RECI-IX

IX Congreso Red Española de
Canales Iónicos

Granada | 13-15 May 2024

Abstract Book

IX CONGRESO DE LA RED ESPAÑOLA DE CANALES IÓNICOS (RECI)

Celebrado en la Facultad de Medicina de la Universidad de Granada

En Granada, del 13 al 15 de mayo de 2024

IX CONGRESS OF THE SPANISH IONIC CHANNEL NETWORK (RECI)

Held at the Faculty of Medicine of the University of Granada

In Granada, from May 13 to 15, 2024

BIENVENIDA AL RECI IX

Estimadas amigas y estimados amigos,

La Red Española de Canales Iónicos (RECI) ha depositado en nosotros el honor de organizar el IX Congreso RECI en la ciudad de Granada del 13 al 15 de mayo. Somos el grupo de Neurofarmacología del Dolor de la Facultad de Medicina y del Instituto de Neurociencias Federico Olóriz de la Universidad de Granada. Asumimos esta tarea con entusiasmo y responsabilidad, con el difícil objetivo de mantener el alto nivel científico alcanzado en los congresos anteriores.

Estamos seguros de que esta reunión será una buena oportunidad para reforzar los lazos científicos y personales de los grupos de investigación que forman parte de la RECI. El programa cuenta con cinco mesas científicas, y tendremos la oportunidad de contar con ponentes internacionales del más alto nivel, con tres conferencias plenarias. Además, uno de nuestros principales objetivos es darle a los jóvenes protagonismo, y para ello, se ha hecho una selección entre las comunicaciones recibidas, para que puedan ser presentadas oralmente como ponencias.

El evento se celebrará en la Facultad de Medicina de la Universidad de Granada, histórica universidad española, fundada por el rey Carlos I (Emperador Carlos V de Alemania) en el año 1531. La actual Facultad de Medicina tiene su sede en un edificio moderno construido hace pocos años, en el Parque Tecnológico de la Salud (PTS), y dispone de todas las comodidades y tecnologías. En el PTS también está la Facultad de Ciencias de la Salud, y diversos Centros de Investigación y empresas biotecnológicas. En la propia sede tenemos previstos momentos de relax para poder hablar y estrechar lazos profesionales y personales durante las comidas, cafés o tomando unas cervezas o unos vinos en el cóctel de bienvenida.

Este evento también es una oportunidad excelente para poder visitar la monumental e histórica ciudad de Granada, destacando el conjunto de La Alhambra y El Generalife, Patrimonio de la Humanidad. Decía Antonio Machado en su cita célebre: “Todas las ciudades tienen su encanto, Granada el suyo y el de todas las demás”. La cena del congreso se celebrará en el Carmen de la Victoria, una edificación típica del Barrio del Albaicín, barrio que también está catalogado como Patrimonio de la Humanidad. Además, Granada cuenta con una situación geográfica privilegiada, con Sierra Nevada a poco más de 30 Km, y otros lugares con encanto muy cercanos, como Las Alpujarras o La Costa Tropical.

El programa final cuenta, además de con las 3 conferencias plenarias, con 15 ponencias invitadas, 16 ponencias cortas y tres sesiones para la discusión de los 43 pósteres presentados.

Confiamos que este evento resulte de interés. Para fomentar la participación, hemos establecido unas cuotas de inscripción asequibles para todo el que quiera asistir.

¡Nos vemos en Granada!

Francisco R Nieto López

Presidente del Comité Organizador

IX Congreso de la Red Española de Canales Iónicos (RECI)

Granada, 13-15 de mayo de 2024

WELCOME TO RECI IX

Dear colleagues and friends,

The Spanish Network of Ionic Channels (RECI) has given us the honor of organizing the IX RECI Congress in the city of Granada from May 13 to 15. We are the Neuropharmacology of Pain group of the Faculty of Medicine and the Institute of Neurosciences "Federico Olóriz" from the University of Granada. We assume this task with enthusiasm and responsibility, with the difficult goal of keeping the high scientific level achieved in previous congresses.

We are sure that this meeting will be a good opportunity to strengthen the scientific and personal connections of the research groups that are part of the RECI. The program has five symposiums, and we will have the opportunity to have international speakers of the highest level, with three plenary conferences. Furthermore, one of our main goals is to give young people a leading role, and to do so, a selection has been made among the communications received so that they can present their work orally.

The event will be held at the Faculty of Medicine of the University of Granada, a historic Spanish university, founded by King Charles I (Emperor Charles V of Germany) in 1531. The current Faculty of Medicine is based in a modern building built a few years ago, in the Health Technology Park (PTS), and has all the comforts and technologies. In the PTS is also the Faculty of Health Sciences, and various research centers and biotechnology companies. At the faculty itself, we have planned moments of relaxation to be able to talk and strengthen professional and personal links during meals, coffees, or having a few beers or wines at the welcome cocktail.

This event is also an excellent opportunity to visit the monumental and historic city of Granada, highlighting the Alhambra and the Generalife, a World Heritage Site. The poet Antonio Machado said in his famous quote: “All cities have their charm, Granada has its own and that of all the others.” The congress dinner will be held at the "Carmen de la Victoria", a typical building in the "Albaicín" neighborhood, a neighborhood that is also listed as a World Heritage Site. In addition, Granada has a privileged geographical location, with "Sierra Nevada" just over 30 km away, and other charming places very close, such as "Las Alpujarras" or "La Costa Tropical".

The final program includes, in addition to the 3 plenary conferences, 15 invited talks, 16 short talks, and 3 sessions to discuss the 43 posters presented.

We trust that this event will be of interest. To encourage participation, we have established affordable registration fees for anyone who wants to attend.

See you in Granada!

Francisco R Nieto López

President of the Organizing Committee

IX Congress of the Spanish Network of Ion Channels

Granada, May 13-15, 2024

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CONGRESS PROGRAMME

Monday - May 13, 2024

15:00 h **REGISTRATION RECI**

16:00-16:30 h **OPENING CEREMONY**

16:30-17:15 h **PLENARY OPENING LECTURE**

Chair: Francisco R. Nieto

Speaker: John Wood (University College London)

From genes to analgesic drugs

17:30-19:30 h **SYMPOSIUM 1. Neuropathies and ion channels**

Chairs: Enrique J Cobos and Miguel A Tejada

Speakers:

17:30 **-Gerard Callejo Martín** (Universitat de Barcelona)

TRESK channels and neuronal excitability: role in pain, itch and peripheral neuropathies

17:55 **-Antonio Rodríguez Artalejo** (Universidad Complutense de Madrid): **Taking advantage of peripheral nervous system plasticity to identify new targets and drugs for neuropathic pain**

18:20 **-Rafael González Cano** (Universidad de Granada)

New tools for the evaluation of pathologic pain in rodents: the role of AI

18:45 **-Victoria Moreno Manzano** (Centro de Investigación Príncipe Felipe, Valencia)

Transplantation of dorsal root ganglia overexpressing the NaChBac sodium channel improves locomotion after complete SCI

18:55 **-Enrique Velasco Serna** (VIB KU Leuven, Belgium)

Sustained action potential firing is produced by membrane potential instabilities generated by NaV1.9 in peripheral sensory neurons

19:05 **- Jorge Fernández-Trillo** (Instituto de Neurociencias, Alicante)

Mechanical sensitivity in MRGPRD nociceptors is mediated by Piezo2

19:15 **- M. Carmen Ruiz-Cantero** (Universidad de Barcelona)

Sigma-1 receptor and pain modulation: a link between TRPV1 and μ-opioid receptor

19:45-21:00 h **WELCOME RECEPTION**

Tuesday - May 14, 2024

9:00-11:00 h **SYMPOSIUM 2. Ion channels and cell biology**

Chairs: Ana Gomis and Juan A. Rosado

Speakers:

9:00 **-Antonio Felipe** (Universitat de Barcelona)

The Kv1.3 channel: a crossroads between cell proliferation and death

9:25 **-Isaac Jardín** (Universidad de Extremadura)

The Role of Orai1 Variants in Calcium Mobilization and Mammosphere Formation in Breast Cancer Stem Cells

9:50 **-Luis Pardo** (Max Planck Institute, Göttingen, Germany)

The role of Kv10.1 during mitosis

10:15 **-Elvira de la Peña** (Instituto de Neurociencias, Alicante)

Modulation of the TRPA1 ion channel by sigma 1 Receptor: role in oxaliplatin-induced peripheral neuropathy

10:25 **-María Teresa Alonso** (Universidad de Valladolid/CSIC)

Direct measurements of luminal Ca^{2+} with endo-lysosomal GFP-aequorin (ELGA) reveal functional IP3 receptors

10:35 **-Marycarmen Arévalo-Martínez** (Universidad de Valladolid)

Kv1.3 and mTOR signaling pathways crosstalk during intimal hyperplasia

10:45 **-Francisco J Taberner Sanchis** (Instituto de Neurociencias, Alicante)

Differential contribution of PIEZO's Blade domain in channel stability and localization

11:00-11:20 h **SPONSORED TALK**

-Diego Fernández, 3Brain AG (Switzerland)

Ion channels and neuronal networks: how high-density microelectrode arrays (HD-MEA) can illuminate beyond single-cell recordings

11:20-12:00 h **COFFEE BREAK AND POSTERS**

12:00-14:00 h **SYMPOSIUM 3. Metabolic and cardiovascular pathophysiologies**

Chairs: Eva Delpón and Mª Teresa Pérez García

Speakers:

12:00 **-Rosa Señaris** (Universidad de Santiago de Compostela)

Role of TRPM8 channels in thermoregulation and energy homeostasis

12:25 **-Marta Pérez-Hernández** (Centro Nacional de Investigaciones Cardiovasculares, Madrid)

Mitochondria unleashed: a novel approach to Arrhythmogenic Right Ventricular Cardiomyopathy

12:50 **-Madeline Nieves-Cintrón** (UC Davis Medical Center, USA)

Regulation of Vascular Cav1.2 channel in diabetes

13:15 **-Paula G. Socuéllamos** (Instituto de Investigaciones Biomédicas Sols-Morreale CSIC-UAM, Madrid)

Lgi3-4 effects on IKur and its electrophysiological consequences in the heart

13:25 **-José Ramón López-López** (Universidad de Valladolid)

Contribution of P2Y6R to essential hypertension through the formation of P2Y6R/AT1R heterodimers

13:35 **-Rebecca Martínez-Moreno** (Institut d'Investigació Biomèdica de Girona)

Molecular insights into the effect of Brugada Syndrome Variants

13:45 **-Manuel F. Navedo** (UC Davis Medical Center, USA)

Vascular smooth muscle Pannexin 1 in diabetic hyperglycemia

14:00-15:00 h **LUNCH**

15:00-16:30 h **POSTER DISCUSSION**

16:30-17:15 h **RECI PLENARY LECTURE**

Chair: Félix Viana

-Thomas Jentsch (Leibniz Institute for Molecular Pharmacology, Germany)

ASOR/TMEM206 – a 'novel' acid-activated Cl channel in cell death and endocytic trafficking

18:00-19:30 h **VISIT TO GRANADA**

21:00-23:00 h **CONGRESS DINNER**

Wednesday – May 15, 2024

9:00-10:30 h **SYMPOSIUM 4. Non-mammalian and artificial ion channels**

Chairs: Vicente Aguilella and José A. Poveda

Speakers:

9:00 **-Joan Cerdà** (Instituto de Ciencias del Mar [CSIC] e Instituto de Biotecnología y Biomedicina [IBB], Universidad Autónoma de Barcelona)

Dissecting the role of aquaporin water channels during sperm osmoadaptation: insights from a fish model

9:25 **-Rafael Giraldo** (Centro Nacional de Biotecnología [CSIC], Madrid). **Engineering a bacterial porin trap for amyloids**

9:50 **-Manuel Nieves-Cordones** (Centro de Edafología y Biología Aplicada del Segura [CSIC], Murcia)

The voltage-gated K+ channel SlSKOR in tomato plants

10:15 **- Héctor Gaitán-Peñas** (Universitat de Barcelona)

Characterization of ClC-1 chloride channels in zebrafish: a new model to study myotonia

10:25 **-Jessica Rojas-Palomino** (Universitat Jaume I, Castellón)

What planar membrane electrophysiology can tell us about the toxicity mechanism of the *P. aeruginosa* effector Tse5

10:40-11:10 h **COFFEE BREAK AND POSTERS**

11:10-13:10 h **SYMPOSIUM 5. Ion channel pharmacology**

Chairs: Asia Fernández Carvajal and Rosa Planells

Speakers:

11:10 **-Rosario González Muñiz** (Instituto de Química Médica [CSIC], Madrid)

TRPM8 antagonists: From basic research to neurocosmetics

11:35 **-Jose Manuel Brea Floriani** (Universidad de Santiago de Compostela)

High Throughput Screening methodologies for ion channel drug discovery. From target-based to phenotypic assays

12:00 **-Raul Estevez Povedano** (Universitat de Barcelona)

Regulatory mechanisms of astrocytic chloride channels

12:25 **-Sara Pérez-Martín** (Universidad Complutense de Madrid. Instituto de Salud Carlos III)

DECA11 selectively increases human INa and IK1 and exerts antiarrhythmic effects in a mouse model of heart failure

12:35 **-David Soto del Cerro** (Universitat de Barcelona)

The Conantokin-Derived Peptide EAR-20 As A Novel NMDA Receptor Positive Allosteric Modulator

12:45 **-Felix Viana** (Instituto de Neurociencias, Alicante)

Macrolide immunosuppressants: a novel class of TRPM8 channel agonists

12:55 **-Magalí Colomer** (Universitat de Barcelona)

Changes in the Kv1.3 pharmacology upon the association with KCNE4

13:15-14:00 h **CLOSING PLENARY LECTURE**

Chair: Francisco R. Nieto

-Marzia Malcangio (King´s College London)

Neuroimmune interactions and arthritis pain in neurodegenerative conditions

14:00-14:15 h **CLOSING CEREMONY**

14:15-15:30 h **LUNCH AND FAREWELL**

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RECI PLENARY LECTURE

Thomas Jentsch

Leibniz Institute for Molecular Pharmacology, Berlin, Germany

SYMPOSIUM 4. NON-MAMMALIAN AND ARTIFICIAL ION CHANNELS

SYMPOSIUM 5. ION CHANNELS PHARMACOLOGY

CLOSING PLENARY LECTURE

Marzia Malcangio

King´s College London, London, United kingdom

PÓSTERS

OPENING LECTURE

FROM GENES TO ANALGESIC DRUGS

SODIUM CHANNELS AND PAIN

John N Wood PhD FRS

Molecular Nociception Group, University College London, London, UK

Pain is a massive problem, particularly amongst the elderly. The revolution in molecular genetics in the late twentieth century has provided powerful insights into how pain works, as well as identifying analgesic drug targets. Peripheral sodium channels are plausible candidates. Today I will discuss recent successes with drugs targetting the voltage-gated sodium channel Nav1.8, found in the peripheral nervous system. Nav1.8 mutations have been linked to cardiovascular problems and even sudden death. The discovery of a role for a small C-terminal fragment of Nav1.8 in cardiac function enables drugs that avoid actions on the heart to be developed. A new orally active drug looks very promising.

In terms of human validation, the sodium channel Nav1.7 is a more compelling target. Humans lacking Nav1.7 are apparently normal but pain-free. However, embyonic pain-free humans and mice lacking Nav1.7 show a distinct mechanism of analgesia from adult knock-out animals. Intriguingly, embryonic Nav1.7 gene deletion enhances endogenous opioid signalling in peripheral neurons, resulting in diminished neurotransmitter release. In contrast, adult gene deletion or channel blocking drugs diminish excitability. Nav1.7 is expressed broadly within the central nervous system, as well as in the autonomic nervous system and some non- neuronal tissues such as the pancreas. Small molecule drug side effects are always a problem, and the broad role of Nav1.7 particularly in the autonomic nervous system means that antagonists of Nav1.7 are unlikely to produce side-effect free analgesic drugs. In contrast, the interaction with the opioid system in embryonic nulls presents a fascinating potential new route to pain treatment.

As well as targetting ion channels, the cell populations expressing particular ion channels can be useful analgesic targets. Chemogenetic silencing or deleting neurons expressing Nav1.8 is a highly effective route to causing analgesia in preclinical studies. In addition, neuro-immune interactions can be interrogated through studies of neuron-depleted mice.

SYMPOSIUM 1. NEUROPATHIES AND ION CHANNELS

TRESK CHANNELS AND NEURONAL EXCITABILITY: ROLE IN PAIN, ITCH AND PERIPHERAL NEUROPATHIES

J. Llimós-Aubach[1], A. Andres-Bilbe[1], A. Castellanos[1], A. Pujol[1], I. Pallás[1,2], N. Comes[1,2], X. Gasull[1,2], G. Callejo[1,2]

[1]Neurophysiology Laboratory, Department of Biomedicine, Medical School, Institute of Neurosciences, Universitat de Barcelona, Barcelona, Spain. [2]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

K2P channels play a crucial role in regulating neuronal excitability through the K^+ background current in neural cells. Particularly, TRESK (K2P18.1) is highly expressed in spinal cord, dorsal root and trigeminal ganglia sensory neurons, influencing their neuronal excitability and action potential firing. We initially described reduced TRESK expression following peripheral nerve injury and suggested its involvement in heightened sensory neuron excitability under such conditions. Sensory neurons from TRESK KO animals exhibited a lower activation threshold reinforcing its role in neuronal excitability regulation. Additionally, recordings of skin nociceptive C-fibers reveled increased activation by cold and mechanical stimuli in KO mice. Consistently, TRESK KO mice displayed reduced mechanical thresholds and heightened cold sensitivity, with no notable changes in thermal sensitivity to warm or hot temperatures. According to several transcriptomic studies, TRESK is expressed in a subset of sensory neurons that also express specific Mas-related G protein-coupled receptors (MrgprA3 and MrgprD), implicated in itch sensation. Our recent findings highlight TRESK as a modulator of non-histaminergic pruriceptor excitability. TRESK KO mice exhibited a heightened scratching response compared to wild-type (WT) mice following subcutaneous chloroquine (MrgprA3 agonist) injection into the cheek. Notably, spontaneous scratching responses were also elevated in KO mice across three skin disease models (psoriasis, allergic contact dermatitis, and dry skin). To further underscore the role of TRESK in sensory neuronal excitability, we have described that mutations in TRESK channels are implicated in mechanical hypersensitivity during migraine pain, likely via a dominant negative effect on TREK-1 channels. Moreover, mutations associated with human small fiber neuropathies disrupt TRESK function, potentially enhancing neuronal excitability in neuropathic disorders. In conclusion, TRESK significantly regulates the excitability of specific sensory neuron populations involved in mechanical and cold pain sensing. Reduced TRESK expression post-injury or dysfunctional mutations, as observed in neuropathies, may contribute to hyperalgesia and allodynia in chronic pain conditions. Additionally, TRESK's role in non-histaminergic itch positions it as a promising therapeutic target for various pathological sensory disorders.

Supported by research grant PID2020-119305RB-I00 (XG & NC), funded by MCIN/AEI/ 10.13039/501100011033 of Spain; LF-OC-22- 001114, Leo Foundation, Denmark (XG); NEA23 CRG184 National Eczema Association, USA (GC); 2021SGR00292 Generalitat de Catalunya (NC); AGORA: Advancing Guidelines with Original Research Achievements in pain, ERA-NET NEURON, EU (XG); Instituto de Salud Carlos III, Maria de Maeztu MDM-2017-0729 and CEX2021-001159-M (to Institut de Neurociencies, Universitat de Barcelona).

TAKING ADVANTAGE OF PERIPHERAL NERVOUS SYSTEM PLASTICITY TO IDENTIFY NEW TARGETS AND DRUGS FOR NEUROPATHIC PAIN

A. Rodríguez Artalejo

Department of Pharmacology and Toxicology. Veterinary Faculty. Universidad Complutense de Madrid, Madrid, Spain

Neuropathic pain is defined as pain resulting from a lesion or disease affecting the somatosensory nervous system, particularly nociceptive neurons. This condition is characterized by functional disturbances that lead to heightened responsiveness to different stimuli (mechanical, thermal, chemical), driven by changes in the expression and/or function of ion channels involved in stimulus transduction and electrical excitability of sensory neurons. Notably, pain triggers a stress response mediated by the sympathetic nervous system, further contributing to these functional disturbances.

We have leveraged these pathophysiological changes to identify potential drug targets and mechanisms suitable for novel chemical compounds and interventions aimed at reducing nociception in the chronic constriction injury model of neuropathic pain. Specifically, we will present data on: i) RGM8-51, a novel TRPM8 antagonist; ii) IQM-PC332, a novel DREAM ligand; iii) SK29661, a selective inhibitor of phenylethanolamine N-methyltransferase; and iv) surgical denervation of the adrenal medulla.

Our findings demonstrate that these approaches effectively modify nociception in neuropathic animals while preserving sensitivity to noxious stimulation in control animals. Consequently, we advocate for continued research into the pathophysiology of chronic neuropathic pain and target-driven drug synthesis to develop novel analgesics potentially beneficial for treating neuropathic pain.

NEW TOOLS FOR THE EVALUATION OF PATHOLOGIC PAIN IN RODENTS: THE ROLE OF ARTIFICIAL INTELLIGENCE

R. González-Cano

Department of Pharmacology and Institute of Neuroscience, University of Granada, Spain

Chronic pain is a widespread public health issue, affecting approximately one in ten adults each year. The persistent challenge of finding effective treatments for numerous chronic pain conditions highlights the urgent need for innovative therapeutic strategies and novel pharmacological targets. Rodent models are essential for understanding human pain mechanisms and advancing the development of new analgesics. However, traditional pain assessment methods in these models, predominantly based on reflex-based tests, often fail to capture the complex and subjective nature of human pain. This discrepancy underscores a critical need for more accurate and humane evaluation techniques in preclinical research.

This presentation focuses on the introduction and validation of several innovative tools that employ Artificial Intelligence (AI) to revolutionize the assessment of pathological pain in rodent models. These tools utilize a combination of advanced machine learning algorithms and computer vision to significantly enhance the accuracy, objectivity, and efficiency of pain measurements. They do so by analyzing subtle behavioral changes or facial expressions in rodents indicative of pain, which are often missed by conventional methods.

A key advantage of these AI-based tools is their capacity to handle vast amounts of data without suffering from human limitations such as fatigue. This capability significantly increases the speed and accuracy of data processing, extends the evaluation periods, and potentially allows for the detection of low-frequency behaviors indicative of spontaneous pain. Furthermore, these tools can help identify new behavioral phenotypes that might previously have gone unnoticed by human observers. This multifaceted approach not only promises to accelerate the development of analgesic drugs but also enhances our understanding of nervous system function.

TRANSPLANTATION OF DORSAL ROOT GANGLIA OVEREXPRESSING THE NaChBac SODIUM CHANNEL IMPROVES LOCOMOTION AFTER COMPLETE SCI

S. Hingorani[1], G. Paniagua Soriano[1], C. Sánchez Huertas[2], E. M. Villalba Riquelme[3], E. López Mocholi[1], B. Martínez Rojas[1], A. Alastrué Agudo[1], S. Dupraz[4], A. V. Ferrer Montiel[3], V. Moreno Manzano[1]

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Spinal cord injury (SCI) is a debilitating condition currently lacking treatment. Severe SCI causes the loss of most supraspinal inputs and neuronal activity caudal to the injury, which, coupled with the limited endogenous capacity for spontaneous regeneration, can lead to complete functional loss even in anatomically incomplete lesions. We hypothesized that transplantation of mature dorsal root ganglia (DRG), genetically modified to express the NaChBac sodium channel, could serve as a therapeutic option for functionally complete SCI. We found that NaChBac expression increased the intrinsic excitability of DRG neurons, promoted cell survival and neurotrophic factor secretion in vitro. Transplantation of NaChBac-expressing dissociated DRGs improved voluntary locomotion seven weeks after injury compared to control groups. Animals transplanted with NaChBac-expressing DRGs also possessed higher tubulin-positive neuronal fiber and myelin preservation, although serotonergic descending fibers remained unaffected. We observed early preservation of the corticospinal tract fourteen days after injury and transplantation which was lost seven weeks after injury. Nevertheless, transplantation of NaChBac-expressing DRGs increased the neuronal excitatory input, by increased number of VGlut2 contacts, immediately caudal to the injuries. Our work suggests that the transplantation of NaChBac-expressing dissociated DRGs can rescue significant motor function retaining an excitatory neuronal relay activity immediately caudal to injury.

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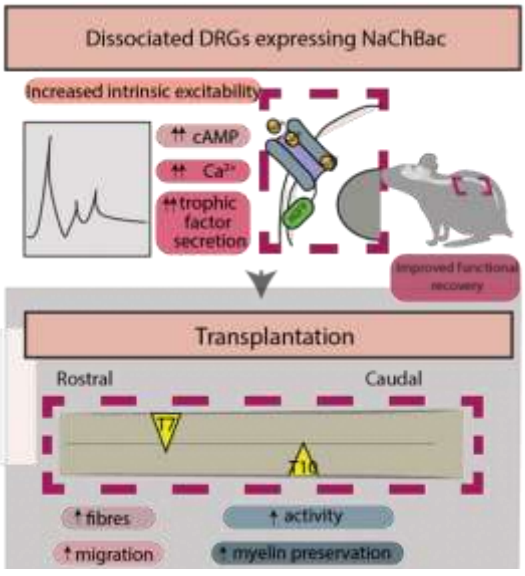

SUSTAINED ACTION POTENTIAL FIRING IS PRODUCED BY MEMBRANE POTENTIAL INSTABILITIES GENERATED BY $NA_V1.9$ IN PERIPHERAL SENSORY NEURONS

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Sustained action potential firing (SAPF) is a feature shared by multiple neuronal types and is crucial for stimulus encoding, neural communication and circuit plasticity. We aimed at unveiling the mechanisms underlying SAPF in Peripheral Sensory Neurons (PSNs), a convenient experimental model for studying single-cell excitability. Current-clamp recordings in primary-cultured PSNs revealed that tonic depolarization elicits Subthreshold Membrane Potential Transients (SMPTs) in 82% of this cells, and statistical analyses indicates that larger and faster SMPTs are causally associated with SAPF. Pharmacological or genetic ablation of the voltage-gated Na+ channel NaV1.9 reduced both SMPTs and SAPF. Computational models of neuronal electrical activity revealed that stochasticity of NaV and KV channels gating is necessary and sufficient for the generation of SMPTs and SAPF. Behavioral tests showed that NaV1.9 KO mice are severely impaired in detecting slow and tonic noxious thermal stimuli. We conclude that stochastic openings of NaV1.9 channels result in SMPTs that support SAPF and, consequently, nocifensive responses to slow and tonic noxious stimuli that are critical for animal adaptation and survival.

