angiogenesis

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Store-Operated Calcium Entry (SOCE) plays critical role in different cellular processes. Within the key elements of SOCE Orai1 is the pore-forming subunit of the store-operated calcium. The aim of this study was to examine the role of SOCE and Orai1 in angiogenesis.

Experiments were conducted using Human Umbilical Endothelial Cells (HUVECs) between 4 and 10 passages. Angiogenesis was evaluated using HUVEC-induced tube formation and by vessels' sprouting using rings of rats' aorta by Matrigel assay and an endothelial cell basal medium enriched with different growth factors (VEGF, FGF, b-EGF, IGF).

We observed that HUVECs tube formation was sensitive to SOCE through the specific inhibitor of Orai1 GSK-7975A. The addition of 20 µm of GSK-7975A didn't inhibit tube formation meanwhile at 50 and 70 µm GSK-7975A prevented angiogenesis, indicating that GSK-7975A effect is dose dependent. Similarly, GSK-7975A was able to inhibit sprouting angiogenesis in aorta rings dose dependently using 50, 70 and 100 µm of GSK. Moreover, Orai1 silencing seems to inhibit tube formation. However, orai1 knockdown only delayed but did not inhibit sprouting.

Our preliminary data suggest that SOCE is required for angiogenesis, although the role of Orai1 is not clear in neovascular formation.

Keywords: Store operated Ca^{2+} entry; Orai1; angiogenesis.

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P030

Functional role of ARC channels in triple negative MDA-MB-231 breast cancer cells.

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In non-excitable cells, the Ca^{2+} permeable plasma membrane channel, Orai1, was initially described to participate in store-operated Ca^{2+} entry (SOCE), a mechanism for Ca^{2+} influx regulated by the Ca^{2+} content in the intracellular reservoirs. Later on, Orai1 was found to be involved in a store-independent mechanism driven by arachidonic acid (AA), the arachidonate-regulated Ca^{2+} (ARC) channels [1]. Two Orai1 variants, Orai1α and Orai1β, generated by alternative translation initiation have been identified [2-3]. Although Orai1α has been found to form ARC channels with Orai3 and the plasma membrane pool of STIM1, the physiological relevance of these channels are poorly investigated. Thus, we aimed to investigate the functional role of ARC channels in triple negative breast cancer MDA-MB-231 cells. Western blotting analysis of cell lysates revealed that MDA-MB-231 cells exhibit a greater Orai1α/Orai1β ratio than the non-tumoral breast epithelial cell line MCF10A. Ca^{2+} imaging experiments revealed that AA-stimulated Ca^{2+} entry is increased in MDA-MB-231 cells as compared with MCF10A cells, thus suggesting a greater number of ARC channels in cancer cells. In mock-treated cells treatment for 24 h with 8 μM AA enhanced MDA-MB-231 cells proliferation. Reconstitution of ARC channels by overexpression of Orai1, Orai3 and STIM1 significantly enhanced AA-induced Ca^{2+} entry but abolished cell proliferation. Summarizing, fine-tuning of ARC-dependent Ca^{2+} entry is essential for MDA-MB-231 cell proliferation, and hence, ARC channels represent a suitable target to develop anti-tumoral pharmacological strategies for breast cancer.

Keywords**:** ARC, Orai1α, MDA-MB-231, cell proliferation.

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P031

Assesing the role of SOCE in proliferation in cancer cells

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Store-operated calcium entry (SOCE) is a major Ca^{2+} entry pathway in non-excitable cells [1]. SOCE and its essential components, STIM, Orai and TRPC, trigger a Ca^{2+} influx from the extracellular medium, upon intracellular Ca^{2+} stores depletion. Those increases in the cytosolic Ca^{2+} levels are required for the activation of several physiological processes, such as proliferation. Deviations in SOCE or the pattern of expression of its components and modulators might lead to various pathological conditions, including cancer [2]. By using molecular biology, biochemistry and fluorescence imaging microscopy techniques we have demonstrated that knockdown of specific components of SOCE in the human promyelocytic leukemia cell line (HL-60), the luminal ER^{+} (MCF7) and the triple negative (MDA-MB-231) breast cancer cells dramatically impaired Ca^{2+} entry. In addition, we present solid evidences demonstrating that SOCE inhibition led to a drastic reduction in proliferation. These data support a role for SOCE and its main components, STIM, Orai and TRP proteins, in the exacerbated proliferation exhibited in tumoral cell, opening the way to the development of tools and therapies that target these proteins, improving the diagnosis and prognosis for acute myeloid leukemia and breast cancer.

Keywords: SOCE; Acute Myeloid Leukemia; Breast Cancer; Orai1, Orai2; TRPC6.

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P032

Sarcoplasmic reticulum calcium release in human induced pluripotent stem cell-derived cardiomyocytes (iPS-CM) is enhanced by triiodothyronine and dexamethasone-induced maturation.

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iPS-CM are an invaluable cell model for studying patient-specific cardiac pathologies. However, they present an immature phenotype with incomplete excitation-contraction coupling, inefficient calcium-induced calcium release, and lack of t-tubules. This compromises the study of calcium handling-related pathologies such as catecholaminergic polymorphic ventricular tachycardia (CPVT). To improve iPS-CM maturity, we treated beating cardiomyocytes with triiodothyronine (T3) and dexamethasone (Dex), as recently described by Parikh and collaborators [1]. The aim of this study was to determine the molecular and physiological changes associated to hormone maturation treatment of iPS-CM.

We generated iPS-CM using a heparin-based differentiation protocol [2] followed by glucose starvation [3]. We treated beating cardiomyocytes monolayers with T3 and Dex from day 16 until day 30 of differentiation. We measured the expression of tubulogenesis markers (*JPH2* and *CAV3*), beta adrenergic receptors (*ADRB1* and *ADRB2*), calcium handling-associated genes (*RYR2* and *PLN*), and the fetal and adult isoforms of *SCN5A* by qPCR. Also, we determined if the sarcoplasmic reticulum (SR) had a higher contribution in electrically-induced calcium transients in hormone treated iPS-CM. Finally, we characterized calcium transients elicited by caffeine pulses in both basal and β-adrenergic stress conditions (100nM isoproterenol).

Our qPCR results showed an increase in the expression of the tubulogenesis marker *JPH2* (2.20-fold), beta adrenergic receptor *ADRB1* (10.11-fold) and calcium handling-related gene *PLN* (1.61-fold). In addition, we found that hormone treatment promotes a switch in the relative expression of the neonatal isoform of *SCN5A* to the adult isoform. Moreover, we found that hormonal treatment induced a higher contribution of the SR in electrically-induced calcium transients. We also found that caffeine sensitivity was increased in hormone-treated *vs.* control cells in the presence of isoproterenol (Kd T3+Dex=1.38±0.06mM; Kd Ctrl=1.92±0.16mM).

In summary, our results demonstrate that the hormone treatment induced upregulation of the *JPH2*, *ADRB1* and *PLN* genes, together with an increased expression of the adult *SCN5A* isoform. The treatment also promoted a more robust contribution of the SR to electrically-induced calcium transient and a higher caffeine sensitivity in β-adrenergic stress conditions. All these changes indicate that using our improved differentiation protocol, iPS-CM can achieve a more mature functional and structural phenotype.

Keywords: iPS-CM; Cardiac maturation; calcium signaling; sarcoplasmic reticulum; β-adrenergic.

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P033

Kv1.3 channel blockade improves insulin resistance and reduces risk of arrhythmia in a type 2 diabetic animal model

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Background: Diabetes is associated with changes in the electrical behavior of the heart, known as cardiac electrical remodeling, that make the myocardium prone to fatal arrhythmias and sudden death. The electrocardiogram of diabetic patients shows changes in the repolarization phase, mostly the lengthening of the heart rate-corrected QT interval (QTc) which correlates with ventricular arrhythmia. Besides, in diabetes a hyper activation of the inflamatory/inmune system also takes place and the released proinflamatory cytokines might be the source of the electrical remodeling. Immunomodulation induced by PAP-1 has been reported to improve insulin resistance, one of the main characteristics of type 2 diabetes mellitus (T2DM), the type that accounts for more than 90% of diagnosed cases.

Purpose: We investigated if immunomodulation, induced by PAP-1, could normalize the ECG alterations and reduce the risk of VT observed in T2DM, as well as improve hyperglycemia by reducing insulin resistance.

Methods: For the T2D model Sprague-Dawley rats were fed for 6 weeks with high fat diet and received an IP injection of STZ (35 mg/Kg) at week 2. Then the animals were treated with either metformin, first therapeutic choice for T2D, or the Kv1.3 channel blocker PAP-1. Concentrations of metformin (50-100 mg/kg daily) or PAP-1 (5-10 mg/kg daily) were adjusted individually to correct hyperglycemia. Baseline electrocardiograms (ECGs) were performed weekly and in vivo arrhythmia susceptibility protocol with caffeine (120 mg/kg) ip. and dobutamine (50 µg/kg) iv. at the end of treatment. Concentrations of the Kv1.3 blocker PAP-1 (5-10 mg/kg daily) and metformin (50-100 mg/kg daily) adjusted individually to correct hyperglycemia. Circulating cytokines wer measured with a BioPlex Elisa kit.

Results: Our T2D model showed insulin resistance, hyperglycemia and the classical alterations in the electrocardiogram. Weekly glucose measurements and the intraperitoneal insulin and glucose tolerance test (IPIGTT) showed that PAP-1 normalized insulin resistance better than metformin. Electrocardiograms to conscious animals showed that, whereas metformin did not normalize ECG alterations, PAP-1 treatment normalized RR interval duration and the prolonged QTc interval. An in vivo arrhythmia susceptibility protocol with caffeine/dobutamine showed that only treatment with PAP-1 reduced the probability of developing ventricular tachycardia

Conclusions: Immunomodulation by PAP-1 treatment normalizes insulin resistance and glucose levels, shows a tendency to normalize QTc interval and reduces the probability of develop severe arrhythmia under cardiac challenge. Kv1.3 channel blockade is a promising new target for T2D treatment with special relevance to cardiac arrhythmia prevention.

Keywords: Low grade inflammation, TNF□, IL1-b.

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P034

Transmural ventricular biopsies to reliably characterize normal human ventricular electrophysiology from living donors

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Introduction: Cardiac tissue slices have been proposed as a research model that combine the advantages of whole organ and isolated cells, offering intact three-dimensional tissue structure, cellular contacts and representative multicellularity in a less expensive and more controlled manner than in vivo studies or ex vivo whole heart analysis. In the case of left ventricular slices, to date these are generally obtained from tissue blocks of failing human hearts or of sacrificed animals.

Methods: In this study, left ventricular transmural tissue blocks (surface area 5×5 mm) were obtained from explanted pig hearts and compared with tru-cut biopsies obtained from the same ventricular area. In addition, tru-cut left ventricular transmural biopsies representative of normal human myocardium were obtained from patients undergoing coronary artery surgery or valve repair. Both tru-cut biopsies and tissue blocks were sliced in a vibratome, providing a large number of viable 350 μm-thick slices, which were used for viability and histological evaluation as well as electrophysiological assessment. Transmembrane potential and intracellular calcium were optically measured and Action Potential Duration (APD) and calcium transient (CaT) characteristics were calculated at pacing frequencies ranging from 1 to 3 Hz. The response to beta-adrenergic stimulation was evaluated by application of isoproterenol.

Results: Viability assays indicated no damage effects associated with biopsy collection and slicing. Histological examination by a pathologist of sections stained with hematoxylin-eosin and Masson's trichrome revealed well-preserved tissue structure. In pigs, mean optically mapped APD and CaT duration presented no significant differences between tissue blocks and biopsies for all tested pacing frequencies, both at baseline and in response to beta-adrenergic stimulation. In humans, APD and CaT duration could be successfully measured at all frequencies and a reduction in their mean values was quantified following beta-adrenergic stimulation.

Conclusions: Tru-cut left ventricular biopsies provide an affordable way to obtain normal human myocardial tissue from a wide range of patients, suitable for electrophysiological assessment and representative of the native myocardium.