References:

1. Velasco, E., Alvarez, J. L., Meseguer, V. M., Gallar, J., & Talavera, K. (2022). Membrane potential instabilities in sensory neurons: mechanisms and pathophysiological relevance. Pain, 163(1), 64–74.
2. Tian, J., Bavencoffe, A. G., Zhu, M. X., & Walters, E. T. (2024). Readiness of nociceptor cell bodies to generate spontaneous activity results from background activity of diverse ion channels and high input resistance. Pain, 165(4), 893–907.
3. Amir, R., Michaelis, M., & Devor, M. (1999). Membrane potential oscillations in dorsal root ganglion neurons: role in normal electrogenesis and neuropathic pain. The Journal of neuroscience :the official journal of the Society for Neuroscience, 19(19), 8589–8596.

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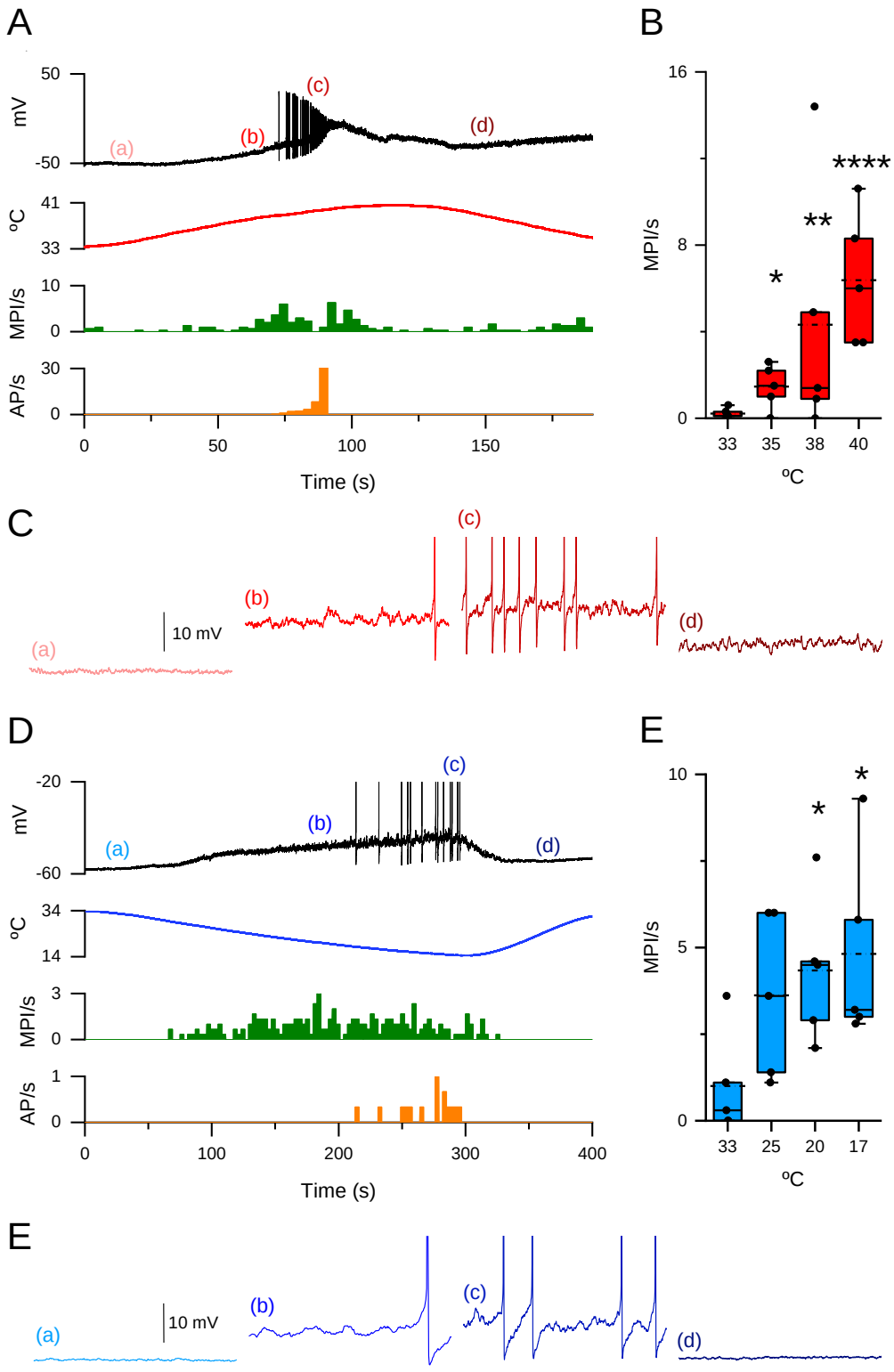
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MECHANICAL SENSITIVITY IN MRGPRD NOCICEPTORS IS MEDIATED BY PIEZO2

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Piezo2 is a well-established mechanotransducer for low-threshold tactile stimuli and proprioception1. The participation of this channel in painful processes such as mechanical allodynia has also been suggested2. Transcriptomic studies show that Piezo2 is expressed in MRGPRD neurons3, a population of non-peptidergic nociceptors that mediate mechanical pain in physiological and pathological conditions. However, the role of Piezo2 in these nociceptors has not been completely elucidated. This study aims to elucidate the functional role of Piezo2 in MRGPRD nociceptors and its potential contribution to mechanical hyperalgesia induced by nerve injury.

Through calcium imaging, we functionally characterized MRGPRD neurons isolated from mouse dorsal root ganglia (DRG) cultures. The analysis revealed two distinct populations: one responsive to β-alanine, an agonist of the Mrgprd receptor, potentially corresponding to polymodal nociceptors, and another unresponsive to β-alanine and previously classified as pure mechanonociceptors. Whole-cell patch-clamp recordings revealed that approximately 50% of neurons from both subpopulations displayed mechanically activated currents with different inactivation kinetics. These currents were almost absent in similarly conducted experiments using DRG obtained from conditional knockout mice lacking Piezo2 specifically in MRGPRD neurons.

Behavioural experiments using the sciatic nerve chronic constriction injury (CCI) mouse model of neuropathic pain demonstrated a reduction in mechanical hypersensitivity in mice lacking Piezo2 specifically in MRGPRD neurons. This observation was more evident when mice were stimulated with high intensity mechanical stimuli, known to activate nociceptors.

Finally, in vivo calcium imaging experiments in the sciatic nerve chronic constriction injury (CCI) model, revealed an increase in the activity of MRGPRD neurons innervating the sciatic nerve in response to the application of mechanical noxious stimuli to the hind paw. The ablation of Piezo2 in conditional knockout mice abolished this increase in activity and also significantly reduced the number of MRGPRD neuros responding to the mechanical stimulation.

In summary, our findings suggest an important role of Piezo2 in the mechanical sensitivity of MRGPRD neurons, both in physiological conditions and in the context of neuropathic pain.

References:

4. A critical role for Piezo2 channels in the mechanotransduction of mouse proprioceptive neurons. Florez-Paz et al., 2016. Sci. Rep. 6, 25923
5. PIEZO2 mediates injury-induced tactile pain in mice and humans. Szczot et al., 2018. Sci Transl Med 10(462).eaat9892
6. Zeisel et al., 2018. Cell 174(4):999-1014-e22

Acknowledgements: *Ministerio de Ciencia e Innovación PID2019-108194RB-I00/AEI/10.13039/50110001103 and PID2022-140961OB-I00/MCIU/AEI/10.13039/501100011033 co-financed by the European Regional Development Fund (ERDF), Generalitat Valenciana PROMETEO/2021/031, and the "Severo Ochoa" Program for Centers of Excellence in R&D SEV-2017-0723.*

SIGMA-1 RECEPTOR AND PAIN MODULATION: A LINK BETWEEN TRPV1 AND μ-OPIOID RECEPTOR

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Introduction: algogenic compounds produced during painful pathologies sensitize peripheral sensory neurons to produce pain hypersensitivity. While prostaglandin E2 (PGE2) and nerve growth factor (NGF) sensitize peptidergic C-nociceptors (TRPV1+), glial cell line-derived neurotrophic factor (GDNF) sensitizes non-peptidergic C-neurons (IB4+) (Woolf & Ma, 2007). Sigma-1 receptor is a Ca2+-sensing chaperone known to modulate analgesia induced by opioid drugs (Sánchez-Fernández et al., 2017). This receptor binds both to TRPV1 and the μ-opioid receptor (MOR), although the functional repercussions of these physical interactions in peripheral sensitization are unknown.

Methods: we tested the effect of sigma-1 antagonism on PGE2-, NGF- and GDNF-induced mechanical and heat hyperalgesia in mice. We used immunohistochemistry to determine the presence of endomorphin-2, an endogenous MOR agonist, on dorsal root ganglion (DRG) neurons. To selectively remove TRPV1+ neurons we used the molecular scalpel resiniferatoxin. Recombinant proteins were used to study the interactions between sigma-1 receptor, MOR and TRPV1. We used calcium imaging to study the effects of sigma-1 antagonism on PGE2-induced sensitization of TRPV1+ nociceptors.

Results: sigma-1 receptor antagonists reversed PGE2- and NGF-induced hyperalgesia, but not GDNF-induced hyperalgesia. The antihyperalgesic effect of sigma-1 receptor antagonism was abolished not only by a sigma-1 agonist, but also by peripheral opioid receptor antagonism (using naloxone methiodide) and the selective MOR antagonist cyprodime. These results indicate that the antihyperalgesic effect of sigma-1 antagonists is mediated by peripheral MOR activation. Endomorphin-2 was detected on TRPV1+ but not on IB4+ neurons, and administration of an anti-endomorphin-2 antibody to a sensitized paw, reversed the antihyperalgesia induced by sigma-1 antagonists, indicating that this effect involves endomorphin-2. We selectively ablated both TRPV1+ neurons and endomorphin-2 labeling by resiniferatoxin, confirming the presence of this opioid peptide on peptidergic C-nociceptors. Sigma-1 antagonism transfers sigma-1 receptor from TRPV1 to MOR, suggesting that sigma-1 receptor participate in TRPV1-MOR crosstalk. Moreover, sigma-1 receptor antagonism reversed, in a naloxone-sensitive manner, PGE2-induced sensitization of DRG neurons to the calcium flux elicited by capsaicin, the prototypic TRPV1 agonist.

Conclusions: sigma-1 receptor antagonism harnesses endogenous opioids produced by TRPV1+ neurons to reduce hyperalgesia by promoting TRPV1 desensitization and increasing MOR activity. Our findings are summarized in Figure 1.

References:

1. Woolf CJ, Ma Q. Neuron. 2007; 55(3):353-64.
2. Sánchez-Fernández C et al. Adv Exp Med Biol. 2017;964:109-132.

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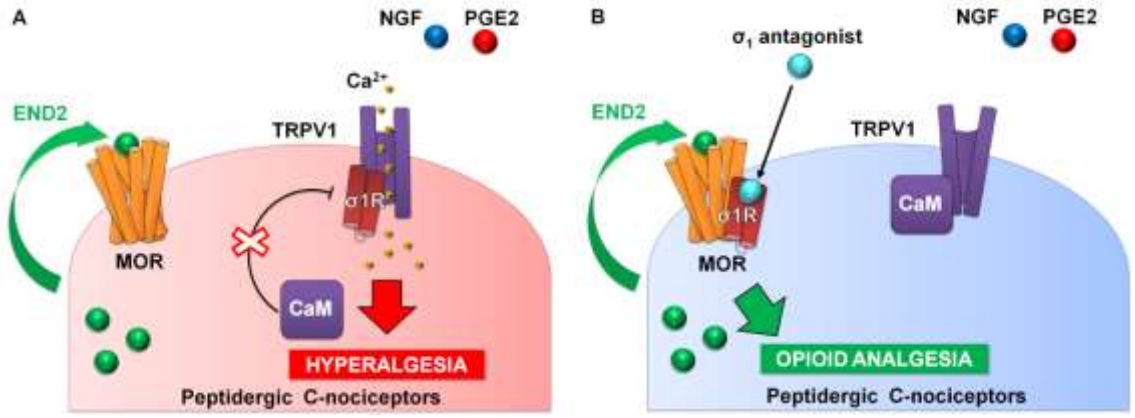

Figure 1. Hypothesis of the mechanism of action of sigma-1 receptor antagonism in the hyperalgesia induced by peptidergic C-neuron sensitization.

SYMPOSIUM 2. ION CHANNELS AND CELL BIOLOGY

THE K_V1.3 CHANNEL: A CROSSROADS BETWEEN CELL PROLIFERATION AND DEATH

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The voltage-dependent potassium channel Kv1.3 is a crucial player in the immune response, impacting leukocyte function. The protein is mainly expressed in the nervous and immunitary systems, but it also is implicated in several other physiological functions body-wide. Kv1.3 significance extends beyond the plasma membrane, as it also operates within the inner mitochondrial membrane. While plasma membrane Kv1.3 drives cellular activation and proliferation, its mitochondrial counterpart (mitoKv1.3) influences cell survival and apoptosis. Consequently, Kv1.3 emerges as a promising target for cancer therapies, given its dual role in cellular processes.

Understanding the trafficking of Kv1.3 to distinct cellular compartments is pivotal. While forward-traffic motifs guide the plasma membrane localization through a COPII-dependent pathway and caveolin association, the mechanisms governing its mitochondrial import remained elusive. Kv1.3 exhibits preferential expression in mitochondria, particularly in the perinuclear region, where it influences the dynamic mitochondrial network throughout the cell cycle. Notably, Kv1.3 expression is integral to the formation of a hyperfused mitochondrial network during the G1/S phase, underscoring its regulatory role in cellular dynamics. In this scenario, we mapped the molecular determinants governing the channel mitochondrial targeting. Thus, an unconventional mitochondrial routing, which involves TIM23 complex to translocate to the inner mitochondrial membrane was deciphered. This mechanism is unconventional because the channel is a multimembrane spanning protein without a defined N-terminal presequence. We found that transmembrane domains cooperatively mediate Kv1.3 mitochondrial targeting and identified the cytosolic HSP70/HSP90 chaperone complex as a key regulator of the process.

The dual physiological functions of Kv1.3—facilitating leukocyte activation and proliferation while promoting apoptosis in tumor cells—underscore its therapeutic potential in immunodeficiency and cancer. The cellular localization of Kv1.3 dictates its functional outcomes; plasma membrane Kv1.3 fosters proliferation, while mitochondrial Kv1.3 modulates apoptotic signaling. Therefore, the balance exerted by these two complementary mechanisms fine-tune the physiological role of Kv1.3 during cell survival or apoptosis.

In summary, Kv1.3 is a pivotal regulator in immune response and cancer pathogenesis, operating through distinct mechanisms at the plasma membrane and within mitochondria. Understanding the intricate interplay between Kv1.3 and its partners and their routing to distinct cellular compartments provides novel insights into therapeutic strategies targeting immunodeficiency and cancer.

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THE ROLE OF ORAI1 VARIANTS IN CALCIUM MOBILIZATION AND MAMMOSPHERE FORMATION IN BREAST CANCER STEM CELLS

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Breast cancer cells remodel the expression and activity of multiple calcium-handling proteins, including Orai and TRPC channels, which contribute significantly to the emergence of tumorigenic hallmarks like augmented proliferation, migration, and resistance to apoptosis. However, there exists limited understanding regarding the phenotypic reconfiguration displayed by breast cancer stem cells (BCSC), a minor cell subset within breast tumors characterized by low proliferative rates, resilience to chemotherapy and radiation, self-renewal capacities, potential for differentiation into diverse cell lineages, and heightened metastatic propensity. Orai1 constitutes the main pore-forming component of calcium-release activated calcium channels, which are responsible for mediating store-operated calcium entry (SOCE) in both excitable and non-excitable cells. Among mammalian cells, two forms of Orai1 have been identified: the full-length variant Orai1α (301 aa), and the truncated version Orai1β, which lacks the initial 63 amino acids. By using different techniques of molecular biology, cell culture and confocal microscopy here we present the expression assessment, at the transcript and protein levels, of the Orai family members, Orai1-3, and various TRPC channels, specifically TRPC1, 3, and 6, within breast stem cells (BSC) derived from the non-tumoral breast epithelial MCF10A cell line, as well as BCSC obtained from the estrogen receptor-positive (ER+) cell line MCF7, the HER2 cell line SKBR3, and the triple negative breast cancer (TNBC) cell line MDA-MB-231. Our findings suggest that Orai1 plays a critical role in mammosphere formation and self-renewal capabilities—distinctive attributes of stem cells—as well as cyclooxygenase (COX) activity in the subset of BCSC derived from various breast cancer subtypes, particularly evident in those derived from TNBC. Moreover, we have demonstrated that both Orai1α and Orai1β equally support the proliferation and spheroid formation of TNBC-derived BCSC.

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THE ROLE OF K_V10.1 IN CALCIUM HOMEOSTASIS DURING MITOSIS

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Cytosolic calcium homeostasis is a crucial factor for many aspects of cell physiology. Calcium entry across the plasma membrane evidently depends on the function of calcium channels, but the membrane potential plays a crucial role in shaping the magnitude of calcium uptake. KV10.1, a voltage-gated potassium channel abundantly expressed in the brain while undetectable in other tissues, has been associated with cancer for many years, but the detailed mechanistic link remains elusive. Excessive or extemporary channel expression of a channel with normal function in cancer or altered electrophysiological function with normal levels of expression in neurodevelopmental diseases (KCNH1 channelopathies) have massive consequences for cell physiology. Besides the distorted excitability expected for altered potassium homeostasis, cells with dysregulated Kv10.1 show resistance to hypoxia, abnormal mitochondria dynamics, and altered calcium homeostasis. Loss of the temporal control of channel expression explains the high detection frequency of Kv10.1 in tumors, which results in a more proliferative and aggressive behavior, resulting in a correlation between the presence of the channel and a bad prognosis of the tumor. We previously showed that Kv10.1 participates in the final steps of cell division and that its expression in healthy cells is limited to a short time window around this crucial event. In contrast, tumor cells often show sustained channel expression throughout the cell division cycle. In mitosis, specifically during metaphase, the correct supply of Ca2+ to the highly dynamic microtubules critically depends on Kv10.1 expression. The channel governs the proper positioning of Ca2+ entry channels, ensuring accurate chromosomal separation. In patients bearing gain-of-function mutations, the combination of the impact of excess of Kv10.1 in neural function together with the predicted alterations in cytoskeletal homeostasis around mitosis explains the pathophysiology, opening a path for the correction of both neurological and morphological alterations in these patients through modulation of Kv10.1. This and its role in cancer increase the therapeutic interest of Kv10.1 targeting and prompt the search for more potent and specific channel modulators.

MODULATION OF THE TRPA1 ION CHANNEL BY SIGMA 1 RECEPTOR: ROLE IN OXALIPLATIN-INDUCED PERIPHERAL NEUROPATHY

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Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent side effect of the treatment with different neurotoxic chemotherapeutics, such as platinum derivatives (cisplatin, oxaliplatin, carboplatin) and taxanes (paclitaxel, docetaxel), which are essential in the treatment of different tumors. In the case of patients receiving oxaliplatin, a key drug in the management of colorectall cancer, neuropathy presents as paresthesias, sensory ataxia due to loss of proprioception, mechanical and thermal allodynia, and pain in both hands and feet as in the oral cavity. In many patients, the symptoms are very disabling, making it necessary to reduce the dose of chemotherapeutics, compromising its effectiveness and patient survival[1]. On many occasions, these symptoms not only present acutely while the treatment lasts, but also persist, resulting in chronic pain that considerably reduces the quality of life of patients.

TRPA1 is a polymodal, non-selective cation channel expressed in nociceptors, activated by physical stimuli and cellular stress products. TRPA1 has been linked to different neuropathic conditions, including CIPN[2]. Sigma-1 receptor is a ligand-regulated chaperone residing at mitochondria-associated endoplasmic reticulum membranes, and expressed in many tissues, including peripheral sensory neurons. Sigma-1 receptors can translocate to the plasma membrane, regulating the expression and function of many ion channels. Sigma-1 receptor antagonists (S1RA), has shown efficacy in a phase II clinical trial for oxaliplatin-CIPN[3]. In a mice model of oxaliplatin neuropathy (3 i.p. injections, 6 mg/kg), we found that TRPA1 is involved in the development of mechanical and thermal hypersensitivity. Notably, the systemic treatment with S1RA, a selective Sigma-1 receptor antagonist, prevented the development of the painful symptoms in these mice. In calcium imaging studies at cellular level, the incubation of cultured DRG primary sensory neurons with S1RA prevented oxaliplatin-induced TRPA1 sensitization. Using biochemical and biophysical approaches in HEK293-TRPA1transfected cells we demonstrate that TRPA1 inhibition by S1RA depends of Sigma-1 receptor expression and is not exerted directly on TRPA1 channels. We also demonstrate that S1RA impairs the formation of TRPA1-Sigma-1 receptor complexes, resulting in reduced TRPA1 expression at the plasma membrane. Alltogether, these findings provide a mechanistic understanding of the role of Sigma-1 receptor inhibitors in the alleviation of painful CIPN by oxaliplatin and suggest new strategies for its prevention and treatment.

References:

1. Gordon-Williams R, Farquhar-Smith P. Recent advances in understanding chemotherapy-induced peripheral neuropathy. F1000Res. 2020;9doi:10.12688/f1000research.21625.1
2. Trevisan G, Materazzi S, Fusi C, et al. Novel therapeutic strategy to prevent chemotherapy-induced persistent sensory neuropathy by TRPA1 blockade. Cancer Res. May 15 2013;73(10):3120-31. doi:10.1158/0008-5472.CAN-12-4370 3.
3. Bruna J, Videla S, Argyriou AA, et al. Efficacy of a Novel Sigma-1 Receptor Antagonist for Oxaliplatin-Induced Neuropathy: A Randomized, Double-Blind, Placebo-Controlled Phase IIa Clinical Trial. Neurotherapeutics. Jan 2018;15(1):178-189. doi:10.1007/s13311-017-0572-5

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DIRECT MEASUREMENTS OF LUMINAL CA^{2+} WITH ENDO-LYSOSOMAL GFP-AEQUORIN (ELGA) REVEAL FUNCTIONAL IP3 RECEPTORS

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Endo-lysosomes (EL) have emerged as relevant players in subcellular calcium signalling alongside the better characterised endoplasmic reticulum (ER) (1). Endo-lysosomal Ca2+ has been implicated in most of these organelle functions, such as late endosome-lysosome fusion, lysosomal exocytosis, or membrane repair. Impaired Ca2+ signalling can cause defects in the endo-lysosomal trafficking and eventually can provoke lysosome storage diseases. Various Ca2+-permeable channels have been localized to membranes of late endosome and lysosome (2). These include Mucolipin transient receptor potential (TRPML1) channels, two pore channels (TPCs) and P2X4 channels.

Much effort has been devoted to developing reliable indicators for monitoring Ca2+ signals in acidic compartmets. However, this task is extremely challenging because most of the fluorescent indicators are sensitive to acidid pH and its fluorescence is quenched. Here we targeted GFP-aequorin (GA) to the endolysosomal lumen and found that a significant fraction of the probe is functional within a mildly acidic compartment. We leveraged this probe (ELGA) to report Ca2+ dynamics in this compartment. We show that Ca2+ uptake is ATP-dependent and sensitive to blockers of endoplasmic reticulum Ca2+ pumps. We find that the Ca2+ mobilizing messenger IP3 which typically targets the endoplasmic reticulum evokes robust luminal responses in wild type cells, but not in IP3 receptor knock-out cells. Responses were comparable to those evoked by activation of the endo-lysosomal ion channel TRPML1. Stimulation with IP3-forming agonists also mobilized the store in intact cells. Our data reveal a physiologically-relevant, IP3-sensitive store of Ca2+ within the endo-lysosomal system.

References:

1.Christensen, K. A., Myers, J. T. and Swanson, J. A. (2002). pH-dependent regulation of lysosomal calcium in macrophages. J Cell Sci 115, 599-607.
2.Yang, J., Zhao, Z., Gu, M., Feng, X. and Xu, H. (2019). Release and uptake mechanisms of vesicular Ca(2+) stores. Protein & cell 10, 8-19.

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KV1.3 AND MTOR SIGNALING PATHWAYS CROSSTALK DURING INTIMAL HYPERPLASIA

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Previous work from our group have demonstrated the efficacy of Kv1.3 blockers as treatment against restenosis, as they inhibit proliferation and migration of vascular smooth muscle cells (VSMCs) reducing intimal hyperplasia (IH)1. Nevertheless, signaling pathways involved in Kv1.3 mechanism remain incompletely understood. It has been reported that Kv1.3 channel modulates proliferation through the MEK/ERK pathway independently of mTOR pathway2. However, the use of mTOR inhibitors in drug-eluting stents represent the gold standard in the clinics for the prevention of restenosis after angioplasty. Thus, we hypothesized that combination of Kv1.3 and mTOR inhibitors could improve IH prevention.

We explore the effects of PAP-1(Kv1.3 blocker) and everolimus (mTOR inhibitor) in IH development using in-vivo (mice carotid ligation) and ex-vivo (human saphenous vein and mammary artery in organ culture) models. Also, their effects on VSMC proliferation and migration was explored in-vitro, using cultured human mammary VSMCs. Both in mice and human vessels, PAP-1 or everolimus alone reduced IH, but combination of both drugs abolished their individual effects, indicating a crosstalk between both pathways with relevant therapeutic consequences. These results were replicated when exploring VSMCs migration, but not with proliferation. The activation of various proteins involved in both pathways was studied, and we found that P70S6K phosphorylation correlated with our functional experiments.