P035

Mutations in KCNQ1, KCNH2 and AKAP9 in a family with congenital long-QT syndrome type 1

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Congenital long-QT syndrome (LQTS) is a hereditary condition characterized by alterations in the ventricular repolarization, increased risk of fainting (syncope) and sudden cardiac death. Diagnosis can be difficult, due to the incomplete penetrance and variable expressivity. Although mutations in 14 genes have been reported to produce LQTS, most of the mutations are found in KCNQ1 (encoding for the Kv7.1 channel, responsible for the I_{Ks} current) and KCNH2 (encoding for the Kv11.1 channel, responsible for the I_{Kr} current). A-kinase anchor protein 9 (AKAP9; Yotiao), another gene associated with LQTS, encodes for a scaffolding protein involved in the regulation of the PKA-dependent phosphorylation of Kv7.1.

The aim of this study is to characterize a family with several members carrying different combinations of mutations in KCNQ1, KCNH2 and AKAP9, and affected by LQTS or Jervell and Lange-Nilsen syndrome, a condition inherited in an autosomal recessive pattern. The index case was a girl who died at the age of 12. She presented a homozygous mutation in KCNQ1 and heterozygous mutations in KCNH2 and AKAP9, associated with a Jervell and Lange-Nielsen syndrome phenotype. Her brother (6 years-old) presents the same genotype and phenotype.

Experiments were performed using molecular biology techniques, patch-clamp, western blot, and microscopy in HEK293 cells transfected with WT and mutant Kv7.1/KCNE1 or Kv11.1 channels.

The mutation in KCNQ1 is intronic, modifies the splicing of the mRNA and produces a deletion of part of exon 3. The resulting DNA codes for a truncated protein with only 264 aa (the WT protein has 676 aa). Homozygous carriers lack functional Kv7.1 channels, and thus I_{Ks} current, whereas heterozygous carriers do not show LQTS phenotype. The missense mutation in KCNH2 is located in the cytoplasmic amino terminus of the channel, and in this family is only presented in heterozygosity. Members carrying only this mutation do not show LQTS phenotype. Electrophysiological analysis of the functional consequences of the KCNH2 mutation is currently being addressed. The missense mutation in AKAP9 is located in the second have of the protein, a part not present in the Yotiao isoform. In this family the mutation is only presented in heterozygosity, and members carrying only this mutation do not show LQTS phenotype. Carriers of combined heterozygous mutations in KCNQ1, KCNH2 and AKAP9 as well as of combined heterozygous mutations in KCNQ1 and AKAP9 show a LQTS phenotype. None of the members of the family presents combined heterozygosity in KCNQ1 and KCNH2.

Our results suggest that AKAP9 and KCNH2 are genetic modifiers of congenital long QT syndrome type 1.

Keywords: arrhythmia; long-QT syndrome; KCNQ1; KCNH2; AKAP9

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P036

Regulation of Kv1.3 to Kv1.5 ratio modulates vascular smooth muscle cell proliferation in human vessels.

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Vascular smooth muscle cells (VSMCs) in the vessel wall are able to switch from a contractile to a dedifferentiated phenotype. This process, known as phenotypic modulation (PM), contributes to the pathogenesis of intimal hyperplasia after vascular surgery. We have previously described that PM associates with a change in the expression of Kv1.3 and Kv1.5 potassium channels: Dedifferentiated VSMC show an increased Kv1.3/Kv1.5 ratio due to Kv1.5 downregulation [1]. As a consequence, there is an increased activity of Kv1.3 channels in synthetic VSMCs, and, more importantly, Kv1.3 blockers inhibit VSMCs proliferation and migration. Here, we explore the efficacy of Kv1.3 channel blockade for the prevention of intimal hyperplasia in human vessels in organ culture and also the mechanisms linking the Kv1.5 to Kv1.3 switch to PM. We used human Mammary Artery (hMA) in organ culture and primary VSMCs cultures obtained from the same samples. We found that Kv1.3 blockers decreases FBS-induced intimal hyperplasia in organ culture, by inhibiting proliferation, migration and extracellular matrix secretion. When exploring the changes in mRNA expression of Kv1.3 and Kv1.5 channels in the different experimental conditions, we found a positive correlation between Kv1.5 and myocardin (MYOCD), the master regulator of the contractile genes expression, while Kv1.3 expression did not show significant variations. These data suggested that Kv1.5 is a MYOCD-regulated gene that acts as a negative regulator of Kv1.3. To confirm this hypothesis, we used viral vectors to overexpress or knock-down Kv1.5 and Kv1.3 channels or MYOCD in VSMCs or in hMA in organ culture. We found that Kv1.5 overexpression decreased intimal hyperplasia in hMA and proliferation of VSMCs in culture to the same extent than Kv1.3 blockers, being both effects non-additive. However, Kv1.5 overexpression did not increase MYOCD. We conclude that Kv1.5 can be considered as a contractile, MYOCD-regulated gene, whose expression prevents Kv1.3 signaling to proliferation possibly by forming heteromultimeric complexes. Upon PM, the loss of Kv1.5 channel expression releases a Kv1.3-dependent proliferative signaling pathway.

Keywords: Kv1.3 channel; Kv1.5 channel; Vascular smooth muscle cells; proliferation; myocardin; Kv1.3 blockers.

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P037

Regulation of vascular tone in a mice model of essential hypertension: role of calcium-dependent chloride channels

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Vascular tone, a key determinant of arterial pressure, is set by the contractile state of vascular smooth muscle cells (VSMCs) and is modulated by many cues affecting all the different mechanisms involved in the process of contraction. Among these mechanisms, membrane potential and $[Ca^{2+}]_i$ are of paramount importance, and changes in the way VSMCs control them are at the core of the development of arterial hypertension, one of the more prevalent pathologies of the cardiovascular system. Using a hypertensive mouse strain (BPH) and its corresponding normotensive control (BPN), our group have identified several changes in the expression of ion channels that are responsible of the depolarized membrane potential and the increased vascular tone of the hypertensive vessels. In this regard, we have described a significantly different functional contribution of voltage dependent Ca^{2+} channels [1], several types of K^+ channels [2] and transient receptor potential (TRP) channels [3] to modulate VSMCs excitability in the hypertensive phenotype. However, changes in the functional contribution to vascular tone of different G-protein coupled receptors (GPCRs) could also be relevant to define the hypertensive phenotype by modulating the TRPC3 channels or the Ca^{2+} activated Cl^- currents (CaCl). Therefore, we explored if changes in the molecular constituents of several G-coupled signaling cascades could contribute to the hypertensive phenotype of the BPN/BPH model.