We conclude that the responses to VSMC migration, but not VSMC proliferation, represent a more reliable indicator of the effects of PAP-1 and everolimus in the development of IH. Also, we found that P70S6K, represents a good biomarker of the of the triggering of the signaling pathways promoting IH, as its activation in response to PAP-1, everolimus or their combination recapitulates the effects of these drugs on VSMCs migration and IH development.

References:

1. Arévalo-Martínez et al.,doi: 10.1161/ATVBAHA.119.313492.
2. Cidad et al., doi: 10.1007/s00424-014-1607-y

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DIFFERENTIAL CONTRIBUTION OF PIEZO'S BLADE DOMAIN IN CHANNEL STABILITY AND LOCALIZATION.

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PIEZO1 and PIEZO2 are major force-gated ion channels, detecting mechanical forces and transducing them into ionic currents in many eukaryotic cell types, serving essential physiological roles. PIEZO channels assemble as trimers with a distinctive large 3-blade propeller shape. The Blade is a large membrane-embedded domain comprising 36 transmembrane fragments organized in 9 Transmembrane Helix Units (THUs). Despite its suggested role in force transduction, the contribution of the Blade domain in other aspects of the channel physiology is not fully understood. In this study, we describe the role of each Blade region on channel localization and stability. We generated truncated PIEZO mutants by sequentially removing one THU at a time, starting from the outermost (THU1) and moving inward. Cycloheximide assays determined protein stability and super-resolution imaging studied the subcellular localization of the truncated channels. Our findings reveal that in both PIEZO channels, the Distal Blade (THU1-3) plays a central role in protein stability and localization. Interestingly, the PIEZO2 Distal Blade contains a region mediating the intracellular retention of the chimeric membrane protein AQP3. These findings indicate that PIEZO1 and PIEZO2 differ not only in their biophysical properties but also in the regulation of their localization.

References:

1. Coste, B. et al. Piezo1 and Piezo2 Are Essential Components of Distinct Mechanically Activated Cation Channels. Science 330, 55–60 (2010).
2. Murthy, S. E., Dubin, A. E. & Patapoutian, A. Piezos thrive under pressure: mechanically activated ion channels in health and disease. Nat. Rev. Mol. Cell Biol. 18, 771–783 (2017).
3. Guo, Y. R. & MacKinnon, R. Structure-based membrane dome mechanism for Piezo mechanosensitivity. eLife 6, (2017).
4. Zhao, Q. et al. Structure and mechanogating mechanism of the Piezo1 channel. Nature 554, 487–492 (2018).
5. Saotome, K. et al. Structure of the mechanically activated ion channel Piezo1. Nature 554, 481–486 (2018).
6. Wang, L. et al. Structure and mechanogating of the mammalian tactile channel PIEZO2. Nature 573, 225–229 (2019).
7 Taberner, F. J. et al. Structure-guided examination of the mechanogating mechanism of PIEZO2. Proc. Natl. Acad. Sci. 201905985 (2019).

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SPONSORED TALK

ION CHANNELS AND NEURONAL NETWORKS: HOW HIGH-DENSITY MICROELECTRODE ARRAYS (HD-MEA) CAN ILLUMINATE BEYOND SINGLE-CELL RECORDINGS

Diego Fernández

3Brain AG, Switzerland

As key factors modulating single cell excitability, ion channels have a profound impact on neuronal network function. High-Density Microelectrodes Array (HD-MEA) allow direct contact between microchips containing 4096 electrodes and biological samples, enabling electrophysiological recording and stimulation with single-cell resolution and at network scale.

Complementing patch-clamp data, this technology allows the simultaneous recording of thousands of cells, providing unprecedented access to network level information. Scientists across different fields use HD-MEAs to:

- Map neuronal circuits and trajectories
- Study neuronal plasticity
- Characterize functional development of iPSC-derived cells
- Conduct in vitro electrocardiograms
- Record light responses in explanted retina

Join us to discover how HD-MEAs can add more value to your ion channel research, bridging the gap from single cell excitability to circuit dynamics.

SYMPOSIUM 3. METABOLIC AND CARDIOVASCULAR PATHOPHYSIOLOGIES

ROLE OF TRPM8 ION CHANNELS IN THERMOREGULATION AND ENERGY HOMEOSTASIS

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The maintenance of homeothermy in changing external environments requires a close link between thermosensation, thermoregulation and energy homeostasis. We found that TRPM8, the main mild cold sensor described so far, plays a key role in thermoregulation and metabolism. The deletion of TRPM8 induces a defective thermoregulation, with a fall of core temperature upon cooling, due mainly to tail heat loss. Furthermore, Trpm8-/- mice raised at mild cold temperatures develop late-onset obesity and metabolic dysfunction, with daytime hyperphagia and reduction of fat oxidation as plausible causal factors. Interestingly, TRPM8 is also expressed in the CNS, especially in thermoregulatory-related areas, and in the retina, principally in amacrine cells and in melanopsin ganglion neurons projecting to the suprachiasmatic nucleus. Finally, this cold ion channel regulates central and peripheral clockwork and the circadian oscillations of body temperature.

In conclusion, our results highlight the relevant role of TRPM8 in the crosstalk between thermoregulation, metabolism and circadian function. Furthermore, they unveil the importance of ambient temperature and cold sensory information as a new influential factor in obesity and metabolism, beyond hypercaloric foods and reduced physical activity, and underlines TRPM8 as a key element and a potential target to combat this epidemic disease.

References:

1. Reimundez, A., Fernandez-Peña, C., Garcia, G. et al. (2018). Deletion of the cold thermoreceptor TRPM8 increases heat loss and food intake leading to reduced body temperature and obesity in mice. *The Journal of Neuroscience*, 38(15), 3643–3656.
2. Ordás P, Hernández-Ortego P, Vara H, et al.. Expression of the cold thermoreceptor TRPM8 in rodent brain thermoregulatory circuits. *J Comp Neurol* (2021) 529(1):234–56.
3. Reimúndez A, Fernández-Peña C, Ordás P, et al.. The cold-sensing ion channel TRPM8 regulates central and peripheral clockwork and the circadian oscillations of body temperature. *Acta Physiol (Oxf)* (2022):e13896.

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MITOCHONDRIA UNLEASHED: NOVEL APPROACH TO ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiac disorder characterized by high propensity to life-threatening arrhythmias and progressive loss of heart muscle. More than 40% of reported genetic variants linked to ARVC reside in the PKP2 gene, which encodes the PKP2 protein (plakophilin-2). PKP2 is classically defined as a protein of the desmosome, an intercellular adhesion structure residing at the intercalated disc (ID).

We have described a comprehensive characterization of the ARVC molecular landscape with heart samples obtained from patients with ARVC with PKP2 mutations and their healthy relatives; and from PKP2cKO mice.

We observed that PKP2 deficiency leads to transcriptional changes in calcium related proteins, affecting calcium homeostasis which facilitate a highly arrhythmogenic state. Moreover, disruption of the ID by loss of PKP2 affects cytoskeleton, which affects nuclear envelope structure leading to DNA damage, and mitochondria. Mitochondrial damage is translated in increased reactive oxygen species. We propose therapies that limit oxidant formation (treatment with sirtuin 3) as a possible intervention to restrict damage in ARVC.

REGULATION OF VASCULAR $CA_V1.2$ CALCIUM CHANNELS IN DIABETES

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Smooth muscle cells are vital for the proper functioning of the vascular system, and L-type CaV1.2 channels are crucial in regulating various physiological functions within these cells. These channels play a vital role in controlling the excitability, proliferation, gene expression, and contraction of smooth muscle cells. Recent studies have revealed that the spatial and temporal properties of CaV1.2 channels depend on the phosphorylation state of serine 1928. This serine is located in the carboxy-terminal of the pore-forming α1C subunit, and its phosphorylation response is triggered by various stimuli. Notably, increased phosphorylation of serine 1928 leads to redistribution of α1C subunits into superclusters, facilitating cooperative activation of CaV1.2 channels. This gating mode amplifies Ca2+ influx and regulates the contractility of vascular smooth muscle. In my talk, I will discuss these exciting findings in more detail and explore their contribution to Ca2+ signaling in vascular smooth muscle.

LGI3-4 EFFECTS ON IKUR AND ITS ELECTROPHYSIOLOGICAL CONSEQUENCES IN THE HEART

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Recent research has revealed the role of leucine-rich glioma-inactivated protein family (Lgi1-4) in the nervous system and its impact on neurological diseases. In neurons, certain Lgi proteins interact with K_V1 and K_V4 channels, modifying their trafficking and/or biophysical properties. However, the expression and impact of these proteins in the heart is still unknown. In cardiomyocytes, activation of $K_V1.5$ channels generates the ultrarapid outward potassium current (I_{Kur}), which is essential for the initial phase of human atrial repolarization. Initial results indicate that Lgi3 and Lgi4 interact with cardiac $K_V1.5$ channels, impairing $K_V1.5/K_V\beta$ association, and partially reversing the $K_V\beta$-induced I_{Kur} amplitude increase. Regulatory proteins associated with ion channels modulate their currents, forming channelosomes. However, the exact composition of these signalling complexes is yet to be elucidated. Changes in the properties or functional expression of some $K_V1.5$ interacting proteins may have crucial pathophysiological consequences. Thus, deciphering the composition of these channelosomes is essential. To study the pathophysiological role of these proteins, we generated a cardiac-specific mouse model expressing Lgi4. On surface ECG, the QRS interval was prolonged, and the conduction system was altered in anesthetized Lgi4 mice. In isolated Lgi4 ventricular myocytes, the duration of early action potential repolarization was prolonged compared to the control. These results correlated with the reduced $K_V1.5$ membrane expression and I_{Kur} density observed in Lgi4 cardiomyocytes, which were similar to the results in heterologous systems. Lastly, we analised the possible changes in the expression of these proteins in atrial samples from patients with atrial fibrillation (AF) compared to sinus rhythm, demonstrating a decrease in Lgi4 at the mRNA and protein levels in AF. This work provides insight into I_{Kur} modulation by Lgi3-4, claiming them as components of the $K_V1.5$ channelosome, as well as possible new therapeutic targets towards AF.

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Contribution Of P2Y6R To Essential Hypertension Through The Formation Of P2Y6R/AT1R Heterodimers

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Hypertension (HT) is the most common modifiable risk factor for cardiovascular disease, Strategies to control HT have a limited success, so there is an unmet need for identification of more efficient treatments for HT. A better understanding of the mechanisms regulating blood pressure could identify novel pathways that can be potential drug targets, so that we can treat HT with a mechanistic-driven approach. We have use a mice model of essential HT (BPN, blood pressure normal, and BPH, blood pressure high) to explore changes in G protein-coupled receptors (GPCR) contractile responses of mesenteric arteries.

Microarrays of vascular smooth muscle cells (VSMCs) from BPN and BPH mesenteric arteries provided differential expression of several elements in GPCR signaling pathways, and some of them were functionally investigated using pressure and wire myography of mesenteric arteries. Differential transcriptome profiling identified P2Y6 purinergic receptor mRNA as one of the top upregulated transcripts in BPH, which correlates with augmented UTP-induced contractions in BPH arteries. Angiotensin-II (AgII)-induced contraction was also higher in BPH mice despite having lower AT1R expression and was sensitive to P2Y6R modulators. Proximity Ligation Assay (PLA) and super-resolution microscopy showed closer localization of P2Y6R and AT1R at the membrane of BPH VSMCs suggesting a functional role for P2Y6R/AT1R complexes in the hypertensive phenotype. In spite of this increased response, we found reduced circulating AgII levels and less hypotensive effect in response to chronic losartan treatment (a ATR1 blocker), indicating that overstimulation of the renin-angiotensin aldosterone system does not contribute to HT in BPH. Intriguingly, BPN but not BPH mice were resistant to AgII-induced hypertension and showed reduced P2Y6R expression in VSMCs. Altogether, we suggest that increased functional coupling between P2Y6 and ATII receptors may contribute to enhanced vascular reactivity during hypertension. In this regard, P2Y6R blockers could represent a novel strategy to treat hypertension.

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MOLECULAR INSIGHTS INTO THE EFFECT OF BRUGADA SYNDROME VARIANTS

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Brugada syndrome (BrS) is an inherited cardiac arrhythmogenic disease that predisposes patients to sudden cardiac death. It is associated with variants in the SCN5A gene, which encodes the cardiac sodium channel alpha subunit (NaV1.5). We recently studied sodium channel currents (INa) in patient-specific human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs). We found that the pathogenic effect of a BrS-related heterozygous variant, SCN5A_c.4573G>A (NaV1.5_p.V1525M), is heavily impacted by the individual's genetic background1. In the present work, we aim to further explore the potential molecular mechanisms that modify the effect of this SCN5A pathogenic variant.

We investigated whether the differences in INa could result from an allelic imbalance expression between the wild type (WT) and the variant. We did not find any differential levels in the RNA expression of each allele, suggesting that the observed current levels must result from functional or trafficking mechanisms.

We then decided to investigate the presence of the recently discovered cardiac-specific short transcript of the SCN10A gene (SCN10A-short/NaV1.8-short)2. We verified, for the first time, the presence of the SCN10A-short transcript in hiPSC-CMs. This transcript was equally found in hiPSC-CMs from the four members of the family and from an independent control, but not in their respective hiPSCs. However, no evidence for the expression of the full length form of SCN10A was found. Next, we investigated the effect of NaV1.8-short on INa, in HEK293T cells co-transfected with NaV1.5_WT or NaV1.5_V1525M. We found that INa was increased with respect to cells expressing only the NaV1.5 channel, as previously reported2. This increment in INa was similar between the WT and mutant NaV1.5. These data suggest that the effect of the NaV1.5_V1525M variant is independent of the NaV1.8-short modulation of the NaV1.5 channel.

References:

1. Martínez-Moreno R, Carreras D, Sarquella-Brugada G, Pérez GJ, Selga E, Scornik FS, Brugada R. Loss of sodium current caused by a Brugada syndrome-associated variant is determined by patient-specific genetic background. Heart Rhythm. 2024 Mar;21(3):331-339. doi: 10.1016/j.hrthm.2023.11.019. Epub 2023 Nov 24. PMID: 38008367.

2. Man JCK, Bosada FM, Scholman KT, Offerhaus JA, Walsh R, van Duijvenboden K, van Eif VWW, Bezzina CR, Verkerk AO, Boukens BJ, Barnett P, Christoffels VM. Variant Intronic Enhancer Controls SCN10A-short Expression and Heart Conduction. Circulation. 2021 Jul 20;144(3):229-242. doi: 10.1161/CIRCULATIONAHA.121.054083. Epub 2021 Apr 29. PMID: 33910361.

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VASCULAR SMOOTH MUSCLE PANNEXIN 1 IN DIABETIC HYPERGLYCEMIA

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Vascular complications are major causes of morbidity and mortality in diabetic patients. Recently, our group identified a novel purinergic signaling pathway involving the AKAP5/$P2Y_{11}$/AC5/PKA/$Ca_V1.2$ protein axis in arterial myocytes that is activated upon diabetic hyperglycemia leading to vasoconstriction. This purinergic complex is activated by the release of nucleotides (e.g. ATP) to the extracellular space in response to hyperglycemic conditions. Pannexin 1 (Panx1) is a channel that mediates ATP efflux, thus inducing purinergic signaling activation. In this study, we hypothesize that Panx1 is a critical regulator mediating ATP release and increasing cAMP synthesis, $Ca_V1.2$ potentiation, and vasoconstriction upon diabetic hyperglycemia. Consistent with this hypothesis, we found that elevated glucose (HG; 20 mM D-glu) induced an elevation in extracellular ATP concentrations, and this was reduced in the presence of the Panx1 inhibitor spironolactone (spiro). Panx1 was found in complex with AKAP5, $P2Y_{11}$, AC5, PKA, and $Ca_V1.2$ in arterial myocytes. This protein complex was strengthened upon HG treatment, and spiro prevented this effect. Spiro and genetic ablation of smooth muscle Panx1 ($iPanx1_{sm}^{-/-}$) blocked cAMP production, $Ca_V1.2$ potentiation, sustained vasoconstriction, and in vivo elevations in cerebral artery myogenic tone and reduced blood flow in response to HG. In a mouse model of type 1 diabetes (e.g. STZ), the increased $Ca_V1.2$ potentiation and enhanced myogenic tone were prevented in arterial myocytes and arteries from $iPanx1_{sm}^{-/-}$ mice. Altogether, these data suggest that Panx1 is part of the AKAP5/P2Y11/AC5/PKA/$Ca_V1.2$ signaling module in arterial myocytes. This Panx1-led complex modulates vascular reactivity in response to diabetic hyperglycemia. Thus, Panx1 could be a new therapeutic target to treat vascular complications during diabetes.

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RECI PLENARY LECTURE

ASOR/TMEM206 – A 'NOVEL' ACID-ACTIVATED CL CHANNEL IN CELL DEATH AND ENDOCYTIC TRAFFICKING

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Since its first description (in Sertoli cells, Auzanneau et al, JBC 2003), a mysterious acid-activated chloride current has been found by many groups in all mammalian cells where it had been looked for. This current was mysterious as it needs both strong external acidification and cytoplasm positive voltages for opening, conditions that are almost never reached at the plasma membrane of mammalian cells. Since the molecular identity of the underlying channel (dubbed ASOR for Acid-Sensitive Outwardly Rectifying channel) had remained unknown, its biological function was enigmatic.

Using a genome-wide siRNA screen, we recently found that it is composed of (ubiquitously expressed) Tmem206 proteins and identified pore-lining residues by mutagenesis and electrophysiology (1). Cryo-EM studies show that Tmem206 proteins, which contain two transmembrane domains, assemble to trimeric channels whose overall topology resemble ASIC cation channels. In collaboration with Steve Long (Sloan Kettering), we determined the cryo-EM structure of ASOR at different pH values and obtained for the first time structures for the open pore (2). These structures are consistent with our previous results and were further validated by functional assays. In contrast to ASIC and most other channels, opening of ASOR involves a drastic rearrangement of transmembrane domains. Their movement is driven by three pairs of extracellular acidic amino-acids which can approach each other only when protons are interposed (2). pH-dependent gating does not involve a histidine residue that was previously implicated in this process.

The strongly acidic external pH needed to activate ASOR is reached only under exceptional conditions in the extracellular space. Accordingly, ASOR plays a role in acidotoxicity and is deleterious in stroke. However, the main function of ASOR is most likely in intracellular vesicles that are sufficiently acidic to activate ASOR. Emerging evidence shows that it plays distinct roles in endocytic processes. We recently examined its role in macropinocytosis, a ubiquitous from of endocytosis that is of particular importance for immune and cancer cells (3). We found that ASOR, in parallel to TPC cation channels previously implicated by the Grinstein lab in this process, is essential for the shrinkage of macropinosomes. Opening of both channels allows the exit of luminal Na and Cl. This leads to osmotic shrinkage which is followed by vesicle budding. Decreased resolution of macropinosomes results in impaired recycling of membrane receptors and luminal content. Accordingly, cancer cell nutrition by albumin uptake was reduced in Tmem206-/- cells (3). ASOR likely modulates several aspects of endocytosis.

References (Own relevant publications)**:**

1. F. Ullrich, Blin S, Lazarow K, Daubitz T, von Kries T, Jentsch TJ, Identification of TMEM206 proteins as pore of PAORAC/ASOR acid-sensitive chloride channels. eLife 8, e49187 (2019).
2. C. Wang, Polovitskaya MM., Delgado BD, Jentsch TJ, Long SB, Gating choreography and mechanism of the human proton-activated chloride channel ASOR. Science Advances 8, eabm3942 (2022).
3. M. Zeziulia M., Blin S., Schmitt F.W., Lehmann M. Jentsch T.J., Proton-gated anion transport governs macropinosome shrinkage. Nature Cell Biol, 24: 885-895 (2022).

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SYMPOSIUM 4. NON-MAMMALIAN AND ARTIFICIAL ION CHANNELS

DISSECTING THE ROLE OF AQUAPORIN WATER CHANNELS DURING SPERM OSMOADAPTATION: INSIGHTS FROM A FISH MODEL

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The ability of spermatozoa to adapt to the osmotic changes when they are ejaculated into the female oviduct, or to the aquatic environment in the case of oviparous vertebrates, is crucial for motility maintenance and fertilization success. Mammalian spermatozoa express four aquaporin water channels, of which some, in conjunction with ion channels, have been inferred to play a role in cell volume regulatory responses. However, in most cases the specific physiological roles of the aquaporins in ejaculated mammalian spermatozoa remain unknown. In mature marine fish spermatozoa, which experience a large osmotic and oxidative stress when they are released into the hyperosmotic seawater for motility activation and fertilization, up to seven aquaporins are expressed. Marine fish spermatozoa therefore represent excellent models to investigate aquaporin-mediated fluid homeostasis in male gametes. Here, I will provide a short overview of the increased diversity of aquaporins in teleosts compared to mammals, and present recent findings showing that selected marine teleost water channels play different roles regulating spermatozoon physiology. This includes facilitating motility activation, mitochondrial detoxification, and in cooperation with ion channels, cell volume regulation. I will also discuss how increasingly sophisticated regulatory pathways evolved to control marine teleost aquaporin trafficking for the maintenance of spermatozoon swimming performance under a high osmotic stress.

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ENGINEERING A BACTERIAL PORIN TRAP FOR AMYLOIDS

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Protein amyloid aggregates are ubiquitous in natural environments and typically originate either from microbial secretions or are released as prion-like proteins from animals, raising concerns about their infectivity and toxicity in contexts such as gut microbiota or soils (1).

We have recently reported (2) the insertion of an amyloidogenic sequence stretch from a model bacterial prion-like protein (RepA-WH1) in distinct extracellular loops of the abundant Escherichia coli outer membrane beta-barrel porin OmpF (3). This approach exploits the self-assembly potential of amyloids: the expression of the grafted porin enables bacterial cells to trap on their envelopes the same amyloidogenic sequence when supplemented as an extracellular free peptide. Conversely, the full-length prion-like protein, including that amyloidogenic peptide, when immobilized as bait on a surface, can capture bacteria displaying the grafted OmpF. Polyphenolic molecules known to inhibit amyloid assembly interfere with peptide recognition by the engineered OmpF, indicating that this is compatible with the kind of homotypic interaction expected for amyloid assembly.

We have now reconstituted OmpF trimers into nanodiscs, which are model membrane nanoparticles of defined composition. These devices will enable the biophysical characterization in vitro of amyloid interactions between target proteins and the porin.

Our studies suggest that synthetic porins may provide suitable scaffolds for engineering biosensor and clearance devices to address the threat posed by prions.

References:

1. Giraldo-Suárez R. (2024) An. R. Acad. Farm. 90: 83–96.

2. Vendrell-Fernández S, et al. (2022) ACS Synth. Biol. 11: 655–667.

3. Horne JE, et al. (2020) J. Biol. Chem. 295: 10340–10367.

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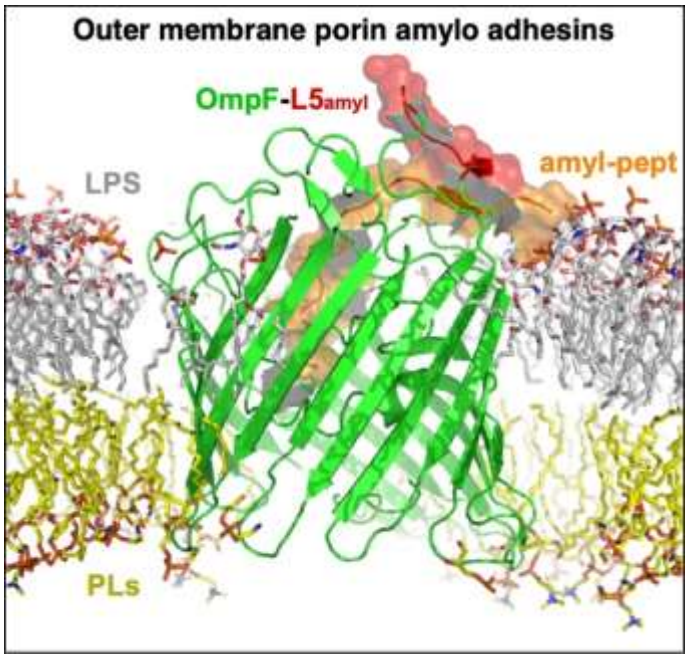

THE VOLTAGE-GATED K+ CHANNEL SLSKOR IN TOMATO PLANTS

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In plants, voltage-gated channels dominate the membrane conductance to K+ in most cell types. Plant voltage-gated channels display structural similarities with animal HCN K+ channels (Nieves-Cordones et al., 2014). They are composed of four subunits with a long intracellular C-terminal containing at least a cyclic nucleotide binding domain (CNBD) connected to the last transmembrane domain by a linker region (C-linker) like HCN channels. Interestingly, different types of rectification are exhibited by plant voltage-gated K+ channels. We have studied the function of the inward-rectifying K+ channel LKT1 and the outward-rectifying K+ channel SlSKOR in tomato plants by using gene-edited knock-out mutants. LKT1 mediates K+ uptake in root cortical cells whereas SlSKOR contributes to K+ load into xylem vessels in root vascular cells (Amo et al., 2021; Nieves-Cordones et al., 2023). CIPK-CBLs are Ca2+-sensor-kinase complexes that decode Ca2+-signals in plant cells. They have a prominent role in the plant's capacity to respond to environmental cues. In plants, voltage-gated K+ channels are targets of CIPK-CBL complexes. We identified the SlCIPK23-SlCBL1/9 complex as an activator of LKT1 and an inhibitor of SlSKOR in two-electrode voltage-clamp experiments in Xenopus oocytes (Amo et al., 2021; Nieves-Cordones et al., 2023). Regulation of SKOR-like channels by CIPK23-CBL1 complexes was also present in Medicago, grapevine, and lettuce but not in Arabidopsis and saltwater cress. Our results provide a molecular framework for coordinating root K+ uptake and its translocation to the shoot by SlCIPK23-SlCBL1/9 in tomato plants. Moreover, they evidenced that CIPK-CBL-target networks have evolved differently in land plants.

References:

1. Amo, J., Lara, A., Martínez-Martínez, A., Martínez, V., Rubio, F., Nieves-Cordones, M., 2021. The protein kinase SlCIPK23 boosts K+ and Na+ uptake in tomato plants. Plant Cell Environ. 44, 3589–3605. https://doi.org/10.1111/pce.14189
2. Nieves-Cordones, M., Amo, J., Hurtado-Navarro, L., Martinez-Martinez, A., Martinez, V., Rubio, F., 2023. Inhibition of SlSKOR by SlCIPK23-SlCBL1 / 9 uncovers CIPK-CBL- target network rewiring in land plants. New Phytol. 238, 2495–2511. https://doi.org/10.1111/nph.18910
3. Nieves-Cordones, M., Chavanieu, A., Jeanguenin, L., Alcon, C., Szponarski, W., Estaran, S., Chérel, I., Zimmermann, S., Sentenac, H., Gaillard, I., 2014. Distinct amino acids in the C-linker domain of the Arabidopsis K+ channel KAT2 determine its subcellular localization and activity at the plasma membrane. Plant Physiol. 164, 1415–1429. https://doi.org/10.1104/pp.113.229757

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CHARACTERIZATION OF CLC-1 CHLORIDE CHANNELS IN ZEBRAFISH: A NEW MODEL TO STUDY MYOTONIA.

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The function of the chloride channel ClC-1 is crucial for the control of muscle excitability. Thus, reduction of ClC-1 functions by *CLCN1* mutations leads to myotonia congenita[1,2]. Many different animal models have contributed to understanding the myotonia pathophysiology. However, these models do not allow in vivo screening of potentially therapeutic drugs, as the zebrafish model does[3]. In this work, we identified and characterized the two zebrafish orthologues (clc-1a and clc-1b) of the ClC-1 channel. Both channels are mostly expressed in the skeletal muscle as revealed by RT-PCR, Western blot, and electrophysiological recordings of myotubes, being clc-1a predominantly expressed in adult stages. Characterization in *Xenopus* oocytes shows that the zebrafish channels display similar anion selectivity and voltage-dependence to its human counterpart. However, they show reduced sensitivity to the inhibitor 9-anthracenecarboxylic acid (9-AC), and acidic pH inverts the voltage dependence of activation. Reduction of *clc-1a/b* expression hampers spontaneous and mechanically stimulated movement, which could be reverted by expression of human ClC-1 but not by some ClC-1 containing myotonia mutations. Treatment of *clc-1* depleted zebrafish with mexiletine, a typical drug used in human myotonia, improves the motor behaviour. Our work extends the repertoire of CLC channels for evolutionary structure-function studies and propose the zebrafish *clcn1* crispant model as a simple tool to find novel therapies for myotonia.

References:

1. Jentsch TJ & Pusch M (2018). CLC Chloride Channels and Transporters: Structure, Function, Physiology, and Disease. Physiol Rev 98, 1493–1590.
2. Pusch M (2002). Myotonia caused by mutations in the muscle chloride channel gene CLCN1. Hum Mutat 19, 423–434.
3. Hernández-Silva D, Alcaraz-Pérez F, Pérez-Sánchez H & Cayuela ML (2023). Virtual screening and zebrafish models in tandem, for drug discovery and development. Expert Opin Drug Discov 18, 903–915.

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WHAT PLANAR MEMBRANE ELECTROPHYSIOLOGY CAN TELL US ABOUT THE TOXICITY MECHANISM OF THE *P. AERUGINOSA* EFFECTOR TSE5

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Pseudomonas aeruginosa uses the contact-dependent type six secretion system (T6SS) to deliver various effectors/toxins in order to manipulate its environment, subvert host cells and gain an advantage in interbacterial competition. Tse5 is one of these effectors. Its full-length structure has been recently determined (González-Magaña et al., 2023), revealing that the hydrophobic C-terminal region (Tse5-CT) is encapsulated by a shell-like structure formed by the protein central domain. It is also known that Tse5-CT detaches from the rest of the protein and is delivered to the target cell membrane, where it exerts a toxic effect by depolarising target cells through the formation of membrane pores (González-Magaña et al., 2022). Here, we use planar bilayer electrophysiology to perform a deep characterisation of Tse5 pore-forming activity using the full-length protein and Tse5-CT. We find that Tse5 induces multi-ionic pores of r ~ 1 nm that prefer cations over anions at neutral pH without exhibiting chemical specificity. Further, the proteolipidic nature of Tse5-induced channels is evidenced by changes in lipid composition, pH, and calcium presence. In experiments reversing the salt concentration gradient or asymmetrical membranes, we find that Tse5 depends on the orientation of the electrical potential across the membrane to form pores, while Tse5-CT induces stable currents in all cases. This result suggests that Tse5 preferably acts from the periplasmic side to deliver its toxic cargo. Although the process of Tse5-CT delivery from full-length is sensitive to the sign of the electric transmembrane potential, Tse5-induced currents are of an equilibrium nature. This comprehensive set of electrophysiological recordings details the properties of Tse5 channels, which helps to understand its toxicity and, consequently, its mechanism to compete with other microorganisms in polymicrobial environments.

References:

1. González-Magaña, A., Altuna, J., Queralt-Martín, M., Largo, E., Velázquez, C., Montánchez, I., Bernal, P., Alcaraz, A., & Albesa-Jové, D. (2022). The P. aeruginosa effector Tse5 forms membrane pores disrupting the membrane potential of intoxicated bacteria. Communications Biology. https://doi.org/10.1038/s42003-022-04140-y
2. González-Magaña, A., Tascón, I., Altuna-Alvarez, J., Queralt-Martín, M., Colautti, J., Velázquez, C., Zabala, M., Rojas-Palomino, J., Cárdenas, M., Alcaraz, A., Whitney, J. C., Ubarretxena-Belandia, I., & Albesa-Jové, D. (2023). Structural and functional insights into the delivery of a bacterial Rhs pore-forming toxin to the membrane. Nature Communications 2023 14:1, 14(1), 1–16. https://doi.org/10.1038/s41467-023-43585-5

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SYMPOSIUM 5. ION CHANNEL PHARMACOLOGY

TRPM8 ANTAGONISTS: FROM BASIC RESEARCH TO NEUROCOSMETICS

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The non-selective cationic channel,Transient Receptor Potential Melastatin type 8 (TRPM8), is activated by different stimuli, like innocuous cool to cold temperatures (15-28 °C), membrane depolarization, cooling agents (i.e. menthol and icilin), and different synthetic molecules.1 Cumulative experimental evidences pinpoint to increased TRPM8 expression in sensory neurons after nerve injury or inflammation, resulting in enhanced sensitivity to cold allodynia and hyperalgesia.1,2 Additionally, overexpression of the TRPM8 channel has been related to the development of various types of cancer, and they are also involved in asthma, cardiovascular, gastrointestinal and neurodegenerative diseases. Therefore, the search for new modulators of TRPM8 has been pursued by either pharmaceutical companies and academic research groups.1,2

In recent years, we have intensely been working on a family of amino acid-derived β-lactams, exhibiting important TRPM8 antagonist activity. Extensive structure-activity relationships have shown the importance of hydrophobic substituents and of the configuration at the different stereogenic centers, among other structural characteristics important for activity. A prototype in this series displays noticeable in vivo activity in different animal models of neuropathic pain, including a model of chemotherapy-induced allodynia. An analogue of this prototype has been developed as a neurocosmetic and is already marketed for alleviating the hypersensitivity signs induced by some chemotherapeutic agents in oncologic patients.

References:

1. Pérez de Vega, M.J.et al., Transient Receptor Potential Melastatin 8 Channel (TRPM8) Modulation: Cool Entryway for Treating Pain and Cancer, J. Med. Chem., 2016, 59, 10006−10029.
2. Fernandez-Carvajal, A.; Gonzalez-Muniz, R.; Fernandez-Ballester, G.; Ferrer-Montiel, A. Investigational drugs in early phase clinical trials targeting thermotransient receptor potential (thermoTRP) channels. Expert Opin. Investig. Drugs 2020, 29, 1209–1222.

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HIGH THROUGHPUT SCREENING METHODOLOGIES FOR ION CHANNEL DRUG DISCOVERY. FROM TARGET-BASED TO PHENOTYPIC ASSAYS

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Drug discovery is a long process where a quick hit identification is critical for advancing in the early preclinical research of novel drugs. Ion channels are involved in multiple physiological processes, being, therefore, a class of attractive drugs targets. However, only 18% of current approved drugs are acting on ion channels (1). This is attributed to the low throughput of the different methodologies that had been employed for screening novel drugs on these different targets. In the last decades, different technological efforts were carried out in order to develop affordable assays with higher throughput that would allow to carry out High Throughput Screening assays for ion channels (1,2). These technologies include radioligand binding assays, ion flux detection, membrane potential-sensitive fluorescent dyes, ion-sensitive fluorescent dyes, microelectrode arrays and automated patch clamp. To obtain a comprehensive overview of each of them, we will review several examples of all these technologies, as well as their application to either target-based or phenotypic assays (3) together with their strengths and weaknesses for being applied to High Throughput Screening.

References:

1. Dallas et al., Advances in ion channel high throughput screening: where are we in 2023? Expert Opin Drug Discov 2024; 19:331-337.
2. Yu et al. High throughput screening technologies for ion channels. Acta Pharmacol Sin 2016; 37:34-43.
3. Martínez et al. In vitro models for neuropathic pain phenotypic screening in brain therapeutics. Pharmacol Res. 2024 Apr;202:107111.

Acknowledgements: *Funded by Agencia Estatal de Investigación (PID2020-119428RB-I00) and Xunta de Galicia (ED431C 2022/20) and European Regional Development Fund (ERDF).*

MOLECULAR CHARACTERIZATION OF MLC MUTANTS REVEALS THE ROLE OF MLC1 AND GLIALCAM IN CONTROLLING GPRC5B SIGNALING

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a rare type of leukodystrophy mainly caused by mutations in two genes that encode for MLC1, a polytopic membrane protein of unknown function, and GLIALCAM, a cell adhesion molecule of the immunoglobulin superfamily. Recent studies indicated that MLC1 downregulates transduction signals, but the molecular mechanisms are unclear. As the orphan G protein-coupled receptor GPRC5B has been identified as a novel GlialCAM and MLC1 interacting protein, and recent studies found MLC patients containing heterozygous mutations in GPRC5B, it has been suggested that GlialCAM and MLC1 might modulate signaling through GPRC5B.

Here we show that GPRC5B activates in a constitutive manner G12/G13, Fyn and beta-arrestin signaling pathways, and MLC1 inhibits GPRC5B-mediated signaling by interfering with GPRC5B oligomerization. In contrast, GlialCAM releases MLC1 inhibition and causes GPRC5B internalization. GPRC5B containing MLC mutations showed increase stability, can activate signaling pathways but are less internalized by GlialCAM. Previously characterized GLIALCAM mutations are also less internalized by GPRC5B due to an increased stability, indicating that reduced GlialCAM internalization by GPRC5B is a common mechanism for several MLC mutations in different genes. Considering the effect of depolarization and hypotonicity in the regulated interaction between MLC1, GlialCAM and GPRC5B, we propose a working model for the functioning of the MLC signaling complex. Importantly, our results suggest that GPRC5B activators could be used as new drugs for MLC disease.

DECA11 SELECTIVELY INCREASES HUMAN I_{NA} AND I_{K1} AND EXERTS ANTIARRHYTHMIC EFFECTS IN A MOUSE MODEL OF HEART FAILURE

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Co-expression of the 132 aminoacids peptide that constitutes the N-terminal domain (NTd) of human cardiac Na^+ channels (Nav1.5) with Nav1.5 or Kir2.1 channels significantly increases the Na^+ (I_{Na}) and inward rectifier K^+ (I_{K1}) currents in heterologous expression systems and adult rat cardiomyocytes [1]. A decrease in I_{Na} and I_{K1} impairs excitability and prolongs refractoriness, enhancing the risk of life-threatening ventricular arrhythmias in patients with heart failure with reduced ejection fraction (HFrEF). Here, we analyzed whether there is a minimal motif within the NTd of Nav1.5 channels capable of increasing I_{Na} and I_{K1}. Serial large- and micro-deletion and point mutations of the NTd demonstrated that the sequence RFTRESLAAIE, named DECA11 (Patent Ref#P202330798), significantly increased I_{Na} and I_{K1} densities in heterologous expression systems and in human cardiomyocytes derived from induced pluripotent stem cells (hiPSC-CM) being Ser6 critical for these effects. DECA11 did not modify the voltage- and time-dependent characteristics of I_{Na} nor the density of its late component (I_{NaL}). Consequently, DECA11 significantly hyperpolarizes the resting membrane potential (-78.4±1.2 vs. -73±1.3 mV n≥8, P=0.01) and increases the action potential (AP) amplitude (108.7±2.5 vs. 95.9±3.4, n≥8, P=0.009), without modifying the duration of the APs recorded in hiPSC-CM. DECA11 did not affect the density of the current generated by Nav1.1, Nav1.2, Cav1.2, Kv4.3, and Kv11.1 (hERG) channels. In isolated cardiomyocytes from mice that were infected or not (control) with adeno-associated particles encoding DECA11 and that underwent or not transverse aortic constriction (TAC), a pressure overload model of HFrEF, DECA11 significantly increased the I_{Na} and I_{K1} that was decreased in TAC control cardiomyocytes. Furthermore, DECA11 significantly decreased the capacitance of TAC cardiomyocytes, which was significantly increased in TAC control cardiomyocytes because of the cardiac hypertrophy. However, it did not modify the ventricular ejection fraction that was compromised in TAC control animals. Importantly, in TAC mice infected with DECA11, the ventricular arrhythmia inducibility and duration were significantly decreased compared with TAC control animals. These results demonstrated that DECA11 significantly and selectively increases the densities of cardiac I_{Na} and I_{K1} and that this augmentation exerts antiarrhythmic effects in a mouse model of HFrEF.

References:

1. Matamoros M, et al. Nav1.5 N-terminal domain binding to α1-syntrophin increases membrane density of human Kir2.1, Kir2.2 and Nav1.5 channels. *Cardiovasc Res* 2016;**110**:279–290.

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THE CONANTOKIN-DERIVED PEPTIDE EAR-20 AS A NOVEL NMDA RECEPTOR POSITIVE ALLOSTERIC MODULATOR

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Glutamate is an essential neurotransmitter in the central nervous system (CNS), coordinating many neurological and physiological processes through the activation of various glutamate receptors. The N-methyl-D-aspartate receptors (NMDARs) represent a crucial subtype of ionotropic glutamatergic receptors, playing a fundamental role in excitatory synaptic transmission. The activation of this ligand-gated ion channel facilitates Ca^{2+} influx through the channel pore, initiating intracellular cascades that modulate synaptic plasticity – a fundamental mechanism underlying learning and memory. A growing evidence of scientific works, spanning from basic science to clinical studies, have linked NMDAR hypofunction to numerous CNS disorders, including autism, schizophrenia, Alzheimer's disease, and intellectual disability, among others. Consequently, there is growing interest in identifying positive allosteric modulators (PAMs) of NMDARs as potential therapeutic options for ameliorating cognitive deficits associated with NMDAR dysfunction. Based on structural insights from the GluN2B subunit of NMDARs and conantokin-G toxin interaction, which selectively targets the aforehead mentioned subunit, a series of peptides (EARs) predicted to modulate NMDAR activity have been designed. In this study, we have evaluated the functional properties of the EAR-20 peptide as a novel PAM for NMDARs. By the use of patch-clamp technique on HEK293T cells transfected with several NMDAR combinations, we demonstrate that EAR-20 robustly potentiates whole-cell NMDAR-mediated currents, exhibiting selective efficacy across distinc heteromeric NMDAR subtypes. Additionally, by single-channel recordings, we demonstrate that the EAR-20 peptide exerts its effects by prolonging the duration of receptor occupancy in the open conformation and also diminishing the desensitization states of the channel, which correlates with our findings in the macroscopic recordings. Finally, this potentiating effect is also observed in hippocampal neurons cultured in vitro, where EAR-20 increases the amount of current driven by spontaneous excitatory postsynaptic currents (EPSCs). Our findings highlight the utility of rational peptide design in yielding potential therapeutc agents for disorders characterized by NMDAR hypofunction.

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MACROLIDE IMMUNOSUPPRESSANTS: A NOVEL CLASS OF TRPM8 CHANNEL AGONISTS

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Macrolides are a class of mostly natural products with a large macrocyclic lactone ring. Important members within this drug class include the immunosuppressant tacrolimus (TAC), an inhibitor of the phosphatase calcineurin, and rapamycin (RAP), an inhibitor of the mTOR kinase. RAP and analogs, called rapalogs, are currently used in the clinic as immunosuppressants to prevent organ rejection, as tumor suppressors and to mitigate ocular inflammatory disorders. In addition, RAP is under intense investigation for some remarkable biological effects; slowing the aging process in mammals.

We found that TRPM8, a polymodal TRP channel involved in cold temperature sensing, thermoregulation and cold pain (1) is activated my macrolide immunosuppressants at micromolar concentrations (2, 3).

In HEK293 cells heterologously expressing mouseTRPM8, TAC or RAP activate non-selective outwardly-rectifying currents and evoke intracellular calcium responses, in a dose dependent manner. At low micromolar concentrations, the macrolides potentiate cold-evoked responses. These effects are conserved in different channel orthologs, including humanTRPM8. The effects of macrolides are direct, activating single-channel currents in purified, reconstituted channels, and cell-attached recordings. Biophysically, TAC and RAP act as type I agonists (i.e. menthol-like), slowing activation and deactivation of the open channel. Surprisingly, despite this similarity to menthol effects, TAC and RAP can readily activate the menthol-insensitive mutant (Y745H), suggesting their interaction with a different binding site.

In mouse primary sensory neurons, TAC and RAP activate TRPM8-expressing cold thermoreceptors. These responses were abolished by TRPM8 antagonists. Selectivity for TRPM8 was further demonstrated by the near suppression of effects in TRPM8 KO mice, while responses to other agonists remained unaltered. In current-clamp recordings, TAC or RAP increased the excitability of cold-sensitive DRG neurons and potentiated their response to cold temperatures.

TRPM8 agonists are currently under development for the treatment of dry-eye disease, a multifaceted condition affecting many individuals, specially of old age. In mice, we found that local application of TAC or RAP increased tearing and blinking, by a TRPM8-dependent mechanism.

These studies show that TAC and RAP, two clinically approved macrolide immunosuppressants, are selective activators of TRPM8. Furthermore, our findings suggest their potential use for the treatment of the symptoms of dry-eye disease.

References:

1. Almaraz et al., Handbook of Experimental Pharmacology 2014; 222:547-579; DOI: 10.1007/978-3-642-54215-2_22.
2. Arcas et al., Journal of Neuroscience 2019, 39:949-969; DOI: 10.1523/JNEUROSCI.1726-18.2018
3. Arcas et al., British Journal of Pharmacology, in press; DOI: 10.1002/BPH.16402

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CHANGES IN THE K_V1.3 PHARMACOLOGY UPON THE ASSOCIATION WITH KCNE4

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Voltage-gated potassium cannel Kv1.3 plays a crucial role during the immune response. Kv1.3 is the main voltage-gated potassium channel expressed in T lymphocytes and is also expressed in a variety of leukocytes. The channel has an important role in mediating T-cell activation, which makes it an important therapeutic target for the treatment of autoimmune diseases and chronic inflammation. Multiple sclerosis and psoriasis can be effectively treated, respectively, with fampiridine and dalazatide, two drugs that target Kv1.3 activity. Functional Kv channels are oligomeric complexes that can associate to ancillary subunits that regulate their function and can modify their pharmacology. For example, Kv7.1 and hERG pharmacology is altered by their association with KCNE1 and KCNE2, respectively. In the immune system, Kv1.3 associates with KCNE family member KCNE4. KCNE4 impairs Kv1.3 plasma membrane trafficking in a stoichiometry-dependent fashion and also contributes to accelerate the inactivation of the channel. In this study, we aim to evaluate the role of KCNE4 in Kv1.3 pharmacology using two drugs: Margatoxin and Psora-4. While Margatoxin binds to Kv1.3 to the outer pore from the extracellular side, Psora-4 targets the intracellular mouth of the channel. Our results show that, while KCNE4 does not alter Margatoxin effects on the channel, Kv1.3 inhibition by Psora-4 is slowed by the presence of KCNE4. We propose how KCNE4-dependent architectural changes in Kv1.3 intracellular structure affect Psora-4 accessibility to its binding sites. Moreover, the Kv1.3/KCNE4 configuration varies among immune cell types and the different stages of the immune response. Rearrangements in channel architecture should be considered during the development of therapeutical approaches to ensure the proper pharmacological targeting of Kv1.3.

References:

1. Vennekamp J, Wulff H, Beeton C, Calabresi PA, Grissmer S, Hänsel W, Chandy KG. Kv1.3-blocking 5-phenylalkoxypsoralens: a new class of immunomodulators. Mol Pharmacol. 2004 Jun;65(6):1364-74. doi: 10.1124/mol.65.6.1364. PMID: 15155830.
2. Grunnet M, Rasmussen HB, Hay-Schmidt A, Rosenstierne M, Klaerke DA, Olesen SP, Jespersen T. KCNE4 is an inhibitory subunit to Kv1.1 and Kv1.3 potassium channels. Biophys J. 2003 Sep;85(3):1525-37. doi: 10.1016/S0006-3495(03)74585-8. PMID: 12944270; PMCID: PMC1303329.
3. Solé L, Roura-Ferrer M, Pérez-Verdaguer M, Oliveras A, Calvo M, Fernández-Fernández JM, Felipe A. KCNE4 suppresses Kv1.3 currents by modulating trafficking, surface expression and channel gating. J Cell Sci. 2009 Oct 15;122(Pt 20):3738-48. doi: 10.1242/jcs.056689. Epub 2009 Sep 22. PMID: 19773357.

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CLOSING PLENARY LECTURE

NEUROIMMUNE INTERACTIONS AND ARTHRITIS PAIN IN NEURODEGENERATIVE CONDITIONS

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A significant proportion of patients with neurodegenerative diseases are affected by alteration of pain sensation. Alzheimer's disease (AD) patients have a compromised ability to report pain and untreated pain contributes to psychiatric symptoms of dementia. Neuronal degeneration provokes an intense reaction by microglia which, depending on their phenotypes of activation, may give rise to pain. I will discuss how inflammatory arthritis affects spinal cord microglial activity and how neuron-microglia communication is altered in the TASTPM transgenic mouse model of AD. Our evidence shows that 6 months old TASTPM display cognitive impairment, brain amyloid pathology and reduced thermal sensitivity compared to WT controls, which is in accordance with the observation that AD patients exhibit reduced pain intensity compared to cognitive-intact individuals. Under conditions of persistent inflammatory pain, spinal microglial activity plays a mechanistic role in central sensitisation, as attenuation of microglial activation correlates with reduced pain-like behaviours. I will show that in persistent inflammatory arthritis, the release of galectin-3 from primary afferent terminals in the dorsal horn mediates inflammatory allodynia via interaction with Toll like receptor 4 (TLR4) in microglia. However, this neuron-to-microglia communication pathway is defective in TASTPM mice that display reduced inflammatory allodynia and reduced spinal cord microgliosis. Indeed, a cluster of TASTPM microglia lacks expression of TLR4 and cannot respond to Gal-3 which is expressed and released by primary afferent fibres in the dorsal horn. Thus, we identified a mechanism through which nociceptors respond to joint inflammation and establish nociception through the activation of microglia. Sensory neuron-derived Gal-3 activates microglial TLR4, and promotes nociceptive signalling via the release of cytokines in the dorsal horn. Intriguingly, in the spinal cords of the TASTPM mouse model of AD, the emergence of a subset of microglia devoid of TLR4 is associated with milder inflammatory nociception.

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POSTERS

LIPIDS AS DRUG DISCOVERY PROBES IN TRPV CHANNELS

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Our research is focused on the transient receptor potential vanilloid 2 (TRPV2) ion channel is a ubiquitous membrane protein responding to several physical stimuli, such as heat, mechanical stretch, osmotic changes, etc. However, few chemical stimuli have been identified for TRPV2, and the few that have been identified are non-specific for this channel [1]. Recently we have defined for the first time the involvement of TRPV2 in vascular physiology [2], simultaneously identifying novel TRPV2 modulators [3]. Here, we expand our research to open a new pharmacological venue on lipid-based drug discovery. TRPV2 and the TRPV channels subfamily are integral membrane proteins, specifcally ion channels governing cellular transport and signalling, which activity is often governed by lipid bilayer composition and properties. Lipids bind to specific sites on these proteins, regulating function, trafficking, and interactions. However, the intricate nature of these interactions, compounded by experimental challenges, impedes full elucidation of lipid-mediated regulation mechanisms. In our study, we utilized coarse-grained molecular dynamics simulations (CG-MD) to investigate lipid-binding sites and interactions across all six TRPV channels, employing a comprehensive asymmetric membrane composition of 10 lipids. CG-MD simulations on TRPV apo structures not only validated known binding sites for cholesterol and phosphatidylinositol-(3,4)-biphosphate but also unveiled potential binding sites for these lipids and others. By correlating known TRPV drug binding sites with identified lipid binding sites, both established and putative, we have assigned specific residues, offering fresh insights for rational drug design targeting TRPV channels. Our research highlights CG-MD simulations' effectiveness in uncovering potential regulatory regions on membrane proteins, providing specific insights into TRPV's lipid-protein interactions. This expansion of our understanding of native membrane protein regulation complexity lays a foundation for future drug development efforts targeting lipid-binding sites on TRPVs as a paradigm for ion channels, which can be extensive to integral membrane proteins.