Endothelial-denuded mesenteric arteries and isolated VSMC from BPN and BPH mice were used to analyze the expression of several GCPRs (P2Y, AGTR, α1, and TP) and CaCl channels (TMEM16A and bestrophin-3) by qPCR. Their functional contribution was explored using electrophysiology and pressure and wire myography. Pressurized mesenteric arteries showed larger vasoconstrictor effects to UTP and UDP in BPH compared to BPN. The pharmacological profile of these responses and qPCR data indicated an increased functional expression of P2Y6 receptors in BPH. Patch-clamp studies showed a significantly larger UTP-induced depolarization in BPH VSMCs which could be abolished by niflumic acid and by Tmem16A inhibitor. Moreover, these blockers induced a significantly higher hyperpolarization in BPH VSMC, pointing to a relevant contribution of CaCl channels to determine VSMC resting Vm. We propose that differences between BPN and BPH UTP-mediated responses are the result of a combination of changes in the expression of P2Y6 receptors, TRPC and CaCl channels. Altogether, these findings could be relevant to explain the increased vascular tone in the hypertensive phenotype.

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P038

Riluzole, BL-1249 AND ML67-33 induce a TREK-like potassium outward current in parasympathetic neurons of the mouse intracardiac ganglion.

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TREK channels (TREK1, TREK2 and TRAAK) constitute a subfamily of the two-pore domain potassium (K2P) channels widely expressed in mammals, both in the central and in the peripheral nervous systems. Our group has shown before that TREK-1 is strongly expressed in the sensitive nodose ganglion and TREK2 in the sympathetic motor superior cervical ganglion of the autonomic nervous system. TREK channels are also expressed in heart cardiomyocytes and they have been related with hypertension or atrial fibrillation, but their presence on neurons of the parasympathetic intracardiac ganglion was unknown. We used the perforated-patch whole-cell modality of the patch-clamp technique to demonstrate the expression of TREK channels in intracardiac neurons in culture. We tested three TREK-channel activators (riluzole, ML67-33 and BL-1249) in voltage-clamp experiments and found that all of them induced a potassium outward current at -30 mV. The riluzole outward current was inhibited by fluoxetine, a strong TREK current inhibitor. Consistently, riluzole induced a hyperpolarization and a reduction of the excitability in adapting intracardiac ganglion. Our results strongly indicate that TREK channels may have an important role in the control of heart rate by modulating the activity of the parasympathetic intracardiac neurons.

Keywords: Parasympathetic neurons; Intracardiac Ganglia; TREK channels.

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P039

Trafficking of the cardiac voltage-gated sodium channel is dependent on N-glycosylation of its β2 subunit.

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The voltage-gated sodium channel is a protein complex playing an important role in cardiac cell contraction. It is mainly composed of a pore-forming α subunit ($Na_V1.5$ in the heart) and two β subunits. β2 (encoded by *SCN2B*) promotes trafficking of α to the plasma membrane, where it performs its function. Pathogenic mutations in β2 are associated with fewer channels at the cell surface, leading to atrial fibrillation and Brugada syndrome (1,2). By biochemical and fluorescence microscopy, we explored the glycosylation profile of β2 in polarized Madin-Darby canine kidney (MDCK) cells. We show that β2 is N-glycosylated at Asn 42, 66, and 74, being sialylated only at Asn 42. Lack of complex glycosylation prevented β2 from reaching efficiently the plasma membrane, leaving the subunit retained in the endoplasmic reticulum. Nonetheless, a fraction of the triple unglycosylated mutant could still be detected at the cell surface. In addition, we demonstrate that wild-type β2 that has not yet undergone full glycosylation still traffics to the plasma membrane bypassing the Golgi apparatus. In addition, we found that β2 glycosylation is essential for its function, as unglycosylated β2 did not promote surface trafficking and localization of $Na_V1.5$. Altogether, we provide evidence at cellular level that N-glycosylation of β2 is key for its localization and for its role on ensuring $Na_v1.5$ trafficking to the cell surface.

Keywords: SCN2B; $Na_V1.5$; cardiac sodium channel; glycosylation; trafficking; MDCK.

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P040

Bisphenol A regulates $Na_V1.7$ ramp currents through activation of estrogen receptor α and PI3K in mouse dorsal root ganglion neurons.

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Bisphenol A is a component found in common household products, such as polycarbonate plastics and epoxi resins, the lining of food cans or thermal paper. Its presence is widespread in the environment. In fact, 93% of the US citizens present measurable amounts of BPA in urine [1] and serum. Many studies suggest that serum BPA concentration in humans is in the range of 1-3 ng/mL [2]. BPA has estrogenic activity and have aroused interest for affecting different physiological systems [3]. While it is well accepted that natural estrogens as 17-β-estradiol may enhance nociception by upregulation of different ion channels and receptors, the implication of BPA in nociception is not well established.

In previous in vivo experiments, we demonstrated that treatment of male C57BL/6 mice with BPA (50 µg/kg/day, an environmentally relevant dose) for 8 days, decreased the latency to pain behavior in response to heat (hot plate at 50ºC), suggesting increased pain sensitivity. We also demonstrated in ex vivo experiments, using the standard whole-cell patch-clamp technique, that incubation of mouse dissociated dorsal root ganglia (DRG) nociceptors with 1 nM BPA, similar to that found in human urine [1], during 24-48h increased the frequency of action potential firing as well as the voltage-gated sodium channel $Na_v1.7$ ramp currents. $Na_v1.7$, which is present in DRG nociceptors and is essential in pain signaling, is the main threshold channel in mouse DRG because its ramp current amplifies small depolarizations, such as generator potentials, and enhances electrical activity.