References:

1. A Perálvarez-Marín, P Doñate-Macián, R Gaudet (2013) What do we know about the transient receptor potential vanilloid 2 (TRPV2) ion channel? FEBS Journal 280, 5471-5487
2. A Perálvarez-Marín, M Solé, J Serrano, A Taddeucci, B Pérez, C Penas, G Manich, M Jiménez, P D'Ocon, F Jiménez-Altayó (2024) Evidence for the involvement of TRPV2 channels in the modulation of vascular tone in the mouse aorta Life Sciences 336, 122286
3. È Catalina-Hernández, M López-Martín, D Masnou-Sánchez, M Martins, VA Lorenz-Fonfria, F Jiménez-Altayó, UA Hellmich, H Inada, A Alcaraz, Y Furutani, A Nonell-Canals, JL Vázquez-Ibar, C Domene, R Gaudet, A Perálvarez-Marín (2024) Experimental and computational biophysics to identify vasodilator drugs targeted at TRPV2 using agonists based on the probenecid scaffold Computational and Structural Biotechnology Journal 23, 473-482

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DAPAGLIFLOZIN AND EMPAGLIFLOZIN INCREASE NA^+ CHANNEL OPEN PROBABILITY AND RECOVER I_{NA} IN CARDIOMYOCYTES FROM A MOUSE MODEL OF HEART FAILURE

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Dapagliflozin (dapa) and empagliflozin (empa) are sodium-glucose cotransporter 2 inhibitors (SGLT2i) used for the treatment of type 2 diabetes (T2D) that significantly decrease mortality in patients with heart failure with reduced ejection fraction (HFrEF), decreasing the incidence of ventricular arrhythmias and sudden cardiac death. The *SCN5A* gene encodes Nav1.5 channels that generate the Na^+ current (I_{Na}), which is responsible for cardiac depolarization and excitability. In HFrEF patients, the expression of Nav1.5 channels is reduced, leading to life-threatening arrhythmias. In human cardiomyocytes derived from induced pluripotent stem cells (hiPSC-CMs), we recently described that 24-h incubation with dapa or empa, at therapeutically relevant concentrations (1 μM), increased the density of the cardiac I_{Na}[1]. Here, we have deciphered the ultimate mechanism responsible for the I_{Na} increase by combining different experimental approaches. Dapa (EC_{50}=0.9±0.09 μM) and empa (EC_{50}=1.0±0.1 μM) produced a concentration-dependent increase of $I_{Nav1.5}$. Single-channel recordings using the cell-attached patch-clamp configuration showed that dapa and empa did not modify the i_{Na} amplitude at any of the voltage tested nor the single-channel conductance which averaged 23.1±2.8 pS in control conditions. Empa, but not dapa, significantly increased the Nav1.5 open time constant (τ_{open}) by up to twice. The increase in the dwell open time produced by empa is reflected in a significant increase in the mean open time (1.4±0.1 vs 0.96±0.04 ms; $P<0.05$), which was not modified by dapa. Conversely, dapa significantly augmented channel re-openings and the number of traces with openings. Because of these effects, both dapa and empa significantly increase the open probability of Nav1.5 channels. Dapa and empa did not modify the transcriptional activity of the human minimal promoter of the *SCN5A* gene while augmented the *SCN5A* mRNA. Flow cytometry experiments showed that dapa and empa significantly enhanced the membrane expression of Nav1.5 channels. Fluorescence recovery after photobleaching (FRAP) analysis demonstrated that empa increased the number of mobile Nav1.5 channels, but neither drug modified the kinetics of the process. Importantly, by the combination of these effects both dapa and empa, significantly increased the diminished I_{Na} density in cardiomyocytes dissociated from mice that underwent transverse aortic constriction, a model of HFrEF.

References:

1. Dago M, et al. Empagliflozin and Dapagliflozin Increase Na^+ and Inward Rectifier K^+ Current Densities in Human Cardiomyocytes Derived from Induced Pluripotent Stem Cells (hiPSC-CMs). *Cells* 2022;11:3707.

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UNDERSTANDING THE ROLE OF TRPM3 CHANNELS IN KIDNEY: IMPLICATIONS FOR BLOOD PRESSURE CONTROL AND HYPERTENSION MECHANISMS

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TRPM3 is a non-selective cation channel activated by pregnenolone sulfate (PS). PS contracts mouse aorta by activating TRPM3 in vascular smooth muscle cells (VSMCs)[1]. However, PS induces vasodilation in mesenteric arteries by activating channels present in the adventitial sensory nerve endings[2]. These opposing effects hinder to interpret the role of TRPM3 channels in the control of blood pressure (BP). Our aim is to understand the underlying mechanisms contributing to essential hypertension for developing effective therapeutic strategies, therefore here we focus on disclose the role of TRPM3 controlling BP by studying TRPM3-KO mice and to extend our knowledge of the vascular effects of TRPM3 channels studying their role in the kidney vasculature, due to its relevant role in BP control.

By measuring BP using a tail-cuff system we observe a hypotensive phenotype in TRPM3-KO mice and resistance to angiotensin II-induced hypertension. BPH kidneys displayed a 2-fold increase in TRPM3 mRNA expression, primarily in the renal cortex, via qPCR. TRPM3 channel location throughout the nephron was studied combining RNAscope *in situ* hybridization and immunohistochemistry with several antibodies. TRPM3 mRNA is highly expressed in the glomeruli, the distal convoluted tubule (DCT) and the collecting ducts (CD), and despite the clear vascular effects, there is no evident expression in the renal vasculature. Therefore, we investigated the crosslink between TRPM3 activation and several renal vasodilation systems throughout the nephron measuring renal vascular flow at constant pressure in isolated kidneys. The blockade of nervous signaling by the CGRP antagonist BIBN 4096, showed an almost complete loss of TRPM3-mediated vasodilatation during phenylephrine stimulation. Similar results were obtained where NOS blocker L-NAME was tested. No effect was observed whit prostaglandin synthesis inhibitor Indomethacin.

Our findings underscore the importance of TRPM3 channels in BP regulation, particularly in modulating vascular flow in the kidney. However, the absence of TRPM3 expression in the renal vasculature suggests a complex crosstalk between the nephron and vessels, suggesting an intricate interplay between TRPM3 channels, tubule-glomerular feedback, nervous signaling, and NO release. Further experiments are needed to seed light on mechanistic pathway.

References:

1. Naylor, J. et al. Pregnenolone sulphate-and cholesterol-regulated TRPM3 channels coupled to vascular smooth muscle secretion and contraction. Circ Res 106, 1507–1515 (2010).
2. Alonso-Carbajo, L. et al. Activation of the cation channel TRPM3 in perivascular nerves induces vasodilation of resistance arteries. J Mol Cell Cardiol 129, 219–230 (2019).

Acknowledgements: *Supported by grants PID2020-118517RB-I00 (MINECO) and VA172P20 (JCyL) and by a predoctoral (JRM) contracts of the Junta de Castilla y León.*

STRUCTURAL-BASED VIRTUAL SCREENING OF IN-HOUSE COMPOUND COLLECTIONS TO IDENTIFY TRPM8 LIGANDS

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The Transient Receptor Potential Cation Channel, Subfamily M, Member 8 (TRPM8), a member of the TRP superfamily, is a nonselective ion channel that is involved in cold perception. This channel is also implicated in diverse physiological processes such as pain signaling, inflammatory responses and cancer, among others. Therefore, there is an interest in the search of molecules capable of modulating these channels.[1] During the last years, significant advances have been made in elucidating the structure of TRPM8 through cryo-electron microscopy studies. These structures had shed light on their 3D structure, as well as revealing the molecular details of ligand recognition. TRPM8 channels are organized as a domain-swapped tetramer, in which each monomer has a transmembrane and a cytosolic domains. In the latter there are four melastatin homology regions. The transmembrane region comprises six α-helices, with four constituting the voltage-sensor-like domain (VSLD), whereas the pore domain (PD) is formed by the remaining two and a pore helix.[2] The VSLD is the binding site of various ligands, including agonist, as menthol or icilin, or antagonist as AMTB or TC-I.[1]

In this communication we present a structural-based virtual screening of several of our in-house compounds collections. Using three homology model of the human TRPM8 channel based on the structures of *parus major* channel (PDB codes and 6O72 and 6O6R)[1] and *mus musculus* channel (PDB code 8E4L),[3] an induced fit docking (IFD) protocol was used to filter 110 compounds. An analysis of the results based on the score, ligand-channel interactions, and visual inspection, allowed the selection of 17 derivatives for further biological testing. The biological evaluation revealed that compounds featuring a nitrogen six-membered heterocyclic core exhibit promising inhibitory activity against menthol binding. Interestingly, three derivatives demonstrated inhibition levels of approximately or greater than 50% at 5 μM concentration. These findings highlight the potential of these derivatives as promising hits for the development of more potent TRPM8 ligands.

References:

1. A. Plaza-Cayón *et al. Med. Res. Rev.* **2022**, *42*(6), 2168. DOI: 10.1002/med.21920.
2. C. Izquierdo *et al*. *Int. J. Mol. Sci.* **2021**, *22*(16), 8502. DOI: 10.3390/ijms22168502.
3. Y. Yin *et al. Science* **2022**, *378*, eadd1268. DOI: 10.1126/science.add1268.

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TRESK CHANNEL REGULATES SENSORY NEURON EXCITABILITY AND ENHANCES ACUTE AND CHRONIC ITCH

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TRESK (K2P18.1) is a background K^+ channel expressed in sensory neurons, where it modulates the resting membrane potential, action potential firing and neuronal excitability. A subset of these sensory neurons, which express specific TRPs and Mas- related G protein-coupled receptors (Mrgprs), are activated by pruritogens and mediate itch sensations. Because TRESK is involved in somatosensation and pain perception, we evaluated the contribution of this channel to pruritic sensitivity and its potential as a target for the treatment of chronic itch pathologies including renal or liver failure, Hodgkin's lymphoma and different types of dermatitis. By combining, RNA in situ hybridization, calcium imaging, electrophysiological and behavioral approaches, we found that TRESK is involved in the modulation of non-histaminergic itch. In situ hybridization experiments show that TRESK coexpresses with mouse MrgprD$^+$ and MrgprA3$^+$ in sensory neurons. At the behavioral level, intradermal injection of chloroquine (CQ) a MrgprA3 agonist, in the cheek model produced an acute scratching response, which was significantly enhanced in mice lacking TRESK.

Interestingly, TRESK knockout (KO) mice also showed alterations in mice models of chronic itch. Induction of Psoriasis, Allergic Contact Dermatitis or Dry Skin showed a significantly higher scratching response in mice lacking TRESK compared to their wild- type (WT) counterparts.

In behavioral tests of acute itch, we corroborate that cloxyquin acts as a specific TRESK activator. Intraperitoneal pre-treatment with cloxyquin did not produce any effect in TRESK KO mice, whereas WT mice showed significantly reduced scratching responses. Furthermore, in a mouse model of imiquimod-induced psoriatic itch, cloxyquin pre-treatment appeared to decrease spontaneous scratching episodes in WT mice compared to TRESK KO mice. Additionally, in situ hybridization of human dorsal root ganglia (DRG), also showed coexpression of TRESK and mice MrgprA3 homologue MrgprX1.

In summary, our data indicate that TRESK is involved in regulating the excitability of sensory neurons that mediate histaminergic-independent itch. Given the prominent role of this neuronal subpopulation in chronic itch diseases, TRESK emerges as a potential candidate for therapeutic intervention.

References:

1. Llimós-Aubach J., et. al. 2024. bioRxiv 2024.01.25.577205
2. Wright P.D., et. al. 2013. Biopchemical and Biophysical Research Communications 441:463-468
3. Han L., et. al. 2013. Nature Neuroscience 16(2): 174-182

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4-*TRANS*-HYDORXYPROLINE DERIVATIVES AS TRPM8 ANTAGONISTS

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TRPM8 channels, or transient receptor potential cation channel subfamily M, member 8, are predominantly expressed in sensory neurons, where they play a crucial role in perceiving cold temperatures and initiating responses to cold stimuli. They are involved in several physiological processes, such as pain perception and thermoregulation, among others, and have been implicated in pathological inflammatory and neuropathic pain conditions and different types of cancer.[1]

We have previously described a family of amino acid-derived β-lactams, having potent TRPM8 antagonist activity, but the preparation of these compounds required long synthetic schemes and the resolution of isomeric mixtures.[2] In the search for other chemotypes to modulate TRPM8 channel, we discovered that the 4-*trans*-hydroxyproline (Hyp) ring is a suitable central scaffold for new TRPM8 antagonists. Modifications at the the three possible positions led to some interesting hits. In the way of hit-to-lead optimization, we explore the combination of the best apendages at *N*- and *C*-terminal positiond in an attempt to find promising leads. This piece of work deals with the synthetic procedures and preliminary biological characterization of new Hyp derivatives **1** as TRPM8 modulators.

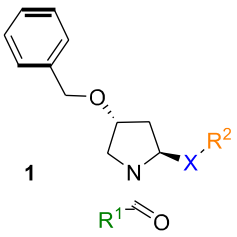

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Figure 1. General structure of 4-*trans*-hydroxyproline derivatives

References:

1. Fernandez-Carvajal, A.; Gonzalez-Muniz, R.; Fernandez-Ballester, G.; Ferrer-Montiel, A. Investigational drugs in early phase clinical trials targeting thermotransient receptor potential(thermoTRP) channels. *Expert Opin. Investig. Drugs* **2020**, *29*, 1209–1222.
2. Martín-Escura, C.; Bonache, M.A.; Medina-Peris, A.; et al. β-Lactam TRPM8 antagonists derived from Phe-penylalaninol conjugates: structure-activity relationships on *N*-monobenzyland *N'*-amide derivatives. *Int. J. Mol. Sci.*, **2023**, *24(19),14894*, and references therein.

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PHARMACOLOGICAL TOOLS TO STUDY THE K_V1.5 CHANNELOSOME: IDENTIFICATION OF NOVEL KCHIP2 LIGANDS

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Ion channels are proteins present in the plasma membrane or in intracellular organelles of the cells, where they play important functions. These proteins form signaling complexes or channelosomes together with other proteins or lipids. The dysfunction of these channels results in a several disorders named channelopathies, which represent a challenge for study and treatment.

In the human heart, K_V1.5 channels deserve special mention since their activation generates an atria-selective current that plays a crucial role in the human cardiac atrial repolarization (I_{Kur}). This channel is encoded by *KCNA5*, and it has been reported that mutations in this gene, inducing both gain- or loss- of function, enhance atrial fibrillation (AF) susceptibility. K_V1.5 is considered a potential target for the development of new drugs useful in the treatment of supraventricular arrhythmias (i.e., AF). However, the great homology between different ion channels makes very difficult the design of selective compounds useful in the treatment of AF.

Valenzuela's group have described the existence of a K_V1.5 channelosome in cardiac rat ventricle, that is absent in atrium, despite K_V1.5 channels are also expressed in the human atria, pointing the importance of the animal species studied. Recently, they have demonstrated that KChIP2 is another new interactor of the K_V1.5 channelosome.[1]

KChIP2 is a potassium channel interacting protein mainly expressed in heart. In our research group, we have a research line focused on the identification of novel KChIP2 ligands as useful tools to understand the role of K_V1.5 channelosome in atrial fibrillation.[2] In this regard, structure-based virtual screening could be an important tool to accelerate their identification. Taking advantage of the single tryptophan residue of KChIP2 located in the ligand binding site, we used tryptophan fluorescence quenching as initial screening technique for the identification of novel KChIP2 ligands.

In this communication, we describe a multidisciplinary approach that starts with a structure-based virtual screening, followed by an iterative process of synthesis/biological evaluation, leading to the identification of new KChIP2 ligands. Electrophysiology assays have allowed to study their role in the K_V1.5 channelosome.

References:

1. a) Macías, A. et al. *Br. J. Pharmacol.* **2014**, *171*, 4914; c) Macias, A. et al. *Int. J. Mol. Sci.* **2021**, *22*, 1336.
2. a) Cercós, P. *et al. Int. J. Mol. Sci.* **2021**, *22*, 1419 ; b) De Benito-Bueno, A. *et al. Int. J. Mol. Sci.* **2022**, *23*, 9170.

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K^+ CHANNEL REMODELING IN COLON CANCER CELLS AND ITS MODULATION BY POLYAMINES

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Colon cancer remains one of the most prevalent forms of cancer and a leading cause of cancer-related deaths in Spain. Most forms of colon cancer are due to loss of APC tumor suppressor that lead to the c-myc oncogene activation and excess polyamine biosynthesis. We have shown that colon cancer cells undergo a large remodeling of intracellular Ca^{2+} homeostasis that is partially reversed by polyamine synthesis inhibition with ornithine decarboxylase suicide inhibitor DFMO. Here, we aimed at investigating possible differences between K^+ channel activity and expression between HT29 colon cancer cells and NCM460 normal colonic cells. In addition, we asked whether polyamine depletion may also reverse the possible differences in K^+ channel activity and expression. For this end, total K^+ currents and currents sensitive to an specific blocker of voltage-gated K^+ channels: Kv10 and Kv11 were monitored in HT29 and NCM460 cells. Additionally, expression of all different types of K^+ channels was tested using microarrays and differential expression analysis. Finally, effects of polyamine depletion on K^+ currents and differential expression of K^+ channels were investigated. Our results suggest a deep remodeling of K^+ channels in colorectal cancer. At the functional level, HT29 tumor cells exhibit lower total K^+ compared to normal NCM460 cells. However, the current mediated by the cancer-linked Kv10.1 and Kv11.1 channels is higher in colon cancer cells. Consistently, differential expression of K^+ channel genes is observed between normal NCM460 cells and HT29 tumor cells. Futhermore, we found that polyamine depletion using DFMO significantly increases the total K^+ current in HT29 cells, an effect associated with the increase in the activity of the Kv10.1 and Kv11.1 channels, which is correlated to changes in the expression of a few genes coding for K^+ channels or their modulators. In summary, our results suggest a remodeling of K^+ channels in colon cancer that could contribute to cancer hallmarks and is potentially linked to excess polyamine biosynthesis.

References:

1. Tajada, S., Villalobos, C., 2020. Calcium Permeable Channels in Cancer Hallmarks. Front. Pharmacol. https://doi.org/10.3389/fphar.2020.00968

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ROLE OF ESTROGEN RECEPTOR β ON ION CHANNEL CURRENTS AND ELECTRICAL ACTIVITY IN HUMAN PANCREATIC β-CELL LINE ENDOC-βH1

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The gonadal hormone 17β-estradiol (E2) is involved on the maintenance of glucose homeostasis by its actions through estrogen receptors (ERs) expressed in pancreatic β-cells. The electrical activity of this cell type plays a critical role in glucose-stimulated insulin secretion. We used the human β-cell line EndoC-βH1 and employed patch clamp technique and qRT-PCR to study the effect of specific agonists of ERs and the endocrine disruptor BPA on ion channel currents and gene expression, and on the cell electrical activity (1). We treated the human EndoC-βH1 cell line with 1nM E2, the ERα agonist PPT, the ERβ agonist DPN, and BPA for 48h. Cells exposed to DPN and BPA exhibited a decrease in K+ currents, while E2 and PPT had no effect. Experiments using specific K+ channel blockers suggested that this reduction in the global K+ current induced by BPA was mediated by inhibition of Kv2.1/2.2 channel type rather than on KCa1.1. Subsequent qRT-PCR analysis revealed down-regulation of Kcnb1 and Kcnb2 genes encoding Kv2.1 and Kv2.2 channels respectively after BPA and E2 treatment. Treatment of cells with BPA in the presence of the ERβ antagonist PHTPP abolished the effect on K+ currents. Electrical activity recordings showed an alteration of the action potential frequency and shape, probably as a consequence of the modification of the K+ current induced by DPN. The altered electrical activity should modify insulin release and suggest that ion channels are an important target of the natural hormone E2 and the endocrine disruptor BPA actions on the excitable pancreatic β-cell.

References:

1. Ignacio Babiloni-Chust, Reinaldo S Dos Santos, Regla M Medina-Gali, Atenea A Perez-Serna, José-Antonio Encinar, Juan Martinez-Pinna, Jan-Ake Gustafsson, Laura Marroqui, Angel Nadal. G protein-coupled estrogen receptor activation by bisphenol-A disrupts the protection from apoptosis conferred by the estrogen receptors ERα and ERβ in pancreatic beta cells. Environ Int, 2022. Jun:164:107250. doi: 10.1016

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NOXIOUS EFFECTS OF ACROLEIN MEDIATED BY DIRECT ACTIVATION OF TRPV1

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Acrolein is a toxic and highly reactive unsaturated aldehyde found in cigarette smoke and vehicle exhaust gases. Acrolein derived from cyclophosphamide is the major culprit of bladder neurogenic inflammation induced by chemotherapy in cancer and autoimmune disease patients. Although the chemosensory cation channel TRPA1 was initially described as the sole target of this compound, recent evidence suggests that TRPV1 may also play a role in acrolein-induced toxicity. Here we show that, acrolein induces bladder irritation and nocifensive responses in wild type and *Trpa1* knockout mice, but was ineffective in *Trpv1* and double *Trpv1*/*Trpa1* knockout mice. Ratiometric Ca^{2+} measurements and patch-clamp recordings in HEK293 cells overexpressing TRPV1 and in primary cultured mouse dorsal root ganglion neurons demonstrate that acrolein activates TRPV1. Unlike TRPA1, which displays quickly desensitizing responses, TRPV1 is activated by acrolein in a rather irriversible manner, with a time course showing an initial slow phase followed by an abrupt avalanche-like phase. Accordingly, we found the N-terminal amino acid residue C157 to be critical for acrolein-induced TRPV1 activation, indicating that the action of this compound is through covalent modification. Notably, single-channel recordings revealed that activation of TRPV1 by acrolein is enhanced by membrane depolarization, indicating for the first example of state-dependent action of an agonist on TRP channels. This type of interaction explains the avalanche-like action of acrolein on TRPV1 currents and suggest that channel sensitizers can dramatically amplify the action of this compound. Taken together, our results reveal a mechanism underlying the major role of TRPV1 as mediator for the acrolein-induced toxicity, unveiling TRPV1 as a potential therapeutic target in a wide spectrum of noxious conditions, such as exposure to smoke and cancer treatment.

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EFFICACY OF STIM1 AND STIM2 ACTIVATING CA^{2+} SIGNALS THROUGH ORAI1α AND ORAI1β

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Store-operated Ca^{2+} entry (SOCE) is orchestrated by the endoplasmic reticulum (ER)-resident proteins STIM1 and STIM2, which interact with and activate the CRAC channels in the plasma membrane, where Orai1 plays a predominant role. Two Orai1 variants have been identified in mammalian cells. The long form, Orai1α, comprises 301 amino acids, and the short form, Orai1β, originates from alternative translation initiation at either methionine 64 or 71. We have investigated the effectiveness of STIM1 and STIM2 in activating Ca^{2+} signals through Orai1α and Orai1β. By epifluorescence microscopy we have found that STIM1/2 double-knockout HEK-293 cells (DKO cells) lack thapsigargin-induced SOCE. Co-transfection with CMV-driven or thymidine kinase-driven Orai1α or Orai1β with STIM1 rescued SOCE in DKO cells. For Orai1α, STIM2 was less effective than STIM1, while the efficacy of STIM2 and STIM1 stimulating Orai1β was indistinguishable. Similar results were observed when Ca^{2+} signals were stimulated with carbachol. By confocal Förster Resonance Energy Transfer (FRET) and immunoprecipitation we examined the interaction of STIM1 and STIM2 with Orai1 variants in HEK293 cells. We found a direct coupling of STIM1 and STIM2 with Orai1α and Orai1β in resting cells, which was significantly enhanced upon stimulation with thapsigargin. In quiescent cells, the interaction between STIM2 and Orai1α is more pronounced compared to the other studied conditions, which might counteract minor leakage from the ER. These findings indicate that STIM2 is less effective inducing SOCE by Orai1α but exhibit a similar efficacy to STIM1 activating Orai1β. Interestingly, the significant interaction between STIM2 and Orai1α at rest suggests a major role for Orai1α in the maintenance of resting ER and cytosolic Ca^{2+} levels.

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ROLE OF ESYT1 IN STORE-OPERATED CALCIUM ENTRY AND VIABILITY OF LUMINAL BREAST CANCER CELLS

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Extended synaptotagmin 1 (Esyt1) belongs to the tethering ER-plasma membrane family of proteins, involved in lipid transport between both membranes [1]; however, Esyt1 presents a unique characteristic with respect to Esyt2 and 3, since it is able to complex Ca^{2+} [2]. Ca^{2+} interaction with the EF-hand domain of Esyt1 has been previously described to be crucial for SOCE activation in some cell types [3]; nonetheless, the role of Esyt1 in breast cancer cells remains unexplored, despite changes in the Ca^{2+} homeostasis controls several cancer hallmarks as previously describe by us and other [6]. Here we show that this protein is highly expressed in luminal breast cancer cells, like the MCF7 and T47D cell lines, as compared to the non-tumoral mammary epithelial MCF10A cell line. Esyt1 expression silencing impairs the activation of SOCE in T47D, while has not effect in MCF7 or MCF10A cells. Finally, the viability of T47D cells was reduced by silencing Esyt1 as denoted by the clearing of calcein stain, which was in parallel with a significant increase in propidium iodide accumulation. Summarizing, we conclude that Esyt1 has a relevant role in Ca^{2+} homeostasis in T47D luminal breast cancer cells, therefore, it may represent a promising target for developing new therapeutic strategies for the treatment of patients with luminal breast cancer.

References:

1. Yu H, et al. Proc Natl Acad Sci U S A. 2016 Apr 19;113(16):4362-7. doi: 10.1073/pnas.1517259113.
2. Kang F, et al. Sci Rep. 2019 Mar 8;9(1):3975. doi: 10.1038/s41598-019-40331-0.
3. Jardin I et al. Sci Rep. 2023 Nov 9;13(1):19471. doi: 10.1038/s41598-023-46946-8.