In this study, we have investigated the mechanism by which BPA increased ramp currents in DRG neurons. For that purpose, estrogen receptor alpha (ERα) and beta (ERβ) agonists and antagonists were used, as well as DRG neurons from ERβ -/- mice. DRG neurons incubated with 1 nM PPT, an agonist of ERα, during 24-48h mimicked the effects of 1 nM BPA on $Na_v1.7$ ramp currents. Moreover, BPA action was blocked using 100 nM MPP, an antagonist of ERα. Additionally, we described that the BPA-induced potentiation of ramp currents was reduced by 100 nM Wortmannin, a broad-spectrum inhibitor of phosphatidilinositide-3-kinase (PI3K), suggesting that the enhancement of $Na_v1.7$ ramp currents may involve PI3K.

In this study, we demonstrate that BPA alters voltage-gated $Na_v1.7$ ramp currents and increases excitability in mice DRG neurons through ERα and PI3K.

Keywords: Nociception; dorsal root ganglion; bisphenol A; $Na_V1.7$ channel; estrogen receptor α.

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P041

Remodeling of mice macrophages induced by trabectedin.

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Immune cells have an important role in the tumor-microenvironment. Macrophages may tune the immune response toward inflammatory or tolerance pathways. Tumor associated macrophages (TAM) have immunosuppressive functions and they are considered a therapeutic target in cancer. The aim of this study was to evaluate the effects of trabectedin, a new class of antitumor agent, on the tumor-microenvironment through the study of electrophysiological and molecular phenotype of macrophages. Experiments were performed using the whole-cell patch-clamp configuration of the patch-clamp technique in resident peritoneal mouse macrophages under different types of polarization. Trabectedin decreased macrophages viability and increased ROS production. Trabectedin does not directly interact with $K_V1.5$ and $K_V1.3$ channels, but treatment (16h) of macrophages with sub-cytotoxic concentrations (0.1-5 nM) increased their K_V current in a concentration-dependent manner due to an upregulation of $K_V1.3$ channels. In vitro generated TAM (TAM_{iv}), by a co-culture of ID8 cells and macrophages, exhibited a M2 phenotype. TAM_{iv} generated a small K_V current, similarly to M2 polarized macrophages, and expressed high levels of M2 markers. In this study, we demonstrated that TAM_{iv} polarization could be re-educated by using sub-cytotoxic concentration of trabectedin. TAM_{iv} treated with sub-cytotoxic concentrations of trabectedin exhibited an upregulation of $K_V1.3$ channels and their M2 phenotype changed towards M1 pro-inflammatory one.

Keywords: Macrophages; tumor associated macrophages (TAM); $K_V1.3$; inflammatory; trabectedin.

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P042

Electrophysiological effects of IQM-266 on K_V1.5 channels.

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The outward potassium current I_{Kur} is the main responsible of the atrial repolarization process and it is generated by the activation of K_V1.5 channels, widely expressed in human atria. It is known that mutations in *KCNA5* gene, which induce both gain- and loss-of-function in K_V1.5 channel, enhance atrial fibrillation susceptibility. Thus, these channels represent a pharmacological target for the development of antiarrhythmic drugs useful in the treatment of supraventricular arrhythmias. K_V1.5 channels assembly with several regulatory subunits such as $K_V\beta$ and KChIPs (K_V Channel Interacting Proteins). It has been described that KChIP2 physically interacts with K_V1.5 and reduces K_V1.5 cell surface expression levels. Our research group has demonstrated that IQM-266 inhibits the current generated by the activation of K_V4.3 and K_V4.3/KChIP2, being the effects more marked when KChIP2 is present. The aim of the present study is to analyze the effects of IQM-266 on K_V1.5 channels.

In order to achieve these objectives, HEK293 cells transiently expressing K_V1.5 were used. Currents were recorded using the whole-cell configuration of the patch-clamp technique.

The effects of IQM-266 on K_V1.5 currents were concentration-dependent with an IC_{50} of 11 μM (n=24). Block induced by IQM-266 (20 μM) sharply increased within the membrane voltage range of the channel activation, arising a maximum degree of block at +20 mV that remained constant at more positive membrane potentials. This compound at 20 μM produced a time-dependent block, inducing a: 1) faster inactivation (τ = 305.8±47.6 ms vs. 168.0±13.4 ms, in the absence and in the presence of IQM-266, respectively, n=9, $p<0.01$), and 2) slower deactivation kinetics, increasing the contribution of the slow component of deactivation to the total process (0.36±0.05 vs. 0.57±0.05, in the absence and in the presence of IQM-266, respectively, n=11, $p<0.01$). These results are consistent with an open channel block mechanism. Finally, IQM-266 (20 μM) enhanced the degree of use-dependent block of the current (25.6±2.7% vs. 77.6±4.9%, in the absence and in the presence of IQM-266, n=5, $p<0.001$). These phenomenon was explained by a slowing of the recovery process in the presence of the compound (991.1±131.8 ms vs. 5132.8±763.6 ms in the absence and in the presence of IQM-266, respectively, n=4, $p<0.05$).

Thus, IQM-266 is able to bind K_V1.5 channels, although with less affinity than that shown in K_V4.3/KChIP2 complexes.

Keywords: K_V1.5; IQM-266; KChIP2; K^+ channels; DREAM.

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P043

Inhibition of muscle-type nicotinic acetylcholine receptors by the alkaloid peimine.

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Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels (LGICs), expressed in central and peripheral nervous system, which are key therapeutic targets since their dysfunction has been associated to several pathophysiological disorders such as muscular myasthenias, neurodegenerative syndromes or epilepsy, among others [1]. We have now looked for new nAChRm modulators by using docking techniques and virtual screening in chemical libraries. As a result, one of the most promising compounds selected was peimine (Pme), a natural alkaloid obtained from *Fritillaria* plant bulbs, whose extracts are widely used to treat cough and asthma in Traditional Chinese Medicine. Recently, some authors have shown that Pme inhibits Nav1.7, Kv1.3 and hERG ion channles with IC_{50}s ranging 44-354 µM [2-3], but so far, none Pme effects have been described on LGICs. The aim of this study was to assess, by electrophysiological techniques, the effects of Pme on *Torpedo* nAChRms microtransplanted to *Xenopus* oocytes.