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MODULATING ROLE OF FILAMIN A 16-24 C-TERMINAL FRAGMENT IN STORE-OPERATED CA^{2+} ENTRY IN COLORECTAL ADENOCARCINOMA CELLS

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Store Operated Ca^{2+} Entry (SOCE) is a major mechanism for Ca^{2+} influx controlled by the intracellular Ca^{2+} stores. The stromal interaction molecule (STIM) protein family, with a predominant role of STIM1, activate Ca^{2+} channels located in the plasma membrane upon store depletion and allow Ca^{2+} entry through CRAC channels, mainly Orai1. Filamin A (FLNA), an actin-binding protein located in the cytoskeletal region of the membrane, has been demonstrated to play an essential role as a negative regulator of SOCE since it modulates Orai1-STIM1 interaction. We first found that FLNA protein is cleaved by calpain in HT-29 colorectal adenocarcinoma cell line, yielding a fragment containing repeats 16-24 ($FLNA^{16-24}$). HT-29 cells exhibit an increased SOCE and Orai1 and STIM1 expression as compared to non-tumoral normal mucosal cells (Sobradillo et al., 2010). Calpain inhibition using calpeptin significantly attenuated FLNA cleaving, which resulted in a decrease of SOCE and Orai1 and STIM1 protein expression. To further demonstrate the role of $FLNA^{16-24}$ as a regulator of SOCE, we expressed in HEK-293 cells dsRED-tagged $FLNA^{16-24}$ and analysed the expression of the key SOCE proteins. Expression of dsRED-tagged $FLNA^{16-24}$ was confirmed by Western blotting and confocal microscopy. Expression of $FLNA^{16-24}$ in HEK-293 cells increased Orai1 and STIM1 protein level without having any effect on the expression of Orai3 and STIM2. As a result, SOCE was enhanced in $FLNA^{16-24}$-expressing HEK-293 cells as compared to mock-treated controls. Summarizing, our results provide evidence for a role for $FLNA^{16-24}$ fragment in the regulation of Orai1 and STIM1 expression and, therefore, in SOCE in colorectal adenocarcinoma cells.

References:

1. Sobradillo, D., Hernández-Morales, M., Ubierna, D., Moyer, M. P., Núñez, L., & Villalobos, C. (2014). J. Biol. Chem, 289(42), 28765–28782.

***Acknowledgements**: Supported by PID2022-136279NB-C21 and PID2022136279NB-C22 MCIN/AEI/10.13039/501100011033 and "ERDF A way of making Europe", and Junta de Extremadura-FEDER (IB20007 and GR18061).*

EXPLORING LOSS OF FUNCTION GENETIC VARIANTS OF HTRPM8 AS A PERSONALIZED TREATMENT FOR HYPERTENSION

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Arterial hypertension (HT) is the single major preventable cause of cardiovascular disease and all-cause mortality. A large number of candidate genes involved in HT traits, including many ion channel genes, have been proposed. We have performed an exome-wide association analysis in two large cohorts to test the association of rare (MAF <1%) and coding variants (with predicted loss-of-function, LoF) with hypertension. We found two LoF variants in TRPM8 associated with increased diastolic blood pressure (R247T and Y251C), and with major adverse cardiovascular event (G921V), present in both cohorts. TRPM8 is a nonselective ion channel activated by diverse stimuli including cold and menthol, which has been implicated in blood pressure regulation in both human and animal models of HT[1-3]. Here we characterize the functional properties of these newly identified LoF variants to determine if they affect TRPM8 channel properties, thus contributing to HT phenotype.

TRPM8 mutations were introduced in the hTRPM8 by PCR-directed mutagenesis. Whole-cell currents from WT or mutant channels expressed in HEK cells were recorded using patch-clamp techniques. $[Ca^{2+}]_i$ was measured with Fluo-4 microfluorometry.

Application of cold (15-18 ºC) of menthol (100µM) activated TRPM8-mediated currents that were significantly smaller in the case of the G921V variant. Also, the increase of $[Ca^{2+}]_i$ upon exposer to the agonists was reduced, confirming *in silico* prediction of G921V as a LoF TRPM8 variant.

Regarding R247T and Y251C, we found larger menthol-activated outward currents compared to TRPM8-WT, together with different desensitization kinetics. Also, R247T TRPM8-mutant exhibited larger unstimulated outward currents However, menthol-induced $[Ca^{2+}]_i$ increase was augmented only in Y251C variant. Contrary to the prediction, both variants represent TRPM8 channels with gain-of-function, albeit with different modulation. The functional characterization of these common variants will help to improve knowledge of HT molecular mechanisms, individual risk stratification and prediction of response to more targeted therapies.

References:

1. Huang et al., 2017. Molecular Medicine Reports. 2017;15(4):1900-1908. doi:10.3892/mmr.2017.6158
2. Zhu et al., 2020. A Randomized, Double-Blind, Placebo Control Trial Comparing Effects and Safety of DANSHU Capsule(Menthol) and Placebo on Blood Pressure and Metabolic Parameters in Prehypertensive and Mild Hypertensive Patients. clinicaltrials.gov; 2020. Accessed March 7, 2023. https://clinicaltrials.gov/ct2/show/NCT01408446.
3. Sun et al., 2014. Hypertension. 2014;63(6):1354-1363. doi:10.1161/HYPERTENSIONAHA.113.02573

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MUTATIONS ASSOCIATED WITH PROPIONIC ACIDEMIA PRODUCE DIRECT ELECTROPHYSIOLOGICAL ALTERATIONS IN HUMAN CARDIOMYOCYTES

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Propionic acidemia (PA) is a metabolic disorder caused by the deficiency of the mitochondrial enzyme propionyl-CoA carboxylase (PCC) and due to mutations in the *PCCA* or *PCCB* genes that encode the two PCC subunits. PA has been associated to some alterations of cardiac electrical activity, including prolongation of the QT interval of the electrocardiogram, life-threatening arrhythmias and sudden cardiac death, although the underlying mechanism is unknown. We analyzed whether the presence of the mutations itself can produce direct cardiac electrophysiological alterations. Using whole-cell patch-clamp we recorded action potentials (APs) and ion currents in ventricular-like induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) obtained from a PA patient carrying two highly pathogenic mutations in the *PCCA* gene [p.Cys616_Val633del and p.Gly477Glufs*9) (PCCA cells) and in hiPSC-CMs from a healthy subject (Healthy cells). In cells driven at 1 Hz, the mutation delayed AP triggering and prolonged the AP duration measured at 20% of repolarization (APD_{20}= 218±37 vs. 120±20 ms; P<0.05), without modifying APD_{50}, APD_{90}, resting membrane potential or AP amplitude. Furthermore, delayed afterdepolarizations appeared at the end of the repolarization phase only in PCCA cells. We next recorded the L-type calcium (I_{CaL}), the Na-Ca exchanger (I_{NCX}), and sodium (I_{Na}) currents in PCCA and healthy cells. The mutation significantly reduced I_{CaL} density (-6.8±1.8 vs -19.3±5.0 pA/pF at 0 mV) and increased the I_{NCX} density measured as the Ni^{2+}-sensitive current (-19.7±2.3 vs -10.9±0.4 pA/pF at -80 mV). Moreover, it significantly reduced peak I_{Na} by 46%, but increased the late I_{Na} measured as the tetrodotoxin-sensitive current (0.65±0.1 vs 0.26±0.06% of peak I_{Na}). Some PA-associated mutations can severely affect the ionic mechanisms controlling cardiac APD and cytosolic Ca^{2+} handling, increasing the risk of arrhythmias independently of the organ damage induced by the systemic acidemia.

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K_V1.3 CHANNEL CONTRIBUTION TO THE OSTEOGENIC DEDIFFERENTIATION OF VASCULAR SMOOTH MUSCLE CELLS IN UREMIA-INDUCED VASCULAR CALCIFICATION

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Vascular calcification (VC) is the result of the pathological deposition of mineral hydroxyapatite on the arterial wall, which associates to increased risk of heart disease, stroke and atherosclerotic plaque rupture1. Phenotypic modulation (PM) of vascular smooth muscle cells (VSMCs) to an osteochondrogenic phenotype is the main factor leading to arterial medial calcification (AMC). AMC is an early event during chronic kidney disease (CKD), triggered by uremia and alterations in mineral metabolism. AMC is preceded by increased arterial stiffness due to elastin degradation. We have previously reported that uremic serum (US) induces Kv1.3 channel upregulation in cultured human VSMCs, which ca be partially reverted by Kv1.3 inhibition2. Here we explore the contribution of Kv1.3 to PM to AMC in aortas from a CKD model both in vivo and ex vivo, in organ culture.

Aortas were obtained from C57-Sham (control) and C57-CKD mice induced by 5/6 nephrectomy. Aortas were either analyzed upon dissection (t=0) or after 10 days incubation in MEM supplemented with control, high phosphate (HP) or US, with or without a Kv1.3 blocker (100nM PAP-1). VC was explored using qPCRs and histological techniques as well as with myography to analyze arterial stiffness.

We found an increased stiffness in vivo in the CKD model, which was reproduced in organ culture with US. PAP-1 application could prevent US-induced stiffness but cannot revert it once established, as in the in vivo CKD model. The histological analysis did not show macroscopic calcification, but we found medial elastin degradation, which associated with an increased expression of MMP2 elastase by qPCR. Altogether, our data suggest that changes in vessel stiffness in the our CKD model could be reflecting early stages of the process of AMC, supporting an important role of Kv1.3 channels at that initial step of VSMCs dedifferentiation towards an osteogenic phenotype.

References:

1. Durham, AL, et al. Cardiovasc Res 114, 590–600 (2018)
2. Cazaña-Pérez, V et al. FUNCTION, 2, (2021)

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ROLE OF MACROPHAGES K_V1.3 CHANNELS IN A MURINE MODEL OF METABOLIC SYNDROME AND T2DM

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Cardiovascular diseases are the leading causes of mortality worldwide. Type 2 diabetes (T2DM) increases the prevalence of vascular disease, with more aggressive forms of disease and worse outcomes. Under T2DM chronic inflammatory signalling in the vasculature sustains endothelial dysfunction, leukocyte infiltration, and a pro-thrombotic environment. This low-grade metabolic inflammation appears to be triggered by the recruitment and activation of macrophages, which exhibit a metabolic-disease-specific phenotype (MMe) different from the classical activated M1 phenotype1.

We have previously found that Kv1.3 blockers inhibit intimal hyperplasia, preventing restenosis. Moreover, systemic application of Kv1.3 blockers also ameliorates metabolic dysfunction in a T2DM model2, independently of their effects on vascular smooth muscle. Because of the importance of the macrophage in the pathogenesis of atherosclerosis in diabetes, we explore if changes in Kv1.3 channels have a functional role in the development of MMe macrophages, contributing to increasing risk of vascular complications in T2DM.

Hypertensive (BPH) mice were fed on a high fat diet (HFD) for 12 weeks to generate a T2DM model. We used bone marrow derived macrophages (BMDM) isolated from male and female control or HFD mice, differentiated to M0 or M1 phenotypes. We obtained outward potassium currents using whole-cell electrophysiology and mRNA levels of macrophages markers (TNFa, iNOs, CD36) and several K channels (Kv1.3, Kir2.1, KCa3.1). Female HFD BMDM showed a higher expression of CD36 (a MMe marker1), in M0 and decreased Kv1.3 expression. In contrast, Kv1.3 mRNA and Kv1.3-mediated currents were significantly increased after LPS stimulation in HFD compared to control. Functional studies showed that Kv1.3 did not contribute to BMDM phagocytosis, but was relevant in cellular respiration and cell migration rate. Altogether, we found gender differences in Kv1.3 functional expression in BMDM, suggesting that MMe Kv1.3 channels could represent a potential target to reduce T2DM-associated vascular complications in females.

References:

1. Kratz M. et al., cell Metabolism, 2014.
2. Arévalo-Martínez M. et al., Molecular Metabolism, 2021.

Acknowledgements: Supported by grants PID2020-118517RB-I00 (MINECO) and VA172P20 (JCyL) and by predoctoral contracts (SME) of the Junta de Castilla y León. DAPP is a Juan de la Cierva postdoctoral.

LGI3 AND LGI4 ELECTROPHYSIOLOGICAL EFFECTS ON K_V4.3 AND K_V4.2 POTASSIUM CHANNELS

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K_V4.2 and K_V4.3 voltage-dependent potassium channels generate the transient potassium outward current (I_{to}), which determines phase 1 repolarization in cardiac action potentials. The characteristics of this current are only observed when these channels interact with auxiliary subunits that develop a modulating function on their activity, forming signaling complexes or *channelosomes.* Abnormal changes in the expression levels of the *channelosome* components and/or in their biophysical properties are related to the development of cardiac pathologies such as atrial fibrillation (AF), the most common type of arrhythmia. Lgi1 and Lgi3, members of the *leucine-rich glioma inactivated* protein family, have proven to modulate the activity of K_V1 and K_V4 channels in neurons, modifying their trafficking, stability, and/or biophysical properties. We have demonstrated that Lgi3 and Lgi4 are the only members of this protein family present in the human myocardium. Consequently, we are interested in unraveling the electrophysiological effects that Lgi3 and Lgi4 induce on the activity of K_V4.2 and K_V4.3 channels. For this purpose, potassium currents were recorded in CHO cells transiently transfected with cDNA encoding the channel and the auxiliary protein of interest, using the whole-cell configuration of the patch-clamp technique. Our results show that Lgi3 and Lgi4 induce important changes in the electrophysiological properties of Kv4.3, while their effects on K_V4.2 channels are not biologically significant. Specifically, the magnitude of the K_V4.3 current in the presence of Lgi3 and Lgi4 is, respectively, 3- and 2-fold greater than that observed in the absence of these proteins. Also, Lgi3 slows down the activation and the inactivation kinetics of K_V4.3; whereas Lgi4 accelerates the recovery kinetics of this channel. Thus, the increase in the magnitude of the K_V4.3 current produced by Lgi3-4 could be explained by: i) the delay in the channel inactivation for Lgi3 and ii) the acceleration of the recovery kinetics for Lgi4. From this study, we establish Lgi3 and Lgi4 as relevant modulators of K_V4.3 channels to be considered in the study of cardiac electrophysiology.

References:

1. Niwa, N. and Nerbonne, J.M. 2010. Molecular determinants of cardiac transient outward potassium current (I(to)) expression and regulation. J Mol Cell Cardiol, 48(1):12-25.
2. Schulte, U., Thumfart, J., Klöcker, N., Sailer, C.A., Bildl, W. et al. 2006. The epilepsy-linked Lgi1 protein assembles into presynaptic Kv1 channels and inhibits inactivation by Kvβ1. Neuron, 49(5):697-706.
3. Kozar-Gillan, N., Velichkova, A., Kanatouris, G., Eshed-Eisenbach, Y., Steel, G. et al. 2023. LGI3/2-ADAM23 interactions cluster Kv1 channels in myelinated axons to regulate refractory period. J Cell Biol, 222(4):e202211031.

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SPATIAL ARRANGEMENT OF THE K_V1.3 CHANNEL AT THE IMMUNOLOGICAL SYNAPSE

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The voltage-gated potassium channel Kv1.3 participates in glucose homeostasis. In this sense, channel inhibition increases insulin sensitivity and glucose uptake playing a role in obesity and type 2 diabetes. Moreover, because of the cross talk between the immune system and some metabolic disorders, Kv1.3 is associated to the onset of metabolic diseases, including autoimmune type 1 diabetes, ulcerative colitis, and Crohn disease. In this context, T-effector memory (T_{EM}) lymphocytes exhibit an exacerbated Kv1.3 expression and activation, and selective channel inhibitors alleviate symptoms. Therefore, proper T cell functions require a controlled Kv1.3 activity, which is regulated by the timing and distribution of the channel at the immunological synapse. In this work, we describe for the first time the heterogeneous arrangement and behavior of Kv1.3 at the synaptic platform. The channel is recruited at early stages of the immunological synapse formation, accumulating at the central and distal supramolecular activation complexes (cSMAC and dSMAC). Interestingly, Kv1.3 accumulation at the dSMAC is potentiated by the activation of the CD2/CD58 axis, which ensures efficient T cell activation and sustained Ca^{2+} signaling. Moreover, Kv1.3 displays different dynamics depending on its localization, moving faster and travelling longer distances at the dSMAC. The decreased mobility at the cSMAC facilitates endocytic and exocytic processes. Hence, once Kv1.3 function is terminated, the channel is further recruited to the cSMAC to be either endocytosed in a clathrin-dependent manner or released in extracellular vesicles. Either mechanism (internalization or release) serves for downregulating Kv1.3 and thus, terminate Ca^{2+} signaling fine-tuning T cell activation. These results shed light on the regulation of Kv1.3 in the immunological synapse, whose distribution, timing, and activation determine the degree of T cell activation triggering autoimmune diseases, such as type I diabetes. Therefore, spatial distribution of the leukocytic Kv1.3 arises as a new therapeutic strategy for treating metabolic disorders.

References:

1. Capera J, Jainarayanan A, Navarro-Pérez M, Valvo S, Demetriou P, Depoil D, Estadella I, Kvalvaag A, Felce JH, Felipe A, Dustin ML. Dynamics and spatial organization of Kv1.3 at the immunological synapse of human CD4+ T cells. Biophys J. 2023 Aug 18:S0006-3495(23)00511-8. doi: 10.1016/j.bpj.2023.08.011. Epub ahead of print. PMID: 37596785.

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RYR2_P.G357S MUTATION ASSOCIATED TO CPVT INDUCED A GAIN OF FUNCTION IN PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES (IPSC-CM)

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an arrhythmogenic disease characterized by adrenergically-induced ventricular tachycardia associated to sudden cardiac death. We studied a CPVT mutation in type-2 ryanodine receptor (*RYR2*), RYR2_p.G357S, found in a large family from the Canary Islands.[1] We obtained skin biopsies from heterozygous and homozygous donors, and iPSC-CM were subsequently derived from those samples.

The objective of this study was to assess the functional impact of this mutation on native RYR2, and to investigate whether the number of mutated alleles can have an additive effect. We generated iPSC-CM using a heparin-based differentiation protocol. Cells derived from three heterozygous and two homozygous patients, and from one non-carrier healthy donor used as control. Calcium imaging techniques were employed to assess RYR2 caffeine sensitivity under basal conditions and β-adrenergic stress induced by 100 nM isoproterenol (ISO).

Calcium transients elicited by 1s-caffeine pulses of increasing concentrations were analysed to construct concentration-response curves. An increase in caffeine sensitivity was observed in all CPVT iPSC-CMs compared to control, suggesting that the mutation promotes a gain of function in basal conditions. RNA and RYR2 protein levels were similar between the CPVT iPSC-CM CPVT and the control. Thus, the heightened RYR2 activity could not be attributed to changes in expression levels. Upon β-adrenergic stimulation, no further increase in caffeine sensitivity was observed in CPVT iPSC-CMs. Caffeine concentration-response curves obtained from iPSC-CM from two heterozygous and one homozygous individuals were not modified by the presence of ISO. Moreover, in the two remaining iPSC-CM cell lines (one from a heterozygous and one from a homozygous individual) the caffeine sensitivity was even reduced.

Collectively, our results suggest that this familial mutation produces a RYR2 gain of function in basal conditions and an aberrant response to β-adrenergic stimulation. qPCR experiments demonstrated that the response to ISO observed in CPVT iPSC-CMs was not caused by differential expression of β-adrenergic receptors. We propose that this abnormal response may underlie the fatal arrhythmic events observed in this family. Finally, no additive effects of the mutation were observed in homozygous iPSC-CM. Thus one mutated RYR2_p.G357S allele is enough to produce a gain of function.

References:

1. Wangüemert, F., Bosch Calero, C., Pérez, C., Campuzano, O., Beltran-Alvarez, P., Scornik, F. S., Iglesias, A., Berne, P., Allegue, C., Ruiz Hernandez, P. M., Brugada, J., Pérez, G. J., & Brugada, R. (2015). Clinical and molecular characterization of a cardiac ryanodine receptor founder mutation causing catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm, 12(7), 1636–1643. https://doi.org/10.1016/J.HRTHM.2015.03.033.

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PIEZO1 AND PIEZO2 BLADE DOMAINS DIFFERENTLY IMPACT CHANNEL FUNCTION

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PIEZO1 and PIEZO2 are the key mechano-gated ion channels for transducing internal and external mechanical stimuli into ionic currents in many eukaryotic organisms. CryoEM and structure-functional studies have revealed that three PIEZO monomers assemble as a 3-blade propeller highlighting essential structural elements for channel function. It contains a central permeation pore surrounded by 3 blade-like domains. The Blade domain is organized in 9 Transmembrane Helix Units (THU) comprising 36 transmembrane (TM) fragments, 4 TMs each THU. Despite its suggested role in force transduction, the contribution of the Blade domain in channel physiology remains unclear. To explore the actual role of each Blade's section in channel function. We have generated a set of PIEZO mutants by sequentially removing one THU each time, starting from the Distal blade and going inward. We then characterize the channels' electrical responses by Poking-membrane displacement (Whole-Cell) and Stretch-negative pressure (Single-Channel). Strikingly, our study indicates that the PIEZO2 Distal Blade (THU1-3) is completely dispensable for proper channel function. However, it is essential for PIEZO1's normal functioning. Suggesting that there are minor structural differences that condition each PIEZO channel function.

References:

1. Coste, B. et al. Piezo1 and Piezo2 Are Essential Components of Distinct Mechanically Activated Cation Channels. Science 330, 55–60 (2010).
2. Murthy, S. E., Dubin, A. E. & Patapoutian, A. Piezos thrive under pressure: mechanically activated ion channels in health and disease. Nat. Rev. Mol. Cell Biol. 18, 771–783 (2017).
3. Guo, Y. R. & MacKinnon, R. Structure-based membrane dome mechanism for Piezo mechanosensitivity. eLife 6, (2017).
4. Zhao, Q. et al. Structure and mechanogating mechanism of the Piezo1 channel. Nature 554, 487–492 (2018).
5. Saotome, K. et al. Structure of the mechanically activated ion channel Piezo1. Nature 554, 481–486 (2018).
6. Wang, L. et al. Structure and mechanogating of the mammalian tactile channel PIEZO2. Nature 573, 225–229 (2019).
7. Taberner, F. J. et al. Structure-guided examination of the mechanogating mechanism of PIEZO2. Proc. Natl. Acad. Sci. 201905985 (2019)
8. Verkest, C. et al. Intrinsically disordered intracellular domains control key features of the mechanically-gated ion channel PIEZO2. Nat. Commun. 13, 1365 (2022).

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TRESK POTASSIUM CHANNEL MODULATES CA3 PYRAMIDAL NEURONS' EXCITABILITY AND HIPPOCAMPAL SYNAPTIC PLASTICITY

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To comprehend the brain's dynamic physiology, we must understand the mechanisms underlying neuronal excitability, crucial for neurons' electrical activation during synaptic communication. Of particular interest is controlled potassium current leakage via two-pore domain potassium (K2P) channels which finely modulate the intrinsic excitability of neurons. TRESK, the latest discovered channel of the K2P family, has a well-characterized pivotal role in nociception. Intriguingly, it also exhibits widespread expression throughout central nervous system1, yet its function within the brain has remained unexplored. We are combining in-situ hybridization RNAscope technique and electrophysiology experiments to examine the contribution of TRESK to hippocampal excitability. We observed TRESK mRNA in excitatory and inhibitory neurons along the CA1-CA3 regions and dentate gyrus. Functionally, field potential and whole-cell patch-clamp recordings in acute slices revealed that TRESK knockout mice exhibit decreased paired-pulse facilitation and impaired long-term synaptic plasticity in the Schaffer Collateral pathway. Additionally, in the absence of TRESK, CA3 pyramidal neurons displayed enhanced excitability via reduced rheobase current and a tendency for a more depolarized resting membrane potential. Accordingly, there was an increased number of cells that were spontaneously active and they had higher firing frequencies compared to control slices. We are currently exploring the role of TRESK in GABAergic cells. Our findings highlight the involvement of TRESK in hippocampal excitability and synaptic plasticity. These data serve as a foundation for future experiments to elucidate the role of TRESK channels in hippocampal intrinsic plasticity, which will provide insights into principles governing the dynamics of neural networks in memory formation.

References:

1. Yoo, S., Liu, J., Sabbadini, M., Au, P., Xie, G. X., & Yost, C. S. (2009). Regional expression of the anesthetic-activated potassium channel TRESK in the rat nervous system. Neuroscience letters, 465(1), 79–84. https://doi.org/10.1016/j.neulet.2009.08.062

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FUNCTIONAL CA^{2+}-PERMEABLE AMPA RECEPTORS MEDIATE HIPPOCAMPAL ASTROCYTIC CALCIUM SIGNALING

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Calcium signaling is key to understanding the active involvement of astrocytes in the nervous system[1]. Over the past few decades, emphasis has been placed on the role that ion channels play in this phenomenon. In this regard, AMPA receptors (AMPARs) play an important role in glutamatergic neurotransmission, being essential in learning and memory processes. However, they are also present in glia and, while the presence of GRIA genes in hippocampal astrocytes is well established[2], the functional expression of AMPARs in these astrocytes remains a subject of debate. In this work, primary astrocytic cultures were prepared from hippocampal tissue and their purity was confirmed using specific astrocytic markers. Calcium-imaging experiments were performed by loading astrocytes with fura-2, a calcium-sensitive dye, to measure intracellular calcium changes upon astrocyte stimulation. Western blotting experiments were performed to confirm the protein expression of AMPAR subunits, and patch-clamp recordings were conducted in whole-cell configuration to investigate AMPAR functionality. Our results demonstrated significant changes in intracellular calcium levels upon stimulation with the selective agonist AMPA, providing evidence for the functional presence of AMPARs in hippocampal astrocytes. Western blot analysis confirmed the expression of GluA1, GluA2, and GluA4 subunits of AMPARs in hippocampal astrocytes while patch-clamp recordings revealed distinct subpopulations with different kinetics and steady-state current, further supporting the presence of diverse functional AMPARs. Additionally, we evidenced that calcium permeable AMPARs are present eliciting fast calcium signaling and we also observed that AMPA stimulation is capable to lead a cross-talking between astrocytes involving ATP release that augment the calcium response. In conclusion, our findings contribute to the ongoing discussion regarding the functional expression of AMPARs, establishing their presence and role in calcium signaling in vitro. Further investigation is warranted to fully understand the contributions of hippocampal astrocytic AMPARs to glutamatergic neurotransmission specially taking into consideration other crucial proteins in AMPAR function such as auxiliary AMPAR subunits.