Co-application of 10 µM ACh with increasing concentrations of Pme showed that nAChRs were reversibly blocked, in a dose-dependent manner. The dose-inhibition curve showed an IC_{50} of 3 µM and a Hill coefficient of 1. Besides, co-application of ACh and Pme enhanced the decay of currents evoked by 10 µM ACh (I_{ACh}), which might be due to an enhancement of nAChRm desensitization, to a slow I_{ACh} blockade, because of its slow binding kinetics, or even to both of them. Nevertheless, the slowing down of I_{ACh} deactivation found when ACh was rinsed while the cell remained in the presence of Pme, indicates that nAChR affinity for ACh is increased and, hence, strongly suggests that Pme is actually enhancing nAChR desensitization. To assess the voltage dependence of nAChR blockade by Pme, short voltage pulses from -120 to +60 mV were applied during the I_{ACh} plateau, either alone or co-applied with either 1 or 5 µM Pme. The corresponding i/v curves showed a slight voltage-dependence of the nAChR blockade by Pme, this increasing when the oocyte was hyperpolarized, suggesting an open-channel blockade. Furthermore, just pre-application of 1 µM Pme, thus acting solely on resting nAChRs, elicited almost no I_{ACh} inhibition, as would be expected if Pme mainly acts on open receptors. Interestingly, we have recently reported that the enhancement of nAChR desensitization by tetracaine is mediated by its binding to a shallow site inside the channel pore [4].

In conclusion, these results indicate that Pme elicits a strong inhibition of nAChRs, acting mainly by an open-channel mechanism of blockade and enhancing desensitization.

Keywords: peimine; nicotinic acetylcholine receptors; *Xenopus* oocytes; allosteric modulation; open-channel blockade; virtual-docking techniques; virtual-screening assays.

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P044

A new family of chiral heterocyclic compounds that blocks the TRPM8 activation.

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Transient Receptor Potential Melastatin type 8 (TRPM8) is a non-selective cationic channel, activated by innocuous cool to cold temperatures (10-28 °C), membrane depolarization, cooling agents, such as menthol and icilin, and different synthetic molecules. Furthermore, it is known that changes or mutations in these channels produce abnormal sensitivity to pain. In addition, overexpression of this channel contributes to the development of various types of cancer, and they are also involved in asthma, cardiovascular, gastrointestinal and neurodegenerative diseases. For these reasons, many pharmaceutical companies and academic researchers are searching for new modulators of TRPM8 [1,2]. In the group of peptidomimetics, we have evaluated several compounds derived from amino acids, and some of them have shown potent and selective TRPM8 antagonist activity. These compounds are β-lactam derivatives that bear at least three hydrophobic substituents needed for TRPM8 blockade [3]. Following these promising results, we proposed the design, synthesis and study of a new chiral heterocyclic scaffold able to bear three hydrophobic substituents. A small library of compounds based on this new scaffold have been synthesized, and different modifications at the three positions were carried out. All components of the library have been characterized in vitro using a HEK cell line stably expressing TRPM8 channels and a fluorimetry essay to measure the entrance of Ca^{2+} through the cell membrane. Some of the prepared compounds showed antagonist activity of TRPM8 channels and merit further optimization.

Keywords: TRPM8; antagonists; chiral heterocycles; SAR.

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P045

IQM-PC332, a novel DREAM ligand with analgesic effect on experimental peripheral nerve injury- and diabetes-induced pain.

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Neuropathic pain is a form of chronic pain arising from damage to the nerves that sense, transmit or process information about environmental stimuli. Given its growing prevalence and common refractoriness to conventional analgesics, the development of new drugs with pain relief effect constitute a prominent clinical need. In this respect, drugs that reduce activity of sensory neurons by modulating ion channels hold the promise to become effective analgesics.

Here we have used two models of neuropathic pain, namely the chronic constriction injury of the sciatic nerve (CCI) and the streptozotocin-induced diabetic neuropathy, to evaluate the mechanical antiallodynic effect of IQM-PC332, a novel Downstream Regulatory Element Antagonist Modulator (DREAM)/KChIP3/calsenilin ligand potentially affecting DREAM-modulated ionic currents like potassium I_A and I_{Cav}.

IQM-PC332 exerted a mechanical antiallodynic effect following intraperitoneal (I.P; DE_{50} of 0.06 µg/kg, E_{max} of 65%) and intraplantar (DE_{50} of 0.24 mg, E_{max} of 68%) administration in the CCI model. Likewise, IQM-PC332 2 µg/kg I.P. significantly reduced mechanical allodynia in diabetic animals. Interestingly, no effect of IQM-PC332 on glucose levels from both CCI and diabetic animal could be observed at analgesic doses. The effects of IQM-PC332 on I_{Nav}, I_{Cav}, I_A, and the electrical excitability in neurons isolated from dorsal root ganglia (L4-L6; DRG) were also studied with the patch-clamp technique. IQM-PC332 reduced peak amplitudes of I_{Cav} from both Control and CCI animals with CI_{50} (@ +10 mV) of 78 µM y 40 µM, respectively. At variance, IQM-PC332 did not exert any effect on I_{Nav} in the concentration range from 0.01 to 50 µM. PC332 10 µM also inhibited I_A in DRG neurons from CCI animals, this effect being larger with membrane depolarization (≈ 50% inhibition at -10 mV). Interestingly, in about 55% of the neurons, IQM-PC332 slowed inactivation of I_A. In accordance with its effects on ion currents, IQM-PC332 10 µM reduced the time to the first action potential (AP) evoked by current injection while decreasing the amplitude and duration of the afterhyperpolarization that followed after APs within a train. However, no clear effect on instantaneous frequency of AP could be observed. It is suggested that inhibition of Ca^{2+} entry through Ca_v channels coupled to neurotransmitter release from peripheral and central terminals of nociceptive neurons may underlie the analgesic effect of IQM-PC332.