References:

1. Bazargani N, Attwell D. Astrocyte calcium signaling: the third wave. Nat Neurosci. 2016 Feb;19(2):182-9. doi: 10.1038/nn.4201. PMID: 26814587.
2. Mölders A, Koch A, Menke R, Klöcker N. Heterogeneity of the astrocytic AMPA-receptor transcriptome. Glia. 2018 Dec;66(12):2604-2616. doi: 10.1002/glia.23514. Epub 2018 Oct 28. PMID: 30370555.

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TRANSCRIPTOMIC ANALYSIS OF CALCIUM CHANNEL REMODELING OF HUMAN, PAIRED COLON PRIMARY TUMORS AND META-ANALYSIS

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We have shown that cultured human colon cancer cell lines show dramatic changes in intracellular Ca2+ homeostasis (Ca2+ remodeling) relative to normal human colon mucosa cells that may contribute largely to colon cancer hallmarks. Specifically, cancer cells show enhanced store-operated Ca2+ entry (SOCE) and decreased intracellular Ca2+ store content that may lead to enhanced cell proliferation and resistance to cell death. Transcriptomic analysis shows differential expression of a large number of genes involved in intracellular Ca2+ transport. However, cell lines studied are not from the same genetic background and differences could be due to reasons other than colon cancer. Concretely, we have used microarrays technology to carry out a comprehensive transcriptomic analysis of 20 paired samples of human colon cancers obtained from a public tumor bank repository. In addition, we have also carried out a meta-analysis with similar public data set from five independent studies. Our results indicate that a few molecular players involved in intracellular Ca2+ homeostasis are differentially expressed in colon cancer samples relative to paired normal colonic tissue from the same patients. We found that 6 molecular players involved in SOCE are differentially expressed. Specifically, colon cancer samples overexpressed ORMDL3, septins 8 and 11 and underexpressed MS4A12 and septins 1 and 6. For transient receptor potential (TRP) channels, colon cancer samples overexpressed only TRPV1 and underexpressed TRPM2 and TRPP5. Regarding Ca2+ release channels at the endoplasmic reticulum, colon cancer samples also overexpressed IP3R3 whereas IP3R1 channels were and downregulated. Regarding Ca2+ extrusion systems, colon cancer samples overexpressed SERCA2, SPCA1 and 2. Finally, regarding mitochondrial Ca2+ transporters, 7 out of all 11 molecular players were upregulated in colon cancer samples including MCU, MICU1, MICU2, MCUR1, NCLX, VDAC1 and 3. Accordingly, our data show that colon cancer samples show overexpression of 15 genes and downregulation of only 6 genes involved in Ca2+ transport. These results suggest that calcium remodeling in paired colon cancer samples may differ from changes previously reported in cell lines. A further meta-analysis carried out show similarities as well as differences with differential expression data obtained in both cell lines and the paired data shown here.

References:

1. Pérez-Riesgo, E., Gutiérrez, L. G., Ubierna, D., Acedo, A., Moyer, M. P., Núñez, L., & Villalobos, C. (2017). Transcriptomic Analysis of Calcium Remodeling in Colorectal Cancer. International journal of molecular sciences, 18(5), 922. https://doi.org/10.3390/ijms18050922

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A THEORETICAL FRAMEWORK FOR POLYMODAL GATING OF SENSORY TRP CHANNELS

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Various transient receptor potential (TRP) channels are notorious for their proposed roles as sensors of chemical, thermal and mechanical stimuli in multiple cell types. While the roles of TRP channels as mechanotransducers in physiological conditions remain debated, it has been proposed that most of them can be activated by mechanical stimulation (Liu and Montell, BBRC, 2015, 460(1):22-5; Startek et al., IJMS, 2019, 20(2):371). Thus, the main objective of this study was to provide a theoretical framework serving to assess the determinants of TRP channel mechanosensitivity. We hypothesized that the mechanically-induced activation is boosted by the weak voltage dependence of TRP channels, as previously proposed for thermal stimuli and chemical agonists. To test this hypothesis, we examined the properties of an existing close-open gating model, which we extended to consider the influence of an external mechanical stimulus, in addition to the known contribution of temperature and the transmembrane membrane potential. The model predicts that the thermal and mechanical responses are determined by the balance between an energy component associated to the protein volume and another associated to the protein-membrane surface, in such a way that the channel volume, the corresponding membrane interface area, their ratio, and their mechanical rigidities emerged as morpho-mechanical determinants along with the gating valence. The model supports the hypothesis linking the mechanical- and voltage-dependent gating by predicting inverse relationships between the mechanical activation and the gating valence. In addition, it serves to explain how TRP channels can act as secondary mechanosensors by being stimulated by second messengers generated upon mechanical activation of signaling pathways. We speculate that, natural selection of ionic channels with low gating change yielded better sensors of thermal, chemical and mechanical stimuli. On the other hand, it may be possible that a TRP channel functioning primarily as thermo- or chemo-sensor is also found to the mechanosensitive in special experimental conditions, by the reason of having a low gating charge, without necessarily having a physiological role as mechanotransducer.

References:

1. Eijkelkamp, N., K. Quick, and J.N. Wood. 2013. Transient receptor potential channels and mechanosensation.Annu Rev Neurosci. 36:519-546.
2. Liu, C., and C. Montell. 2015. Forcing open TRP channels: Mechanical gating as a unifying activation mechanism. Biochem Biophys Res Commun. 460:22-25.
3. Surface Tension, Surface Energy, and Chemical Potential Due to Their Difference. C.-Y. Hui† and A. Jagota. Langmuir 2013, 29, 36, 11310–11316.
4. Surface Energy and Surface Tension in Solids and Liquids. E. Orowan. Proceedings of the Royal Society of London. Series A, Mathematical and Physical Sciences , May 12, 1970, Vol. 316, No. 1527 (May 12, 1970), pp. 473-491.
5. Peripheral thermosensation in mammals. Vriens, J., B. Nilius, and T. Voets. 2014. Nat Rev Neurosci. 15:573-589.
6. Temperature Sensation: From Molecular Thermosensors to Neural Circuits and Coding Principles. Xiao, R., and X.Z.S. Xu. 2021. Annu Rev Physiol. 83:205-230.

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TGFβ SIGNALING AND THE METABOLIC CHALLENGE IN GESTATIONAL DIABETES MELLITUS

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Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. It is the most common metabolic disorder of pregnancy[1]. The prevalence of GDM has experienced a marked increment in the last two decades due primarily to an increase in obesity at childbearing age. GDM develops when pancreatic β-cells fail to overcome maternal insulin resistance, a physiological condition that develops to maintain the supply of foetal energy throughout gestation. Potential causes of inadequate β cell function in GDM are myriad and not fully described.

The aim of the study was to explore the role of TGFβ signalling pathway during pregnancy and in the etiology of GDM. We also aim to evaluate the cross-talk regulation with the hormonal pregnancy-environment which is key for the normal functioning of pancreatic β-cell and its adaptive response. To this end we have established an animal model based on nutritional manipulation which resembles the pathology of GDM. Appropriate non-pregnant control animals have been included in the study.

We have characterized the metabolic changes including glucose homeostasis, insulin sensitivity as well as the functional adaptations at the endocrine pancreas level which take place during pregnancy in control and GDM mice. We found that GDM mice developed severe glucose intolerance, aggravated insulin resistance as well as impaired pancreatic β-cell function and α-cell response to glucose, hallmark features of GDM. Furthermore, ex vivo, we observed that the islets from GDM mice exhibited decreased glucose-stimulated insulin secretion and content and altered expression of certain genes which are known to be important for β-cell function and identity such as insulin, Mafa or Hnf4α among others. As a correlate of function, calcium signalling and electrical activity including voltage-dependent calcium currents (VDCCs) and voltage dependent potassium currents were also characterized. In addition, we have identified a number of key elements of the TGFβ signalling pathway which are differently regulated in the pancreatic β-cells in GDM compared to normoglycemic pregnant mice.

The results obtained may provide new insights into the pathophysiological mechanisms involved in the etiology of GDM.

References:

1. Buchanan, T. A., Xiang, A. H. & Page, K. A. Gestational diabetes mellitus: risks and management during and after pregnancy. Nature reviews. Endocrinology 8, 639-649, doi:10.1038/nrendo.2012.96 (2012).

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THE SARS-COV-2 ENVELOPE PROTEIN CHANNEL: EXPLORING ITS ION SELECTIVITY AND OLIGOMERIZATION-DEPENDENT ACTIVITY

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The envelope (E) protein of SARS coronavirus 2 (SARS-CoV-2), the pathogen that caused the recent COVID-19 pandemic, and its predecessor SARS-CoV-1, are found in the endoplasmic reticulum–Golgi intermediate compartment (ERGIC), where virion budding takes place, and are considered viroporins. It has been claimed that E protein promotes the formation of “cation-selective channels”. However, whether this term represents specificity to certain ions (e.g., potassium or calcium) or the partial or total exclusion of anions is a matter of controversy. Herein, we discuss this claim based on the available data for SARS-CoV-1 and -2 E and on new experiments performed using the untagged full-length E protein from SARS-CoV-2 in planar lipid membranes of different types, including those that closely mimic the ERGIC membrane composition. Our experimental evidence shows that the selectivity of E-induced channels is very mild and depends strongly on lipid environment. Thus, despite past and recent claims, we found no evidence that the E protein forms cation-selective channels impermeable to anions, and even less that E protein forms bona fide specific calcium channels. In fact, the E channel maintains its multi-ionic non-specific neutral character even in concentrated solutions of Ca^{2+} ions. Also, in contrast to previous studies, we found no indication that SARS-CoV-2 E channel activation requires a particular voltage, high calcium concentrations or low pH, in agreement with previous data from SARS-CoV-1 E pointing to a mildly-selective proteolipidic channel. The available atomic resolution channel 3D structures cast doubts about the E protein oligomerization state in the membrane and the involvement of lipids in the actual channel conformation. We run sedimentation velocity experiments suggesting that the E channel population is mostly pentameric, but very dynamic and probably heterogeneous, consistent with the broad distribution of conductance values typically found in functional experiments. We also performed additional electrophysiology measurements using E-enriched liposomes at different protein-lipid ratios to investigate whether peptide oligomerization affects E channel activity and modulates its conductance and selectivity. Our results are promising in the aim of reconciling the apparent inconsistency between available structural and functional data regarding SARS-CoV-2 E channel activity.

References:

1. W. Surya, E. Tavares-Neto, A. Sanchis, M. Queralt-Martín, A. Alcaraz, J. Torres, V. M. Aguilella. The complex proteolipidic behavior of the SARS-CoV-2 envelope protein channel: weak selectivity and heterogeneous oligomerization. Int. J. Mol. Sci. 24 (2023) 12454.

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ROLE OF SCN5A-SCN10A HAPLOTYPE COMPOSITION IN BRUGADA SYNDROME

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Brugada syndrome (BrS) is a rare inherited cardiac disorder characterized by ventricular arrhythmias and a high risk of sudden death. It often displays with a family history and arrhythmogenic symptoms typically emerging in the fourth decade of life. It is 8-10 times more prevalent in men than women, with varying rates depending on the geographical region. Historically, BrS has been considered a monogenic disease, being SCN5A the main gene associated to BrS. SCN5A encodes the α subunit of the major cardiac voltage-gated sodium channel isoform $Na_V1.5$. About 25% of BrS cases carry deleterious single nucleotide variants (SNVs) in SCN5A. Another gene associated with BrS is SCN10A, which encodes the α subunit of neuronal sodium channel $Na_V1.8$. Altogether, variants in cardiac ion channel genes account for 30-35% of the cases, while for the rest (65-70%) the etiology remains unknown. Multiple studies suggest a complex inheritance pattern and heterogeneous genetic basis, warranting investigation into molecular mechanisms affecting cardiac sodium currents. Our studies have demonstrated that the composition of a haplotype comprising 7-SNVs in the SCN5A-SCN10A locus is associated with different susceptibilities to BrS (Pinsach-Abuin et al. 2021). Among these, the most common haplotype in the population and found in homozygous BrS patients is the so-called haplotype 1 (Hap1), followed by Hap2 and Hap3. In order to determine the haplotype composition of the SCN5A-SCN10A locus in European BrS cases and control individuals, we use long-read nanopore sequencing. Additionally, we are also examining the functional effects of the SCN5A-SCN10A haplotype composition in cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs) to determine the association between BrS risk/protection and haplotype composition at a functional level. To this end, we are converting a risk (Hap1/Hap1) to a protective (Hap2/Hap3) haplotype using a CRISPR/Cas9 genome editing technique. The cell line generated will be then differentiated into cardiomyocytes. This should allow us to study cardiac gene expression levels and sodium currents in a valuable cellular model preserving the patient's genetic background.

References:

1. Pinsach-Abuin et al. 2021. Analysis of Brugada syndrome loci reveals that fine-mapping clustered GWAS hits enhances the annotation of disease-relevant variants. Cell Reports Medicine 2 (4): 100250 (PMID: 33948580) https://doi.org/10.1016/j.xcrm.2021.100250.

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THE KIR2.1E299V MUTATION INCREASES ATRIAL FIBRILLATION VULNERABILITY WHILE PROTECTING THE VENTRICLES AGAINST ARRHYTHMIAS IN A MODEL OF SQTS TYPE 3

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Aims: Short QT syndrome type 3 (SQTS3) is a rare arrhythmogenic disease caused by gain-of-function mutations in *KCNJ2*, the gene coding the inward rectifier potassium channel Kir2.1. We used a multidisciplinary approach and investigated arrhythmogenic mechanisms in an *in-vivo* model of *de-novo* mutation Kir2.1E299V identified in a patient presenting an extremely abbreviated QT interval and paroxysmal atrial fibrillation.

Methods and results: We used intravenous adeno-associated virus-mediated gene transfer to generate mouse models, and confirmed cardiac-specific expression of Kir2.1WT or Kir2.1E299V. On ECG, the Kir2.1E299V mouse recapitulated the QT interval shortening and the atrial-specific arrhythmia of the patient. The PR interval was also significantly shorter in Kir2.1E299V mice. Patch-clamping showed extremely abbreviated action potentials in both atrial and ventricular Kir2.1E299V cardiomyocytes due to a lack of inward-going rectification and increased IK1 at voltages positive to −80 mV. Relative to Kir2.1WT, atrial Kir2.1E299V cardiomyocytes had a significantly reduced slope conductance at voltages negative to −80 mV. After confirming a higher proportion of heterotetrameric Kir2.x channels containing Kir2.2 subunits in the atria, *in-silico* 3D simulations predicted an atrial-specific impairment of polyamine block and reduced pore diameter in the Kir2.1E299V-Kir2.2WT channel. In ventricular cardiomyocytes, the mutation increased excitability by shifting INa activation and inactivation in the hyperpolarizing direction, which protected the ventricle against arrhythmia. Moreover, Purkinje myocytes from Kir2.1E299V mice manifested substantially higher INa density than Kir2.1WT, explaining the abbreviation in the PR interval.

Conclusion: The first *in-vivo* mouse model of cardiac-specific SQTS3 recapitulates the electrophysiological phenotype of a patient with the Kir2.1E299V mutation. Kir2.1E299V eliminates rectification in both cardiac chambers but protects against ventricular arrhythmias by increasing excitability in both Purkinje-fiber network and ventricles. Consequently, the predominant arrhythmias are supraventricular likely due to the lack of inward rectification and atrial-specific reduced pore diameter of the Kir2.1E299V-Kir2.2WT heterotetramer.

References:

Cardiovascular Research, cvae019 (https://doi.org/10.1093/cvr/cvae019)

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APPLICATION OF SHANNON INFORMATION THEORY TO THE STUDY OF SENSORY ION CHANNELS

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Sensory ion channels, such as TRPs and Piezos, mediate cellular responses to thermal, chemical and mechanical stimuli. Elucidating how these channels transduce environmental stimuli into electrical and biochemical signals is crucial to understand their roles in physiological processes and human diseases. We used a two-state gating model to assess, theoretically, the molecular determinants of polymodal gating in sensory ion channels. We found that the equations of channel sensitivities to different stimuli (*X*), determined as the first derivative of the open probability, have the same structure. This is, the variance of the open probability $\sigma^2 = P_O(1 - P_O)$ multiplied by the negative derivative of the opening free energy with respect to the stimulus: $\frac{\partial P_O}{\partial X} = \sigma^2 \cdot \left(-\frac{\partial \Delta \mathcal{G}}{\partial X}\right)$. Multi-state models yield a similar equation, with σ^2 as a recurrent factor, indicating that the channel sensitivity is proportional to how much the channel can alternate between states of distinct conductance. This is reminiscent of a cornerstone result of Shannon Information Theory stating that the efficiency of a communication channel depends on how variable (or entropic) the output of the channel is. The calculated steady-state Shannon entropy, which quantifies the average information produced at the channel output, equals to the sum of two terms. One, is a positive term related to the standard deviation of the open probability, while the other is a negative term related to how much the energy of the stimulus biases the gating of the channel towards the open or closed states. Application of this rationale to experimental data on TRP and Piezo channels reveal how channel gating stochasticity and physical sensitivity contribute distinctively in different ranges of applied stimuli. These results illustrate that sensory ion channels can be viewed as information channels encoding input stimuli into output ionic currents and that they can be studied with Shannon Information Theory, a meta-disciplinary formalism originally conceived to assess the efficiency of communication channels. Future studies using this framework may shed light into other intriguing aspects of these channels, such as their regulation mechanisms, the relevance of polymodality and their interactions with other components of cellular signaling pathways.

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TETRODOTOXIN REDUCES MECHANICAL AND THERMAL HYPERSENSITIVITY IN A MODEL OF RHEUMATOID ARTHRITIS

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Introduction: Pain associated with rheumatoid arthritis (RA) is highly prevalent and the main concern of patients with this disease [1]. However, there are no specific drugs for treating this pain, and classic analgesics, mainly NSAIDs, are used. These are often ineffective and have adverse effects that limit their use [2]. Tetrodotoxin (TTX), a voltage-gated sodium channel blocker, has shown efficacy in various animal models of pain and in clinical trials (phase III) for cancer-associated pain [3]. The aim of this study is to evaluate the effect of tetrodotoxin treatment on pain associated with RA.

Methods: The collagen-induced arthritis (CIA) model was employed in female Wistar rats. Peripheral inflammation was evaluated by measuring paw thickness with an electronic caliper, mechanical allodynia with the von Frey test, and heat hyperalgesia with the Hargreaves test. TTX was administered subcutaneously on day 13 after immunization. Doses of 6 and 9 µg were used, and behavioral assessments were performed 0.5, 1, and 24 h after administration.

Results: Paw inflammation began on day 11-12 and reached its maximum value on day 18. However, mechanical allodynia and heat hyperalgesia appeared earlier, on day 8, and by day 13, they were nearly maximal. Acute treatment on day 13 with TTX reduced mechanical allodynia and heat hyperalgesia in a dose- and time-dependent manner. The effect completely disappeared 24 h after administration.

Conclusion: Acute treatment with TTX reverses mechanical allodynia and thermal hyperalgesia associated with collagen-induced arthritis. These results suggest that TTX could be useful for treating pain associated with rheumatoid arthritis.

References:

1. Hewlett S, Sanderson T, May J, et al. 'I'm hurting, I want to kill myself': rheumatoid arthritis flare is more than a high joint count--an international patient perspective on flare where medical help is sought. Rheumatology (Oxford). 2012;51(1):69-76. doi:10.1093/rheumatology/keq455
2. Allen A, Carville S, McKenna F; Guideline Development Group. Diagnosis and management of rheumatoid arthritis in adults: summary of updated NICE guidance. BMJ. 2018;362:k3015. doi:10.1136/bmj.k3015
3. Huerta MÁ, de la Nava J, Artacho-Cordón A, Nieto FR. Efficacy and Security of Tetrodotoxin in the Treatment of Cancer-Related Pain: Systematic Review and Meta-Analysis. Mar Drugs. 2023;21(5):316. doi:10.3390/md21050316

Acknowledgements: *FPU Program (Ministry of Universities). Research project PID2021-123058NA-I00 funded by MCIN/AEI/10.13039/501100011033/ and by ERDF A way to make Europe, and the Andalusian Regional Government (grant CTS-109).*

THE IMPACT OF EPILEPSY ASSOCIATED MUTATIONS IN GLUN2B SUBUNIT ON BK-NMDAR FUNCTIONAL COUPLING

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The large conductance calcium-activated potassium channels (BK channels) are characterized by responding to two distinct physiological stimuli: intracellular Ca^{2+} and membrane depolarization. In neurons, these channels are activated in a coordinated manner by both stimuli, but elevated concentrations of intracellular Ca^{2+} (between 1 and 10 μM) are required for them to respond to physiological voltage changes. Therefore, it is believed that most BK channels are located in nanodomains (about 20-50 nm distance) near sources of Ca^{2+}, such as N-methyl-D-aspartate receptors (NMDAR). Isaacson and Murphy's work in 2001 first demonstrated NMDAR-BK channel coupling in the olfactory bulb (1). Since then, new evidence has emerged regarding the existence of functional coupling between NMDAR and BK channels in other regions of the brain (2), as well as their physiological relevance in neuronal function (3). Several inherited and de novo mutations in genes encoding NMDAR subunits have been directly associated with neurodevelopmental disorders, such as intellectual disability, epilepsy, and autism spectrum disorders. An example is the V15M and V618G mutations in the GRIN2B gene, which encodes the GluN2B subunit of NMDAR, linked to the pathology of EIEE27 (early infantile epileptic encephalopathy). By employing a combination of electrophysiological, immunohistochemical, and imaging techniques, we further characterized the effect of these GluN2B mutations on NMDAR-BK channel coupling. Overall, our results suggest that: i) despite the V15M mutation reduced membrane expression, these channels are capable of efficiently couple with BK channels, and ii) the V618G mutation shows an increase in membrane expression and has a Ca^{2+} permeability comparable to GluN2B(WT); however, it selectively disrupts functional coupling with BK channels. Future studies will be necessary to determine the molecular basis responsible for the effects on BK channel coupling produced by the V618G mutant. Taken all together, our findings reveal some disease-related GluN2B mutations that reduce the NMDAR-BK interaction, either by altered interactions with the BK channels and/or by functional uncoupling the NMDAR-BK complexes. This research highlights the importance of elucidating the molecular mechanisms underlying such mutations, providing crucial insights into the pathophysiology of neurodevelopmental disorders and offering potential avenues for therapeutic intervention.

References:

1. Isaacson JS, Murphy GJ. Glutamate-Mediated Extrasynaptic Inhibition: Direct Coupling of NMDA Receptors to Ca2+-Activated K+ Channels. Neuron 2001;31(6):1027–34.
2. Zhang J, Guan X, Li Q, Meredith AL, Pan HL, Yan J. Glutamate-activated BK channel complexes formed with NMDA receptors. Proc Natl Acad Sci USA 2018; 115(38):E9006–14.
3. Gómez R, Maglio LE, Gonzalez-Hernandez AJ, Rivero-Pérez B, Bartolomé-Martín D, Giraldez T. NMDA receptor-BK channel coupling regulates synaptic plasticity in the barrel cortex. Proc Natl Acad Sci USA 2021;118(35):e2107026118.

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MOLECULAR PLAYERS INVOLVED IN INTRACELLULAR CALCIUM HOMEOSTASIS IN PRIMARY CULTURES OF HUMAN GLIOBLASTOMA CELLS

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Evidence indicate that cancer cells show changes in intracellular Ca^{2+} homeostasis (Ca^{2+} remodeling) that may contribute to cancer hallmarks. Understanding of these changes may lead to new markers and/or treatments for cancer. Here we aimed at investigating Ca^{2+} remodeling in glioblastoma, the most malignant form of human brain tumors. For this end, fresh samples of human glioblastoma were quickly obtained at the time of surgery and pooled into two parts. A part was frozen for transcriptomic analysis and the remaining tissue was used to establish a primary culture. After 24-48 h, cultured tumor cells were used for calcium imaging experiments. Other, less malignant tumors were also used for comparison. Calcium imaging experiments in glioblastoma cells and cells from other brain tumors indicate that glioblastoma cells show intermediate levels of resting intracellular [Ca^{2+}], very low Ca^{2+} responses to activation of voltage-gated Ca^{2+} channels but large responses to ATP and to activation of store-operated Ca^{2+} entry (SOCE) that increases with tumor malignancy. Mitochondrial depolarization inhibits SOCE, suggesting mitochondrial Ca^{2+} uptake prevents SOCE inactivation in glioblastoma. Consistently, mitochondrial depolarization and SOCE antagonist BTP2 inhibit glioblastoma cell proliferation. Transcriptomic analysis was carried out using illumina to identify genes involved in intracellular Ca^{2+} homeostasis in glioblastoma. Our results show that glioblastoma cells express six different types of voltage-gated Ca^{2+} channels including Cav1.2, Cav1.3, Cav2.1, Cav2.3, Cav3.1 and Cav3.2; seven purinergic receptors including ionotropic P2X4, 6 and 7 and metabotropic P2Y1,6,12 and 13; fourteen different types of transient receptor (TRP) channels including TRPC1,3,4 and 6, TRPV1-3, TRPM2,3,4 and 7, TRPML1 and TRPP1 and 2. Glioblastoma cells also express most players involved in SOCE including ORAI1-3, STIM1-2 and the following modulators CRACR2A, STIMATE, ORMDL3, SARAF and Septins2 to 11. Glioblastoma cells express also three different Ca^{2+} release channels at the endoplasmic reticulum, IP3R1-3 and RYR1 and 3. Regarding Ca^{2+} extrusion systems, glioblastoma cells express six different types of Ca^{2+} pumps including PMCA1,2, and 4, SERCA2 and 3 and SPCA1; two types of Na^{+}/Ca^{2+} exchangers, NCX1 and 3; and, finally, all tested mitochondrial Ca^{2+} transport systems. These results may provide new insights on Ca^{2+} remodeling in glioblastoma.