Keywords: I_A; I_{Cav}; DREAM; KChIP3; Calsenilin; IQM-PC332; neuropathic pain.

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P046

First evidence of voltage-gated sodium channel blockade by the marine toxins azaspiracids.

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Azaspiracids (AZAs) are marine toxins responsible for human intoxications discovered two decades ago. Although the levels of these toxins in fisherey products are regulated in the European Union their mechanism of action is still unknown. This group of toxins has been reported to be neurotoxic in several in vitro systems. The first human intoxication documented occurred in The Netherlands after ingestion of contaminated mussels harvested in the Ireland coast in 1995 [1] and nowadays these compounds are distributed worldwide. AZAs reach human consumers through ingestion of contaminated shellfish and cause a variety of gastrointestinal, cardiovascular and neurological symptoms [2-4]. Preliminary studies on the mechanism of action of these compounds reported inhibition of the spike rate in spinal cord neurons [5] but this effect was apparently not related with a blockade of voltage-gated sodium channels, a fact that could be related with the small number of cells evaluated in this initial report. Further on, using automated patch clamp, the activity of AZA1 was analyzed in Nav1.6 sodium channels subunits and this study reported no effect of AZA-1 alone on voltage-gated sodium channels but an inhibition of Nav1.6 channels in the simultaneous presence of AZA1 and glutaric acid [6]. Thus, with the aim of clarifying additionally the purposed effect of azaspiracids on sodium channels, the action of this toxin on different voltage-gated sodium channels subunits expressed in HEK cells was evaluated. Surprisingly, in voltage clamp experiments using manual patch clamp, recordings revealed that AZA1 alone inhibited voltage-gated sodium channels in a concentration-dependent manner, with a maximal effect of about 50 % inhibition at nanomolar concentations (50-200 nM). Additional studies on the effect of the AZA2 analogue on voltage-gated sodium channels are also presented. All together, the findings reported here indicate that voltage-gated ion channels could be related with some of the symptoms observed in humans after consumption of shellfish contaminated with these compounds and point out that additional studies assessing their action on ion channels are needed in order to protect human health. Furthermore, the reported blockade of sodium channels by AZA1 deserves further assessments of combined toxicity due to the risk of additive toxic effects between AZAs and other common marine toxins that are very potent blockers of voltage-gated sodium channels such as paralytic toxins (saxitoxins) and tetrodotoxins.

Keywords: Azaspiracid; European mollusks; ion channels; risk assessment; toxicity; voltage-gated sodium channels.

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P047

Comparative effects of synthetic ciguatoxin CTX3C, gambierone and 44-methylgambierone (MTX3) on voltage-gated sodium channels.

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Gambierdiscus species are the producers of the main toxins causative of the foodborne illness known as Ciguatera Fish Poisoning (CFP). These toxic dinoflagellates are the producers of highly complex polypeptide toxins detected worldwide nowadays. CFP affects annually 10000 to 500000 people worldwide [1] although the prevalence of this disease could be underestimated [2] and at present blooms of these dinoflagellates have expanded worldwide reaching European coasts. In fact, the presence of *Gambierdiscus* species and the related toxins and CFP intoxications have been repetitively identified in Europe during the last decades, especially in the Canary Islands [3-4] and Madeira [5]. Besides ciguatoxins, which are known as activators of voltage-gated sodium channels [6], the structure of two additional ciguatoxin-related toxins named gambierone and 44-methylgambierone (MTX3) has been recently elucidated [7-8]. Initial reports on the biological activity of gambierone and 44-methylgambierone described an effect similar to that of the synthetic ciguatoxin CTX3C although of much lower potency [7-8]. With the goal of further investigating the relative toxicities and activities of these three compounds additional experiments were performed. First, the neurotoxic effect of the three compounds has been evaluated using a human neuronal cell model based on the incubation of SH-SY5Y with ouabain and veratridin together with ciguatoxin or ciguatoxin-like compounds to evaluate their *in vitro* toxic potency [9]. The results presented here indicate that while CTX3C exacerbated the ouabain and veratridin neurotoxicity (in a concentration dependent manner), neither gambierone nor 44-methylgambierone mimicked this effect. Furthermore, while CTX3C at nanomolar concentrations hyperpolarized the activation of voltage-gated sodium channels and decreased current amplitude the effect of both gambierone and 44-methylgambierone was negligible. Therefore, the current results indicate that the ciguatoxin-related compounds gambierone and 44-methylgambierone, widely identified in *Gambierdiscus* extracts, are less potent than CTX3C *in vitro* and thus suggest that their contribution to the human symptoms elicited by CFP may also be minor.

Keywords: ciguatoxin; gambierone; 44-methylgambierone (MTX3); neurotoxicity; voltage-gated sodium channel

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P048

Cisplatin induces apoptosis by targeting mitochondria in human promyelocytic leukaemia HL-60 cells

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Cisplatin is one of the most important and efficacious chemotherapeutic agents for the treatment of cancer. Cisplatin forms inter- and intrastrand crosslinked DNA adducts and its cytotoxicity is mediated by propagation of DNA damage recognition signals to downstream pathways, which ultimately results in apoptosis. This study was intended to investigate whether mitochondria are involved in the cytotoxic effect of cisplatin in human promyelocytic leukaemia HL-60 cells. For this purpose, sub-confluent cultures of HL-60 cells were challenged with different doses (5-100 μM) of cisplatin for 24 h and cytotoxicity was then checked by means of MTS assay. Cisplatin presented enhanced cytotoxicity, with a half-maximal (50%) inhibitory concentration (IC_{50}) of 11.3 μM for HL-60 cells. Mitochondrial staining with the cationic dye TMRM revealed that cisplatin produced a rise in the proportion of cells with depolarized mitochondria, which was independent of mitochondrial reactive oxygen species (ROS) overproduction. The increase in damaged mitochondria induced by cisplatin was consequently followed by released of pro-apoptotic factors from mitochondria, which translated into activation of the initiator caspase-9 and subsequent triggering of caspase-3-dependent apoptosis. Finally, nuclear staining with Hoechst 33342 revealed that cisplatin treatment produced nuclear condensation and DNA fragmentation, thus confirming that the main form of cell death was apoptosis. The present study provides a new mechanistic insight into cisplatin-induced cell killing and may lead to the design of novel therapeutic strategies to improve anticancer drug efficacy.