References:

1. Hernando-Pérez E, Pérez-Riesgo E, Cepeda S, Arrese I, Sarabia R, Villalobos C, Núñez L. Differential Ca2+ responses and store operated Ca2+ entry in primary cells from human brain tumors. Biochim Biophys Acta Mol Cell Res. 2021 Jul;1868(8):119060. doi: 10.1016/j.bbamcr.2021.119060. Epub 2021 May 14. PMID: 33992673.

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EXPLORING THE LINK BETWEEN CPT1c DEFICIENCY, AMPA RECEPTORS EXPRESSION AND NOCICEPTION

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In recent years, pain research has experienced an exponential growth. However, despite these advancements, our understanding of this very complex subject remains far from comprehensive. The majority of research efforts in the field of nociception have focused on elucidating the role of ion channels and receptors in sensory and spinal cord (SC) neurons. Conversely, there has been relatively less emphasis on exploring the involvement of intracellular proteins in this phenomenon. One such protein is carnitine palmitoyltransferase 1C (CPT1c), which plays multiple roles in different processes ranging from learning and memory to lipid metabolism. Particularly relevant to our study is CPT1c's ability to modulate the surface expression of AMPA-type glutamate receptors by interacting with them within the endoplasmic reticulum, specifically with the GluA1 subunit. In this study, we employed a combination of molecular biology techniques, electrophysiology, calcium imaging and behavioral assessments in mice to explore the potential role of CPT1c in modulating nociception through the regulation of AMPARs in both sensory neurons from the dorsal root ganglia (DRGs) and spinal cord neurons.

Electrophysiological recordings of small-diameter DRG neurons show hyperexcitability with no changes in action potential (AP) threshold, particularly in non-peptidergic nociceptors, which highly express GluA1 under wild-type conditions. Analyses of APs also indicate an elevated afterhyperpolarization phase in CPT1c-deficient nociceptors, suggesting potential alterations in K^+ conductance. Intracellular calcium mobilization in response to AMPA and glutamate stimulation are enhanced in CPT1c KO SC neurons in culture. Consistent with these findings, AMPA-evoked currents from cultured SC neurons are also slightly increased in the KO group. Western-blot analyses show sex-differences in AMPAR subunit expression. We found a decrease in GluA1 expression in the SC of CPT1c female animals, but not in males. Additionally, samples from KO males exhibit a significant reduction in GluA2 expression. Lastly, behavior assessments of CPT1c-deficient animals exhibit enhanced sensitivity to mechanical stimuli, especially in females.

Taken together, our findings suggest that the increased sensitivity observed in KO animals may result from enhanced nociceptive input from the periphery combined with alterations in AMPAR composition in the spinal cord, ultimately impacting neurotransmission and nociception.

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DIFFERENTIAL EXPRESSION OF THE $CA_V2.3$ SUBUNIT OF R-TYPE CALCIUM CHANNEL IN THE BRAIN OF THREE MOUSE MODELS OF ALZHEIMER'S DISEASE

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Calcium plays fundamental roles in many facets of neuronal physiology such as gene expression, growth and differentiation, synaptogenesis, dendritogenesis, membrane excitability, neurotransmitter release, synaptic plasticity and learning and memory (1). Voltage-gated calcium (Ca_V) channels are required for many of those brain functions. Among the Ca_V channel family, R-type calcium ($Ca_V2.3$) channels play important roles in hippocampal functions including synaptic plasticity (2), a neuronal process known to be altered in neurodegenerative diseases. However, no information is available about the potential alteration of $Ca_V2.3$ in Alzheimer´s disease (AD). The goal of this work is to determine the possible alteration of $Ca_V2.3$ in Aβ and tau pathology. Here, we provide a quantitative description on the expression and distribution patterns of $Ca_V2.3$ in three transgenic mice models (APP/PS1, P301S and 5xFAD mice) of Alzheimer´s disease (AD), combining histoblots and immunoelectron microscopic approaches. Using the histoblot technique we revealed differences in the expression of $Ca_V2.3$ that is directly dependent on the transgenic mouse model. Thus, the expression of $Ca_V2.3$ was significantly reduced in the hippocampus of 5xFAD mice in a laminar-specific manner at 10 months of age but was unaltered in APP/PS1 mice at 12 months or in P301S at 10 months compared to age-matched wild type mice. This reduction in the expression of $Ca_V2.3$ protein was confirmed using western blot analysis. Ultrastructural approaches using the pre-embedding immunogold technique, demonstrated that the subcellular localization of $Ca_V2.3$ was significantly reduced along the neuronal surface of CA1 pyramidal cells. Altogether, our findings provide evidence of specific alterations of $Ca_V2.3$ in the hippocampus in Aβ pathology, suggesting the involvement of Cav2.3 in the mechanisms causing abnormal network activity of the hippocampal circuit and cognitive impairment characteristic of 5xFAD mice.

References:

1. Kawamoto EM, Vivar C, Camandola S. (2012) Physiology and pathology of calcium signaling in the brain. Front Pharmacol. 3:61. doi: 10.3389/fphar.2012.00061.
2. Parajuli LK, Nakajima C, Kulik A, Matsui K, Schneider T, Shigemoto R, Fukazawa Y. (2012) Quantitative regional and ultrastructural localization of the Ca(v)2.3 subunit of R-type calcium channel in mouse brain. J Neurosci. 32(39):13555-67.

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ZINC IMBALANCE POTENTIATES TRPM7-MEDIATED MOTOR NEURON DEATH

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Zinc is known to participate actively in the functioning of the nervous system. Its distribution is tightly regulated, predominantly found in glutamatergic neurons tuning the excitability. The excessive release of zinc has been demonstrated to contribute to excitotoxicity upon transient ischemic insults or epileptic seizures, partly facilitated by TRPM7 activity, a zinc-permeable cation channel. Zinc has been shown to increase reactive oxygen species (ROS) production via the NADPH oxidase and mitochondrial dysfunction.

Amyotrophic Lateral Sclerosis hallmarks include glutamatergic excitotoxicity, ROS generation, and metal imbalance. Studies have shown that zinc levels are elevated in the cerebrospinal fluid of ALS patients. Moreover, a genetic association between TRPM7 and ALS has been established. We hypothesize that increased zinc levels can potentiate TRMP7 activity, leading to relevant pathological processes in the context of motor neuron disease. In this study, we show electrophysiological data of TRPM7 in HEK293 cells illustrating that TRPM7 activity is enhanced by pathophysiological concentrations of zinc. Using NSC-34 cells, a motor neuron-like cell model, we show that TRPM7 activation causes cell death in a zinc-dependent manner. To elucidate the pathological mechanism, we characterize zinc movements in the mitochondria, we measure the mitochondrial membrane potential and we assess the generation of ROS upon activation of TRPM7. Overall, our findings confirm that zinc imbalance and its effect on TRPM7 causes the death of NSC-34 cells by disrupting mitochondrial physiology. The validation of these results in other motor neuron models and in vivo models will be essential. Currently, there is no cure for ALS and treatment options are limited. Further confirmation of these results could open the door for novel pharmacological interventions for ALS patients.

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NOVEL HETEROCYCLES FOR NEUROPROTECTION OF RAT HIPPOCAMPAL NEURONS AGAINST EXCITOTOXICITY AND ALZHEIMER´S DISEASE: ROLE OF INTRACELLULAR CA^{2+}

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Alzheimer´s disease (AD) is the most common dementia affecting nearly hundred of thousand patients only in Spain. Unfortunately, a few treatments are available and none of them is efficient in terms of interrupting the progression of the disease. The most important risk factor for developing AD is aging. However, aging is most frequently not taken into account when designing models and/or strategies for developing new treatments. AD is associated to excess of amyloid β peptide and excitotoxicity that may lead to synaptic dysfunction and neuron cell death by a mechanism that may involve changes in intracellular Ca^{2+} homeostasis that are exacerbated by aging. We have introduced recently long-term cultures of newborn rat hippocampal neurons as cell models for studying Ca^{2+} remodelling in aging and AD. Here we used these cell cultures to investigate the effects of novel compounds based in heterocycles on cell death induced by glutamate receptor agonists and amyloid β peptide oligomers (Aβo). We found that several heterocycles failed to inhibit neuron cell death induced by NMDA and Aβo. However, two novel compounds named AB1 and AB2 significantly prevented neuron cell death induced by NMDA and Aβo as assessed by Annexin V assay. In addition, we also found that NMDA and Aβo induced large increases in intracellular Ca^{2+} in aged neurons in vitro. We studied the possible effect of these compounds on Ca^{2+} responses induced by activation of glutamate receptors, voltage-gated Ca^{2+} channels and Ca^{2+} rises induced by oligomers of amyloid β peptide (Aβo). Accordingly, we present novel selected heterocycles that prevent neuron cell death induced by excitotoxicity and Aβo and the possible role of Ca^{2+} responses in neuroprotection against AD.

References:

1. Núñez L, Calvo-Rodríguez M, Caballero E, García-Durillo M, Villalobos C. Neurotoxic Ca2+ Signaling Induced by Amyloid-β Oligomers in Aged Hippocampal Neurons In Vitro. Methods Mol Biol. 2018;1779:341-354. doi: 10.1007/978-1-4939-7816-8_20. PMID: 29886542.

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TRESK IN MRGPRD$^+$ NEURONS-MEDIATED COLD SENSITIVITY

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TRESK background potassium channel plays a critical role in modulating the action potential firing and excitability of primary sensory neurons. It is selectively expressed in populations of neurons involved in the perception of touch (low-threshold mechanoreceptors) and pain (nociceptors) and its depletion results in enhanced pain sensitivity. Recent studies indicate that TRESK channel reduces mice's cold and mechanical sensitivity. Nevertheless, it is not clear whether it is involved in the cold sensing mediated by neurons from the dorsal root ganglia (DRG).

Using wild type and TRESK knock-out mice, we have explored the role of the channel in a population of DRG nociceptors expressing the MrgprD receptor (MrgprD$^+$ neurons). In a multidisciplinary study, we have validated that TRESK is expressed in 70% of MrgprD$^+$ neurons and that it modulates their excitability, reducing the number of neurons activated by the MrgprD specific agonist β-alanine in male and female mice, and by cold temperatures in female mice. Moreover, we have also observed that TRESK modulates the cold responsiveness of other populations of DRG nociceptors and TRPM8-expressing primary sensory neurons.

Although we have found that TRESK seems to modulate mice's MrgprD$^+$ neurons-mediated cold sensitivity, knocking out the channel does not influence DRG-mediated mice's cold sensitivity. Moreover, we have found that TRESK inactivation by the calcineurin inhibitor Tacrolimus does not affect DRG-mediated mice's sensitivity to cold temperatures. Nevertheless, activating DRG MrgprD$^+$ neurons with β-alanine seems to increase the cold sensitivity of TRESK KO but not WT mice.

In summary, TRESK modulates the excitability of MrgprD$^+$ nociceptors and their activation by cold temperatures. Moreover, the channel seems to participate in the mice's cold sensitivity mediated by MrgprD$^+$ neurons, which is not sufficient to impact mice's DRG-mediated cold sensitivity in physiological conditions and in a model of calcineurin inhibitor-induced pain syndrome.

References:

1. Castellanos, Aida, Anna Pujol-Coma, Alba Andres-Bilbe, Ahmed Negm, Gerard Callejo, David Soto, Jacques Noël, Nuria Comes, and Xavier Gasull. 2020. "TRESK Background K+ Channel Deletion Selectively Uncovers Enhanced Mechanical and Cold Sensitivity." Journal of Physiology 598(5): 1017–38. doi:10.1113/JP279203.
2. Guo, Zhaohua, Chang Shen Qiu, Xinhua Jiang, Jintao Zhang, Fengxian Li, Qin Liu, Ajay Dhaka, and Yu Qing Cao. 2019. "TRESK K+ Channel Activity Regulates Trigeminal Nociception and Headache." eNeuro 6(4). doi:10.1101/679530.
3. Llimós-Aubach, Júlia, Alba Andres-Bilbe, Anna Pujol-Coma, Irene Pallás, Josep Maria De Anta, Concepció Soler, Núria Comes, Gerard Callejo, and Xavier Gasull. "TRESK Background Potassium Channel in MrgprA3+ Pruriceptors Regulates Acute and Chronic Itch." Preprint paper. doi:10.1101/2024.01.25.577205.

Acknowledgements: *European Union, Fondo Europeo de Desarrollo Regional (FEDER), Ministerio de Ciencia e Innovación (PID2020-119305RB-I00), Ministerio de Ciencia e Innovación and Instituto de Salud Carlos III (FIS PI1700296), Generalitat de Catalunya (2017-SGR-737, 2021-SGR-00292), Maria de Maeztu (MDM-2017-0729, CEX-2021-001159-M), RETIC (RD16-0008-0014), Pain-Net Network (H2020-MSCA-ITN-2016) and Universitat de Barcelona PREDOCS-UB (2019-2020).*

ENHANCING THE ANALGESIC EFFECTS OF PREGABALIN BY THE SIGMA-1 RECEPTOR BLOCKADE WITHOUT INCREASING THE SIDE-EFFECTS

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Chronic pain, such as neuropathic pain, is very disabling and very prevalent. Pregabalin modulates the α2δ auxiliary subunits of some voltage-gated calcium channels and is one of most-used drugs for neuropathic pain, but unfortunately, it is not fully effective and produces many side effects[1]. We studied the modulation of pregabalin-induced analgesia by sigma-1 receptor (σ_1-R) inhibition in order to improve its therapeutic range (without increasing side effects) in two translational models of preclinical pain.Experiments were performed on female CD-1 mice. To induce mechanical allodynia we used intraplantar capsaicin (1µg); we also evaluated mechanical allodynia and thermal hyperalgesia in a model of neuropathic pain. Pregabalin (0.312-60 mg/kg) and S1RA (5-120 mg/kg), a σ_1-R antagonist, were injected alone or in combination subcutaneously (s.c.). To test the influence of σ_1-R inhibition, the σ_1-R agonist PRE-084 (5-40 mg/kg; s.c.) was administered. To investigate whether the association of S1RA and pregabalin potentiates the adverse effects of pregabalin, we evaluated the effect of these drugs on two frequent adverse effects produced by pregabalin: motor incoordination with the rotarod test and constipation by measuring the inhibition of gastrointestinal transit. The association of sub-analgesic doses of pregabalin and S1RA markedly potentiated both capsaicin- and neuropathic-induced mechanical allodynia, and produced additive effects on neuropathic-induced thermal hyperalgesia, without alteration of motor coordination or gastrointestinal transit. The administration of PRE-084 together with S1RA and pregabalin completely abolished the potentiated effects on capsaicin-induced pain and neuropathic pain, without affecting that of pregabalin per se. The results of this study show that the association of the σ_1-R antagonist S1RA and pregabalin is very advantageous since it increases the therapeutic range of pregabalin, inhibiting the painful hypersensitivity associated to capsaicin and neuropathic pain at low doses, without producing the typical adverse effects produced by pregabalin at higher doses. The association of pregabalin and S1RA might represent a new therapeutic strategy in neuropathic pain.

References:

1. Meaadi J, Obara I, Eldabe S, Nazar H. The safety and efficacy of gabapentinoids in the management of neuropathic pain: a systematic review with meta-analysis of randomised controlled trials. Int J Clin Pharm 2023;45(3):556-565.

Acknowledgements: *Research project funded by Junta de Andalucía (CTS-109) and Esteve Pharmaceuticals.*

NA_V CHANNELS AS KEY THERAPEUTIC TARGETS FOR OXALIPLATIN-INDUCED PERIPHERAL NEUROPATHY: ALTERATIONS IN MALE AND FEMALE RAT SMALL SENSORY NEURONS

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Oxaliplatin is one of the most common chemotherapeutic agents prescribed for digestive tract tumors. Unfortunately, it induces a severe peripheral neuropathy (OIPN) that can provoke acute and chronic pain, drastically limiting its clinical use. It is well-known that this neuropathy originates from the functional alteration of peripheral sensory neurons. Among the molecular mechanisms involved, Na_V channels are potential key therapeutic targets due to their crucial role in neuronal excitability. However, the contribution of Na_V channels from nociceptors to oxaliplatin-induced sensitization is poorly understood. In this study, we investigated the oxaliplatin direct effect on the electrical and Na_V activities of small-diameter dorsal root ganglion (DRG) neurons. To do this, we developed an OIPN *in vitro* preclinical model by incubating the DRG neuronal cultures for 48 hours with oxaliplatin. As a result, the oxaliplatin treatment resulted in a potent and selective increase in the excitability of the IB4(+) population of neurons. The hyperexcitability detected correlated with a potentiation of the somatic Na_V currents. Pharmacological dissection of these currents showed a major effect on tetrodotoxin (TTX)-resistant currents, where oxaliplatin treatment caused an increase in the sodium current amplitude, an 8-mV hyperpolarizing shift in its activation curve, and a faster recovery from inactivation. Furthermore, the potential sex differences were examined by analyzing male and female rat DRG cultures separately. While both sexes exhibited increased action potential firing frequency and reduced current rheobase following oxaliplatin treatment, females displayed greater sensitivity, with augmented spontaneous activity and depolarized resting membrane potential. Male and female TTX-resistant currents showed a higher amplitude and hyperpolarizing shift, appearing as major contributors to the nociceptor hyperexcitability caused by oxaliplatin in both sexes. Nevertheless, we could not discard a minor contribution of TTX-sensitive Na_V channels in the hyperexcitability of male nociceptors due to the slower inactivation kinetics found in TTX-sensitive currents and the hyperpolarizing shift detected in the voltage dependence of $Na_V1.6$. Altogether, our findings underscore TTX-resistant channels as potential therapeutic targets for attenuating OIPN symptoms and highlight the utility of *in vitro* preclinical models for investigating signaling pathways and sexual dimorphism in peripheral neuropathies.

References:

1. Villalba-Riquelme, E., de la Torre-Martínez, R., Fernández-Carvajal, A., & Ferrer-Montiel, A. (2022). Paclitaxel *in vitro* reversibly sensitizes the excitability of IB4(-) and IB4(+) sensory neurons from male and female rats. *British journal of pharmacology*, *179*(14), 3693–3710. https://doi.org/10.1111/bph.15809

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INVESTIGATION OF GABAERGIC COMMUNICATION WITHIN THE AXONS OF PRIMARY SENSORY NEURONS IN A MICROFLUIDIC CULTURE SYSTEM

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The GABAergic cross-talk between the cell bodies of primary sensory neurons within the Dorsal Root Ganglia (DRGs), has been shown to modulate the transmission of painful stimuli[1,2]. However, the possibility of crosstalk between the nerve fibers within the peripheral nerve, is yet to be explored. To address this aspect, we recapitulated the sensory neuron axonal morphology in a novel compartmentalized microfluidic system[3]. This setup included co-culturing DRG and dorsal horn (DH) neurons, which allowed not only to better mimic the physiology and morphology of the peripheral neuron *in vitro*, but also to carry out investigation of signaling processes at distinct segments of somatosensory axon. Our observations reveal a subset of DRG neurons exhibiting typical pseudo-unipolar morphology with T-junction and polarized axonal branches, traversing both, 'peripheral' and DH compartments. Immunocytochemical investigations revealed the expression of $GABA_A$ receptor subunits, including γ_2, α_1 and α_2, along cultured DRG neurons axons and selective axonal stimulation with GABA (100 mM), evoked calcium events associated with action potentials (APs) at the cell bodies. Interestingly, Iodide imaging of HEK_{GABAA}-EYFP reporter cells co-cultured with DRG neurons axons, showed that Capsaicin and KCl^- application at the peripheral compartment triggered GABA release both from axons themselves and from cell bodies, likely via vesicular exocytosis. These experiments reveal new level of axonal crosstalk within the peripheral nerves. The microfluidic culture system constitutes a valuable tool for exploring axonal dynamics contributing to the modulation of the transmission of sensory information.

References:

1. Du, X., Hao, H., Yang, Y., Huang, S., Wang, C., Gigout, S., Ramli, R., Li, X., Jaworska, E., & Edwards, I. (2017). Local GABAergic signaling within sensory ganglia controls peripheral nociceptive transmission. *The Journal of Clinical Investigation*, *127*(5), 1741–1756.
2. Hao, H., Ramli, R., Wang, C., Liu, C., Shah, S., Mullen, P., Lall, V., Jones, F., Shao, J., & Zhang, H. (2023). Dorsal root ganglia control nociceptive input to the central nervous system. *PLoS Biology*, *21*(1), e3001958.
3. Vysokov, N., McMahon, S. B., & Raouf, R. (2019). The role of NaV channels in synaptic transmission after axotomy in a microfluidic culture platform. *Scientific Reports*, *9*(1), 12915.

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FUNCTIONAL ANALYSIS OF KCNK18 GENETIC VARIANTS ASSOCIATED WITH NEUROPATHIC PAIN

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Neuropathic pain (NeuP) arises from lesions or diseases affecting the somatosensory nervous system, with origins ranging from trigeminal neuralgia to diabetic neuropathy and small-fiber neuropathy (SFN). The complex pathophysiology requires a multidisciplinary approach to study NeuP effectively. Patients commonly report a wide range of abnormal sensations such as stabbing or burning pain, itching, numbness, hyperalgesia, or allodynia. Since pharmacological treatments often offer limited relief, understanding the genetic basis of NeuP is crucial for advancing its management. We have recently identified novel potentially pathogenic gene variants in KCNK18, which encodes the leak K^+ channel TRESK, and the transient receptor potential channels TRPA1, TRPM2, TRPM8, TRPV1, and TRPV3, along with ANO3 encoding a Ca^{2+}-gated Cl^- channel in SFN patients. It highlights the involvement of these ion channels in sensory processing and pain perception[1,2]. TRESK, encoded by KCNK18, exhibits a predominant expression in the dorsal root ganglia and the trigeminal ganglia, where leaky K^+ currents act as a regulatory mechanism to attenuate neuronal activation. Thus, TRESK KO mice manifest an enhanced mechanical sensitivity and cold allodynia[3]. The p.(Met370Cysfs*?) and p.(Ser252Leu) KCNK18 variants have emerged as two of the most relevant and potentially pathogenic mutations in the analyzed patient cohorts. Specifically, the heterozygous thymine deletion at c.1107 (p.(Met370Cysfs*?)) results in a frameshift mutation, leading to an extended out-of-frame C-terminus. By quantifying current density with whole-cell patch clamp, we observed a significant reduction of basal current density in the p.(Met370Cysfs*?) TRESK compared to the wild-type TRESK. It may heighten neuronal susceptibility to stimuli, potentially contributing to increased excitability. These findings are further supported by the reduced expression of the p.(Met370Cysfs*?) TRESK at the plasma membrane, as revealed by our confocal microscopy studies. In addition, the p.(Ser252Leu) KCNK18 exhibits a notable reduction in basal current density, possibly attributed to altered channel phosphorylation, suggesting a potential compromise in neuronal excitability. Notably, we have found larger response to intracellular Ca^{2+} concentration in the p.(Ser252Leu), indicating an altered calcineurin-dependent modulation. We currently aim to elucidate genotype-phenotype correlations to deepen our understanding of the genetic basis of NeuP and to improve its management, ultimately seeking for enhanced treatment outcomes.

References:

1. Ślęczkowska M, Almomani R, Marchi M, Salvi E, de Greef BTA, Sopacua M, Hoeijmakers JGJ, Lindsey P, Waxman SG, Lauria G, Faber CG, Smeets HJM, Gerrits MM. Peripheral Ion Channel Genes Screening in Painful Small Fiber Neuropathy. Int J Mol Sci. 2022 Nov 15; 23(22):14095.
2. Ślęczkowska M, Misra K, Santoro S, Gerrits MM, Hoeijmakers JGJ; PainNet Study Group. Ion Channel Genes in Painful Neuropathies. Biomedicines. 2023 Sep 29; 11(10):2680.
3. Castellanos A, Pujol-Coma A, Andres-Bilbe A, Negm A, Callejo G, Soto D, Noël J, Comes N, Gasull X. TRESK background K+ channel deletion selectively uncovers enhanced mechanical and cold sensitivity. J Physiol. 2020 Mar; 598(5):1017-1038.

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THERMO TRP ION CHANNELS AS A KEY MOLECULAR AND FUNCTIONAL LANDMARK FOR NEUROPATHIC PAIN TRANSDUCTION IN SUBSETS OF SOMATOSENSORY NEURONS

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Paclitaxel-induced peripheral neuropathy (PIPN) is a challenging side effect arising from treatment of several anti-cancer agents that constitutes a major medical and societal problem because there is no effective prevention or treatment method. The direct impact paclitaxel on sensory neuron excitability and potential gender differences remains unclear.

We employed a long-term (15 days in vitro) primary culture of mice dorsal root ganglion (DRG) neurons to examine how two consecutive administrations of paclitaxel influence the electrical activity of IB4(+) and IB4(-) sensory neurons of male and female adult mice during sensitization and resolution. Paclitaxel was found to enhance spontaneous activity and amplify tonic firing of action potentials in both IB4(-) and IB4(+) neurons. Although these effects vanished 96 hours post-initial treatment, they remained consistent after the second administration. Paclitaxel also decreased the current rheobase for action potential firing by expediting the after-hyperpolarization phase. Furthermore, the drug influenced Na^+ ion currents, notably increasing the activity of TRPV1, TRPM8, and TRPA1 channels upon the second dose. Intriguingly, female DRGs neurons exhibited greater sensitivity to paclitaxel-induced sensitization compared to their male counterparts.

Our findings suggest that paclitaxel enhances the electrogenicity of sensory neurons by altering the activity of thermoTRPs and unveil sex-related differences. Our in vitro, pre-clinical PIPN model serves as a valuable tool for exploring the dynamics and molecular mechanisms underlying nociceptor sensitization and desensitization by chemotherapeutics and algesic agents, as well as for evaluating modulators of neural excitability with high clinical translational potential.

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