Keywords: leukaemia, apoptosis, caspases, mitochondrial transmembrane potential, cisplatin.

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P049

Pro-apoptotic effects of a thiazoline-containing Pd(II) complex in human promyelocytic leukaemia HL-60 cells

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Evading apoptosis represents the third hallmark of cancer. Cisplatin is a common chemotherapeutic agent used to treat cancer given that it can restore apoptotic mechanisms in cancer cells. However, cisplatin produces serious side effects as it also affects healthy cells, and certain cancers may acquire resistance to cisplatin. Thus, synthesis of new Pt(II) compounds as an alternative to cisplatin is warranted to avoid resistance and undesirable side effects. Pd(II) could be a Pt(II) surrogate given the similarity of coordination chemistry between them, thus widening the spectra of available anticancer drugs. The objective of this study was to test the potential cytotoxic and pro-apoptotic actions of a Pd(II) complex coordinated with the ligand PyTT (2-(2-pyridyl)imine-N-(2-thiazolin-2-yl)thiazolidine), a thiazoline derivative, with formula [PdCl2PyTT]. Its potential anticancer ability was evaluated in human promyelocytic leukaemia HL-60 cell line. To this aim, sub-confluent cultures of HL-60 cells were challenged with different doses (5-100 μM) of PyTT and PdPyTT for 24 h and cytotoxicity was then checked by means of MTS assay. The complex PdPyTT presented enhanced cytotoxicity, with a half-maximal (50%) inhibitory concentration (IC_{50}) of 20.7 μM for HL-60 cells. Nonetheless, the thiazoline derivative PyTT by itself did not showed cytotoxic effects. Moreover, nuclear staining with Hoechst 33342 revealed that the complex PdPyTT produced nuclear condensation and DNA fragmentation, thus indicating that the main form of cell death was apoptosis. These findings were in line with activation of caspase-9 and caspase-3 observed after treating HL-60 cells with the complex PdPyTT for 24 h. On the other hand, exacerbated reactive oxygen species (ROS) production was also observed when HL-60 cells were incubated with 20.7 μM PdPyTT, ROS being mainly of mitochondrial origin. Although further studies are required to understand the underlying mechanisms, the present findings suggest that thiazoline-based Pd(II) complexes are promising alternatives to hamper cancer proliferation when common anticancer drugs fail.

Keywords: leukaemia, apoptosis, reactive oxygen species, chemotherapy, thiazoline-based Pd(II) complex.

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P050

Brugada Syndrome and long QT syndrome can be caused by mutations in the gene encoding the Tbx5 transcription factor

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Background: Tbx5 is a transcription factor that plays a key role in cardiac development. It is also expressed in adult cardiac cells, although its role its unknown. The *SCN5A* gene encodes the Nav1.5 channel responsible for the cardiac sodium current (INa). Brugada syndrome (BrS) is frequently associated with loss-of-function mutations in the *SCN5A* gene that reduce the INa density. On the other hand, the Long QT syndrome type 3 (LQT3) is associated with gain-of-function *SCN5A* mutations that increase the sustained component of the INa (INaL). In the context of the ITACA Consortium, we identified two missense mutations in the *TBX5* gene in two families with some members diagnosed with BrS (p.F206L) or with LQT3 (p.D111Y). Interestingly, none of the affected members carry any mutation in the *SCN5A* gene nor display any structural cardiac alteration. In this work, we aimed to characterize the effects of both mutations on the INa and INaL to unravel whether they can be associated to BrS and LQT3.

Material and methods: INa and INaL were recorded using the whole-cell patch-clamp in HL-1 cells transfected with human native (WT) and mutated Tbx5. INa, INaL and action potentials were recorded in ventricular myocytes from cardiac–specific transgenic-like mice created on the basis of adeno-associated virus gene transfer and in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) infected with lentivirus encoding human WT and mutated Tbx5.

Results: Transfection of HL-1 cells with WT and p.D111Y Tbx5 significantly increased the peak INa density (from −52.6±5.5 to −78.7±11.3 and −71.0±11.0 pA/pF, respectively, n≥15, P<0.05), leaving unaffected the time- and voltage-dependent properties of the current. Conversely, p,F206L Tbx5 strongly reduced the peak INa density (-21.0±4.3 pA/pF, n=13, P<0.01). p.D111Y Tbx5, but not WT or p.F206L Tbx5, significantly increased the INaL density (from −2.9±0.5 to −4.6±0.8 pA/pF at -20 mV, n≥15, P<0.05). These results were completely reproduced in mouse ventricular cardiomyocytes and in hiPSC-CM expressing WT, p.D111Y or p.F206L Tbx5. Furthermore, in both cell types, p.F206L reduced action potential amplitude, whereas p.D111Y significantly prolonged action potential duration. In luciferase-reporter assays we demonstrated that WT and p.D111Y Tbx5 similarly enhanced the expression of Nav1.5. Conversely, p.F206L Tbx5 did not increase the expression of Nav1.5 and, more importantly, blunted the increase produced by Tbx5 WT in cells expressing both forms (WT+p.F206L; 1:1 ratio). Nav1.5 channels phosphorylation by βIV-spectrin–targeted calcium/calmodulin-dependent kinase II (CaMKII) increased the INaL and the QT duration. Tbx5 WT represses the expression of CaMKII, and βIV-spectrin, whereas p.D111Y Tbx5 did not reduce but enhanced the expression of CaMKII, and βIV-spectrin leading to an increased Nav1.5 phosphorylation.

Conclusions: These results demonstrate that in human adult cardiac cells Tbx5 WT regulates the expression of the genes encoding Nav1.5, CaMKII and βIV-spectrin, which cooperatively modulate the cardiac INa. A failure of this Tbx5-regulatory role may produce a decrease in the INa that can lead to the BrS or an increase in the INaL that can account for the QT prolongation. Thus, *TBX5* could be a novel gene associated with these genetically-determined arrhythmogenic syndromes.

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